ANNUAL REPORT 1981

COLD SPRING HARBOR LABORATORY

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Cold Spring Harbor Laboratory
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1981 Annual Report
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Front and back covers: Sammis Hall, new residençe facility at the Banbury Conference Center. Photos by Korab, Ltd.

COLD SPRING HARBOR LABORATORY

COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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DIRECTOR'S REPORT 1981

The daily lives of scientists are much less filled with clever new ideas than the public must imagine. To be sure, we have largely been educated with the best thoughts of the past, but as we all learn in school, the reading of history does not let us predict the future. It is not that we are much too satisfied with the earlier triumphs of our youths. Even the most profound leaps forward become boring if lectured on too often. I remember that after giving only some six lectures on the double helix, I had gone stale and desperately needed some new thought to emerge so as to regain the feeling of being alive. But wanting to be clever and achieving it are not the same. During those post-DNA semi-blues, I had moved to Caltech and become friendly with Dick Feynmann, then the brightest star on its or any other physics scene. Then he liked to tell others how hard it was for him to develop a first-rate idea and that nothing of value had come easily. There was always the possibility that Dick was exaggerating his struggles, to make those of us less intellectually endowed more at ease with his brilliance. It seemed wisest, however, to take his words at face value. Success would not necessarily come with hard work, but without its pain true advances were unlikely to fall out. In the now almost 30 years that have since elapsed, I have seen almost no exceptions to this generalization and find that I quickly lose interest in a scientist if I discover that he lacks virtual monomaniacal interest in his work.

Most of our days are thus spent not being clever about the future, but somehow hopefully positioning ourselves so as to be thought clever at some later period. To start with we must know what we want to solve. If we start out to conquer a minor goal, then the odds are hopelessly stacked against us. On the other hand, if we set our sights on an important objective merely because it is there, we should not assume that its intrinsic importance in any way justifies inevitable failure. We all know too many people who have set out to crack the mysteries of the human brain and have only invented new words to describe our continuing deep ignorance of perception, memory, and learning. It is therefore essential to gain somehow a feeling as to whether a given problem might now be solvable or whether we must await the reception of some new facts that as yet do not exist. Here we generally think ahead for a period of one to several years, knowing we are likely to lose our grants or potential for further fellowships if we show no progress. Even more important, failure itself is not pleasant to live with, and our very sanity is likely to demand a move to a more realistic objective.

The question thus should be asked as to whether this past decade's massive preoccupation with the nature of cancer cells was premature given that even today we know so little about normal cells. Most fortunately, we had not bitten off more than we could chew. Our apparent gamble in pushing such large masses of federal monies into research on cancer, as opposed to, say, into developmental biology, was never that far out a bet. For already by the early 1960s unambiguous connections between cancer and many different viruses had been established. It was not that viruses were then thought to be the cause of most cancer. What we liked best about them was that they were the perfect tools to probe the molecular genetics of higher vertebrate cells. Viruses gave us a logical handle to use our brains, as opposed to our emotions, in deciding what to do next.

I first became aware of the possibility of using viruses to study cancer in the fall of 1947 when, as a beginning graduate student at Indiana University, I took Salva Luria's course on viruses. Not until 1959, however, did I see any way to rationally connect viruses and cancer. Then I first learned of Seymour Cohen's recent research showing that the DNA molecules of the T-even bacteriophages coded for proteins involved in DNA synthesis. This discovery opened my mind to the possibility that animal viruses might have similar genes, and soon I was telling everyone within reach that the cancer-causing (oncogenic) capacity of the DNA tumor viruses must arise through their possession of genes that turn on DNA synthesis. But my idea failed to excite anyone else, in large part because there was no way to test it. After a politely cool reception before a large audience at Massachusetts General Hospital, I returned to thinking about protein synthesis. This was a wise move. In contrast to cancer, there were hard facts to think

about, and we would soon be searching successfully for messenger RNA.

Soon afterwards, the exciting molecular nature of the newly discovered DNA tumor viruses, polyoma and SV40, became known. They were among the smallest of any known viruses, having virtually no more genetic information than the smallest bacteriophages. And they could be propagated in cells growing in culture, thereby allowing the routine preparation of radioactively labeled virus. Moreover, through the work of Dulbecco, Stoker, and Sachs these tiny tumor viruses were shown to be capable of converting cultured normal cells into their cancerous ("transformed") equivalents. The stage was thus set to test directly whether the transformed cells contained new genetic information acquired from their respective inducing tumor viruses. These crucial experiments, first done (1965-1968) in Dulbecco's lab at the Salk Institute, revealed the presence of polyoma (SV40)-specific DNA (genes) within the transformed cells. There they had become inserted in the host-cell chromosomal DNA and most likely coded for cancer-inducing proteins.

By this time (the late 1960s) the basic features of the genetic code had been worked out, and with the isolation of the first bacterial repressors there was a widespread feeling among molecular biologists that the moment had arrived to tackle the structure and functioning of higher eucaryotic genes. And given that the most successful route into the genetic essence of *E. coli* had been through its viruses, the phages, the most sensible way to approach the vastly more genetically complex higher eucaryotic cells had to be through intensive molecular characterizations of their viruses. Here, because of their small sizes, the most obvious viruses with which to start were SV40 and polyoma. And so the Dulbecco and Stoker labs began to receive an increasing number of visiting scientists who wanted to learn the essentials of animal virology as the first step in their move into the field of eucaryotic gene regulation.

The migration of so many leading molecular biologists into the DNA tumor virus field between 1968 and 1972 thus in no way reflected the feeling that the cancer problem in any sense was solvable over the short term. Rather, it reflected, as now perceived, a highly intelligent assessment of where the next major advances in molecular genetics would lie.

We did have the worry, however, that growing animal cells in culture would be far more expensive than equivalent work with bacteria, and that barring some unexpected good luck, our molecular attack on tumor viruses would move much more slowly than we would like. In addition, it would consume massive sums of money. So in the summer of 1970 I went to Washington to lobby in favor of a great increase in monies for the NCI (that was to be the War on Cancer), arguing that our goal of identifying viral cancer genes and their functions was now feasible given sufficient funding.

In retrospect, I was far more optimistic than I should have been given the hard facts at my disposal. Even then, almost 20 years after the discovery of the double helix, our tools for directly analyzing DNA were primitive, with most of our past successes with *E. coli* the result of sophisticated genetic analyses that we saw little chance of soon duplicating with higher cells like those of the mouse or humans. On the other hand, I knew well that a rash of unexpected new discoveries had allowed the genetic code to be elucidated much sooner than any of us thought possible when we took on this objective after the discovery of the double helix. And so I hoped that during the 1970s many powerful new experimental procedures would become available if our science continued to be well-funded.

But at first the SV40 work here, as in the other labs that had gone into the DNA tumor virus field, as expected went painfully slowly. Wisely, we did not ask ourselves too often where we were likely to have real answers that might excite us, much less others. Then, almost without warning, the restriction enzymes of Werner Arber were realized to be the almost magical sequence-specific DNA-cutting enzymes that we had so long dreamt about but never seriously thought would exist. And happily there turned out to be many, many cutting enzymes, with Richard Roberts here quickly changing the face of DNA chemistry through his isolation, from a wide variety of bacteria, enzymes that cut at a very large number of unique DNA sequences. Then came recombinant DNA and the easy possibility of the cloning of the DNA fragments made by the specific restriction enzyme cuts. With the development of these cloning procedures, work with tumor virus genes now could move almost as fast as that with bacterial genes, and our initial speculations about the existence of viral cancer genes have turned into the hardest of hard facts.

Now most exciting are powerful recombinant DNA experiments here, at the MIT Cancer Center, at the Sidney Farber Cancer Center of Harvard Medical School, and at the NCI that have resulted in the isolation of several human genes whose misfunctionings lead to cancer. And the next several months will most likely witness the isolation of their respective oncogenic protein products. The pace of our research has thus changed over the past 10 years from that of an impatient snail to that of an almost uncontrolled tornado.

The odds nevertheless remain high that we still have a very long way to go before we can honestly say to the outside world that we understand the chemical essences of many cancer cells. For up till now most of our success has come from analyses at the DNA level, with our knowledge of the fundamental biochemistry of any cell, be it normal or cancerous, woefully incomplete. So while we may soon clearly say that the overabundance

or mistimed appearance of a given protein has led to this or that cancerous state, we shall likely still be a very, very long way from understanding the cellular consequences of this overproduction (mistiming). In pursuing these facts, however, we shall most probably be making one unanticipated discovery after another as to how the vast multitudes of chemical reactions within cells are harmoniously regulated.

Happily the morale of scientists now looking to spot the exact chemical changes that characterize cancerous transformations is very high. They know just enough about normal cells to come up frequently with new hypotheses as to what has gone wrong in cancer cells. And because they now can run very precise 2-D gels and will soon have monoclonal antibodies of ever-increasing specificity, their new ideas can be more quickly checked than ever before. The realization that their latest brainstorm might well be wrong matters much less than the realization that hard facts about cancer cells are rapidly accumulating and that the next decade should witness a virtual flood of relevant information.

As an example, many cancer cells move in much-less-coordinated fashion than their normal equivalents, and common sense tells us to look at the musclelike proteins within cancer cells. Now our Cell Biology group is very excited by changes in amounts of the several tropomyosinlike molecules that accompany cancerous transformations induced by SV40 and adenovirus 2. The significance, however, of these observations is impossible to assess as long as we remain so ignorant as to why our cultured cells each possess several types of tropomyosin. We need to learn more about the tropomyosins themselves, an endeavor that is likely to divert us, at least temporarily, from the cancer cell itself.

The outside world should not, however, be unhappy with the thought that the scientists who now study cancer cells may still have so far to go. For now when I am asked how the recent striking successes of scientists in identifying cancer genes might lead to ways of selectively killing cancer cells, I can give no encouraging response. Without further facts we must guess that most of those proteins whose overproduction (mistiming) leads to cancer are vital cellular constituents that cannot be dispensed with. It thus may not be possible to selectively block their action in cancer cells without simultaneously adversely affecting their respective vital functioning in normal cells.

When, however, we understand the proteins of the cancer cell at the deep level at which they will soon be able to characterize its genes, we may see the cancer problem in a very different light. Now there is no point in being inherently pessimistic on the basis of still hopelessly inadequate information. Instead, our only sensible course of action is to continue to encourage the best scientists we can find to work on the nature of cells both normal and cancerous, and to see that they have all the resources that can be wisely utilized. In so helping them, we should in no way try to plot their course of action. That approach could only be successful if we ourselves knew where the truths of the future will lie.

We must, therefore, have the courage of continuing to face the inherently unknown, trusting that our best younger scientists have been so trained that the unexpected will not disturb but instead be seized for the opportunity to reveal the scientific cleverness that they all hope so badly to display. Only in this way will they have a fighting chance to banish someday from the human condition the dread that the word cancer still brings to the human heart.

HIGHLIGHTS OF THE YEAR

Renewal of Our Program Project Support for Tumor Virus Research

The main financial backing for our tumor virus research comes not from a multitude of relatively small grants to individual scientists, but through a large, single, program project grant from the NCI. It not only provides supply and equipment monies, but also the salaries of many key scientists on our staff. Funds coming to a group as opposed to individuals give us much-needed salary stability. If a given scientist leaves, the monies that support him do not follow him, but remain with us to let us appoint a replacement. Program project grants generally run for five years, and our first such grant commenced in January of 1972. Through it we had the opportunity to start our Nucleic Acid Chemistry Section and attract Richard Roberts to lead it. The very large contribution we made to tumor virus research by the finding and use of new restriction enzymes was possible only through our program project support, as was the development in James Lab of new gel electrophoretic methods for separation of the DNA fragments created by restriction enzyme cuts. So we considered our initial program project monies well spent, as did the Site Visitors who reviewed us and recommended the second five years of support that commenced in January of 1977. Soon afterwards we more than justified this support through our many collective efforts that resulted in our independent discovery of RNA splicing. The many consequences of splicing dominated our work over the next several years, with the pace of this research increasing following the introduction of recombinant DNA methodologies when the NIH Guidelines were loosened to permit work on tumor virus genomes (early 1979).

We thus had confidence that we would again be judged well when our big grant came up for formal renewal in the late fall of 1981. Given, however, the increasingly tight federal funding situation, we were necessarily worried as to whether we would be given sufficient funds to let

us work at our past pace. So, great efforts went into the preparation of our grant proposal, as well as into the way we would present our data to the Site Visitors who looked us over in late May. Happily the final verdict was most favorable, and we now have guaranteed, starting January 1, 1982, the resources that should give us another five years of productive DNA tumor virus research.

Supervising this grant application was Joe Sambrook, who deserves much credit for a job that needed to be very well done and was so accomplished.

The Cloning of Human Cancer Genes

The capacity of purified DNA to transfer genetic traits from one cell to another was first demonstrated using bacteria almost 40 years ago. Those experiments, done by O.T. Avery, Colin Macleod, and Macyln McCarty at the Rockefeller Institute, provided the first hard proof that DNA is the primary genetic material. Soon there were attempts to transfer functional DNA into the cells of higher organisms, but the first such experiments were at best ambiguous. A French group claimed to have transformed the plumes of ducks by injecting DNA into their eggs, but when it became known that the eggs had been acquired from a local farmer's market and did not behave reproducibly, the episode was regarded as an example of hapless "transduction." Later, claims were made by Allex Fox that *Drosophila* DNA could produce quasi-stable genetic changes, but little attention was paid to his work. Only after purified animal viral DNA (SV40, polyoma, adenoviral) was shown to be infectious, and Ca^{++} shown to greatly enhance this infectivity, was the transfer ("transfection") of eucaryotic genes seriously sought for. By 1977, reproducible, high-level success with the herpes thymidine kinase gene by Michael Wigler and Richard Axel of P & S solidly established DNA transfection as a workable line of research. Particularly important was their unexpected observation that those rare cells that become transfected take up very large numbers of DNA molecules that they quickly link together in very long linear concatenates.

The characterization of the optimal circumstances for DNA transfection quickly led to experiments to see whether DNA from cancer cells could transform normal cells into their cancerous equivalents. The first successful such research, using DNA from a chemically transformed mouse cell, was done in Bob Weinberg's lab at the MIT Cancer Center. Soon afterwards the phenomena was extended to DNA obtained from cell lines arising from several types of human tumors. Among the first starting such experiments was Michael Wigler, who moved here from P & S late in 1978 to head up our Mammalian Cell Genetics Section. The cancer transfection experiments of his group started in earnest some 18 months ago and soon showed that several human tumor cell lines (lung, colon, bladder, and neuroblastoma) yield DNA that transforms mouse NIH3T3 cells into their cancerous equivalents. Given this result, the next step obviously was to clone the respective "cancer" gene(s) using recombinant DNA methodologies. Instead of using the more conventional brute force method of ponderously screening a very large library of human tumor cell DNA, Wigler's group opted for cloning procedures that select for a bacterial supressor tRNA gene that they enzymatically linked to the tumor cell DNA used to transform normal mouse cells. Already this selective procedure has been used most successfully by Wigler and his most talented collaborators Kenji Shimizu and Mitch Goldfarb to clone a putative human bladder cancer gene.

Final Stages in the Construction of the Reginald Harris Building

The tiny "mouse house" next to McClintock Lab that has served as our animal holding facility has with each passing year become more and more inadequate. We have thus been anxiously awaiting the arrival of the new and much larger modern animal facilities that our new Reginald Harris Building will provide. For reasons apparently only clear to our outside contractor, the pace of construction went very slowly until the middle of the summer. The Harris Building, however, is not a simple building, containing a multitude of devices to save and reuse energy. So we were not surprised when by late fall it was clear that the newly compromised finishing date of January 1982 would not be met. Then we guessed that only by mid-April would we have the opportunity to begin to expand the number of experimental mice, rats, guinea pigs, and rabbits needed to meet our research demands. Now we are at last in possession of an essentially complete building. The formal dedication is scheduled for May 27th to coincide with our annual RNA Tumor Virus Meeting.

Adjacent to the Harris Building are greatly expanded parking facilities that can handle some 100 cars. This construction has momentarily created a large treeless area which by late May shall be transformed back to a sylvan appearance through the planting of some 60 trees in addition to masses of appropriate shrubs.

Our Symposium Centered on the Cytoplasm

The preoccupation of this lab with molecular genetics has, not surprisingly, given a strong genetic basis to many of our Symposia. In chosing "The Organization of the Cytoplasm" for our June 1981 topic, we initially excluded any discussion of matters relating to the inside of the nucleus. We knew, for example, that if we discussed the mechanics of cell division, we would again be focusing on chromosome structure, a topic that we come back to over and over. We instead tried to play it safe by emphasizing speakers working on the

Reginald Harris Building

structures of the filamentous systems that give cells their shape and bring about their coordinated movements. In choosing them we consulted countless outside advisors, with the final program a product of Guenter Albrecht-Buehler's and my desire to emphasize the research directions that seem likely to dominate cytoplasmic research over the next several years. As the Symposium drew near we found that many of our projected discussions would make sense only if we learned the newly discovered facts about the genes that code for the cytoskeletal proteins. Recombinant DNA methodologies thus became an unexpectedly important aspect of our meeting, which represented the most complete overview of the cytoplasm that has yet been attempted.

To set the framework for the Symposium, we asked Ephraim Racker, Frank Solomon, Dan Branton, and Gunther Blobel to present opening night overviews of cytoplasmic organization and function. These proved most incisive, as was the concluding summary given so well by Bill Brinkley.

Schematic Drawings Are Now in Hand for Our New Auditorium

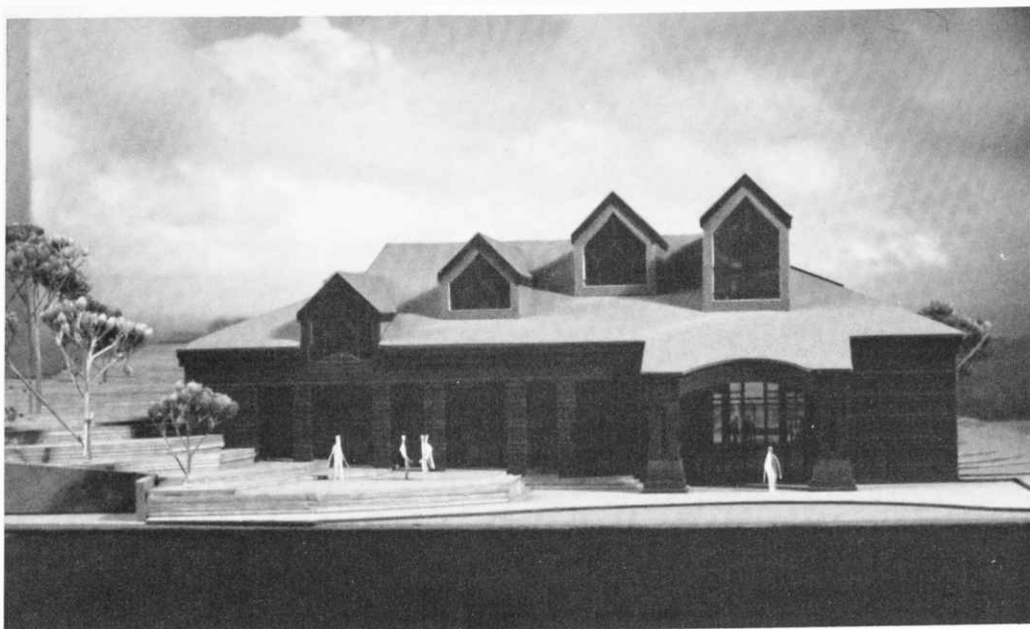
Over the past year, our Building Committee headed by Edward Pulling has had numerous discussions with our architects Moore, Grover, and Harper concerning the details of the new auditorium building that we have so long felt necessary for the maintenance of our position as the leading international meeting site for molecular biology. We are very satisfied by the schematic plans now in hand and hope to initiate soon a major fund drive for the two million dollars that the building will require. At the same time we are planning the renovations that need to be made to Blackford Hall to give it the kitchen facilities required for serving the 300–400 people that we must feed each day during our summer meeting season. Our final objective is an integrated meetings complex containing the new Auditorium, Blackford Hall, and the Vannevar Bush building, which we intend to connect directly to Blackford so that it can give us additional indoor dining space and at long last do away with the need for the large tents that we must have on hand in case of rain.

Optimally, this complex will be ready for the summer of 1985, when we shall be holding special celebrations to mark our 50th Symposium on Quantitative Biology.

Continued High-level Popularity of Our Summer Courses

The number of applicants for our 14 summer courses totaled 563, almost identical in number to the 556 applicants for the 1980 courses. Of these, 200 (vs 193 in 1980) students applied for our eight offerings in neurobiology. Particularly impressive was the 31 applications for our new advanced course on Single-channel Recording and we are repeating this workshop-type effort in 1982. Key to this offering and to our Brain Slice Course were funds provided by the Klingenstein Fund. They came to our aid at the last moment when expected federal funding fell victim to the preferential utilization of NIH funds for research grants as opposed to short-term training.

The remaining 363 applicants were for our courses in Cancer and Genetics, with again our Molecular Cloning Course drawing the most applicants (159 vs 172 in 1979). Two new courses were given, Plant Molecular Biology taught by Fred Ausubel and John Bedbrook and Introduc-



Architect's model of prosposed auditorium

tion of Macromolecules into Mammalian Cells taught by Mario Cappechi and Geoffry Cooper. Both were heavily oversubscribed and will be given again in 1982.

The fact that the number of applicants effectively remained the same despite severe decreases in the individual grant funds that pay for so many of the stipends reflects well on the continued perceived high potential contribution of these courses to our students' intellectual futures. We award as much scholarship aid as our funds permit, usually in the form of ½ stipends that require a corresponding vote of confidence from our students' home institutions.

To Give Us the Proper Balance, the Scope of Our Year-round Effort in Neurobiology Must Be Increased

We now run the largest summer advanced training program in neurobiology in the world, a remarkable achievement under any circumstances but one made more impressive considering the tiny size of our year-round neurobiology program. Even if the outside funding for our courses were easy to obtain, and now just the opposite situation holds, the major responsibility of organizing these courses requires more time than Bill Udry and I have to give, as well as a great diversion from research of Birgit Zipser, who until last year was our only resident neurobiologist. We thus must develop here a significant year-round neurobiology research program if we are to remain a potent factor in the summer training of neurobiologists. Toward this end last year we appointed Susan Hockfield to our staff and strongly encouraged Ron McKay to make the transition to being a full-time neurobiologist. Our situation, however, is still very metastable, with our house of cards capable of falling down if one or more members of our year-round staff leaves. We must recruit more neurobiologists to our staff and help them acquire the postdoctoral fellows who would benefit by working here with them. Toward that end I have approached a major foundation to see whether they would give us the seed money to let us make a modestly big neurobiological splash using our recombinant DNA and hybridoma methodologies. A positive response would not only be most welcomed news obviously to us, but also we feel to the future of neurobiology on the worldwide scene.

Dedication of the Max Delbrück Laboratory

The final touches to the new addition that transformed our more than somewhat decrepit Davenport Lab into our marvelous new Delbruck Laboratory occurred in mid-summer. The "post-modern colonial" design of our architects Moore, Grover, and Harper is universally thought a striking success, greatly enhancing the pleasure of a walk along Bungtown Road. The waterside of Delbrück Lab has been most imaginatively transformed into an almost Japanese garden environment by Hans Triede and his most-competent grounds staff, and the tiny bridge that cuts across the little pond brings delight to the eye with each passage. The new labs provided by Delbruck give our Yeast Group for the first time a real home in which to pursue the molecular details of their cassette model for mating-type interconversions. Also available now for the first time are growth chambers for plant cells, giving the occupants of

Delbrück Laboratory, rear view

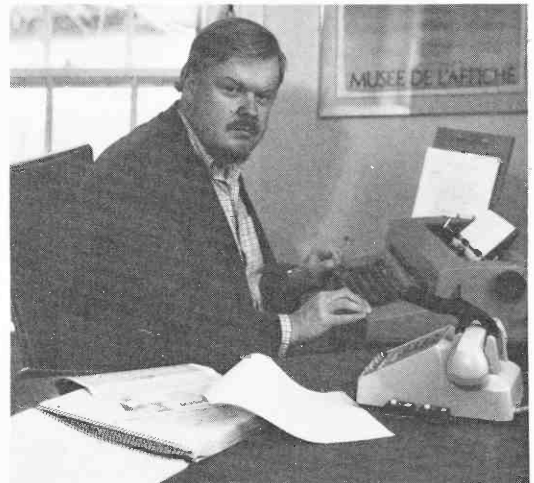
Delbrück the chance to try their hands at the genetic engineering of higher plant cells.

The Dedication Ceremony for Delbrück, held during our annual Phage Meeting in August, was a most-splendid occasion, with Manny, Jonathan, Nicola, and Tobby Delbrück attending, as did many of the scientists whose intellectual lives Max helped form here in Cold Spring Harbor. The memory-filled dedication booklet, most ably put together by Susan Gensel, Ellen DeWeerdt, and Tom Broker, has recently been expanded to include the highly personal dedicatory addresses given by Rollin Hotchkiss, Gordon Sato, John Cairns, and Al Hershey. Concluding the dedication day was a traditional square dance on the big lawn below Davenport House (Carnegie Dorm) that brought back memories of similar occasions in the early days of phage.

High-level Employment of Our Banbury Facilities

We now possess at the Banbury estate given to us by Mr. Charles S. Robertson a uniquely wonderful center for small conferences. This past year it hosted a most intellectually diverse smorgasbord of high-level meetings and courses. Particularly important was the meeting arranged in late March on the "Quantification of Occupational Cancer" that brought together many of the leading authorities on cancer causation to discuss whether it is possible to assess correctly that fraction of cancer that has an occupational origin. This is a field not lacking in strongly held diverse views, and the very fact of this meeting was an achievement in itself. We collected together speakers who were not prone to face each other, and their opposing arguments are faithfully reproduced in the semi-massive 752-page Banbury Report that was published eight months later.

Our late October meeting on the "Patenting of Life Forms" was also a notable occasion, providing an intimate view of patent examiners, lawyers, and judges, all enthusiastically displaying the wares of their trades. We have also begun to use Banbury for the holding of select, small scientific meetings, with much praise marking the holding of intellectually spirited gatherings on "Gene Amplification and Aberrant Chromosomal Structures" in October and "Construction and Use of



Victor McElheny

Mammalian Eukaryotic Viral Vectors" in December.

The initial hopes that we had for the Banbury Center have thus been more than achieved in the now less than four years that Victor McElheny has functioned as its director. It is thus with much regret that I must report that as of May 1, 1982 Victor will leave the Banbury Center to become the director of a new program for science journalists that he will organize at MIT under the auspices of its Program on Science, Technology, and Society.

Now we are searching for a new Banbury Director, knowing that duplicating Victor's intelligence, dedication, and infectious conversational charm will not be easy. The Banbury Center will necessarily have a different style when his replacement arrives.

Sammis Hall Comes into Use

Sammis Hall, our new residence at the Banbury Conference Center, was formally dedicated on July 19, 1981. Charles S. Robertson's mother was a Sammis, a name associated with early Huntington, being one of its first settlers in the 1650s. For many generations the Sammis family farmed the land where the Banbury Center resides, and it was this family association that led Charles and Marie Robertson to build their home here in 1936. Charles Moore, in designing Sammis Hall, drew on the work of Palladio, in particular his Villa Poiana, and Sammis Hall is appropriately covered with stucco of the terra cotta hue that dominates the northern Italian countryside.

Its first occupancy occurred in late March during our meeting on "Quantification of Occupational Cancer," and from its initial use it has been greatly praised by its residents. The dedication event itself was a most special event, with my wife Liz preparing the handsome dedication booklet and with Charles Moore present to give a talk on the "Mind of the Architect" and Gunther Stent speaking on the "Architecture of the Mind." Mr. and Mrs. Robertson's children, Mimi, Ann, and William, were on hand, as was his sister Mrs. Charles Rose. It was an occasion of which we are sure Charles Robertson would have been proud, and we continue to miss him greatly

The Use of Davenport House for Musical Concerts

In late April Davenport House became recipient of a gift of a marvelous new Baldwin grand piano, thus opening up the very real possibility of concerts in its newly renovated Music Room. The first such concert was given on a mid-May evening with the splendid duo-pianists Cosmo Buono and David Bradshaw giving us their polished pyrotechnics. They also most kindly arranged with my wife Liz a 1981-1982 series of Sunday afternoon concerts. Cosmo and David opened this series late in September, followed by a splendid October recital by the violinist Helen Armstrong accompanied by David and a mid-November concert by the very talented younger pianist Garah Landes.

Our new Baldwin gives to our staff a wonderful instrument to practice upon, and it will surely enrich our cultural environment for many, many years to come.

Our Publishing Program Continues at a Brisk Pace

The activities of our publishing group in Nichols steadily gained momentum as 1981 progressed. Newly appearing this year were a second edition

Davenport House concert

of Bob Pollock's most successful *Readings in Mammalian Cell Culture,* as well as two volumes arising from our Neurobiology Program, *Monoclonal Antibodies to Neural Antigens* edited by Ron McKay, Martin Raff, and Louis Reichardt and *The Neurobiology of the Leech* edited by Ken Muller, John Nicholls, and Gunther Stent. We also published a new lab manual on *Hybridoma Techniques* that came out of an EMBO course given by George Köhler and his colleagues at the Basel Institute of Immunology. It quickly proved in great demand, and we are now in the third printing.

Our most major publishing effort continues to be the proceedings of our annual Symposium. *Movable Genetic Elements,* our 1980 Symposium product, came off the presses in May, later than we wanted, but nonetheless a notable achievement considering that it is a two-volume set containing more than 1000 pages. It continues to sell well, reflecting the great excitement that characterizes this rapidly developing focal point of modern genetics. The production of our eighth volume in our Cell Proliferation series was also a major event. Entitled *Protein Phorsphorylation,* this two-volume set, edited by Ora Rosen and Edwin Krebs, contains more than 1400 pages that detail the current widespread excitement about protein kinases and their roles in cellular metabolism.

Great efforts were also spent in preparing books to appear in 1982. Scheduled for appearance in the first half of 1982 are four titles in our monograph series, *The Molecular Biology of the Yeast Saccharomyces, RNA Tumor Viruses, Mitochondrial Genes,* and a revised paperback version of *DNA Tumor Viruses.* We are also initiating a new series of rapidly produced digests of the small scientific meetings held at the Banbury Center. The first such titles are *Gene Amplification* and *Eukaryotic Viral Vectors.* Also scheduled to appear before the mid-year is our new manual *Molecular Cloning,* edited by Tom Maniatis, Ed Fritsch, and Joe Sambrook. Already more than 3000 advance orders have been placed for what is bound to be a best seller.

This year now makes the ninth that Nancy Ford has directed our publication program, and she deserves great credit for seeing that our books are now among the most handsome scientific books produced anywhere in the world. During these years she has been most ably assisted by Annette Kirk, who has just received a most deserved promotion to the newly created position of Assistant Director of Publications.

The volumes sold during 1981 totalled 16,056, a number very similar to sales in 1980 (16,041) and in 1979 (16,300). In 1982 we shall witness a significant increase in sales, reflecting the many fine new books to be published over the next several months.

Major Changes in Our Scientific Roster

The fact that our staff positions have no hard monies behind them makes virtually inevitable

Guenter Albrecht-Buehler

the decisions by key scientists to move on to positions that offer more long-term security. We have suffered a particularly major loss this year with the departure of Keith Burridge, just following our Symposium, to take up a position in the Cell Biology Department of the University of North Carolina Medical School at Chapel Hill. Keith came to us in late 1973 as a postdoctoral fellow from the MRC Laboratory of Molecular Biology in Cambridge, later joining our staff and serving as a focal point in the development of our Cell Biology Group. A further major loss for our Cell Biology effort is the impending (late May 1982) of Guenter Albrecht-Buehler for a tenured professorship at Northwestern University Medical School. For eight years Guenter has generated many innovative ideas about cell motility and we shall miss the deep intelligence that he brings to bear on intelligent behavior at the cellular level.

Leaving us in November was John Smart, who came here to most effectively set up our Protein Chemistry Section. John's move was to the genetic engineering firm Biogen, where he will be in charge of the Protein Chemistry Section of their new lab in Cambridge. An equally important loss occurred with the departure of Kurt Drickamer in September to the University of Chicago, where he has been appointed Assistant Professor of Biochemistry.

Leaving us during 1981 upon the completion of their postdoctoral periods here were Fred Asselbergs, who has moved to Switzerland for a position in the Biogen Geneva lab; Bob Blumenthal, to be an Assistant Professor of Biochemistry at the Toledo University Medical School; George Chaconas, for an Assistant Professorship of Genetics in the Cancer Research Laboratory at the University of Western Ontario; Richard Frisque, to

become an Assistant Professor of Virology at Pennsylvania State University in University Park; Shiu-lok Hu, to join Molecular Genetics, Inc. in Minneapolis; Bing-dong Jiang, to return to Shanghai, China; Thomas Meyer, to return to Heidelberg University in Germany; Kim Nasmyth, to take up a staff position at the MRC Laboratory of Molecular Biology in Cambridge; Keith Willison, to join the staff of the Cancer Research Council in its London Labs; and Clifford Yen, to help form a new biotechnology firm here on Long Island.

Promoted to positions as Senior Staff Investigator are Fuyuhiko Tamanoi, to help start our new DNA Synthesis Section in Demerec Lab; Mitchell Goldfarb, who first came here from Bob Weinberg's MIT Lab to postdoc in Mike Wigler's Lab; and Ron McKay, who after his degree at Edinburgh, was a postdoc in Walter Bodmer's Oxford Lab before coming to James Lab. Rasika Harshey and Bruce Stillman moved up from their postdoc slots to Staff Investigator positions.

A New Addition to Our Senior Scientist Ranks

Acting in their spring meeting, our Trustees approved the most well deserved promotion of Michael Wigler to the rank of Senior Scientist beginning January 1, 1982. This post carries a rolling commitment of five years of salary support. Given that we must largely live on short-term federal funds, the "Rolling 5" position represents the closest approximation to a tenured position that we can offer and signifies our faith that the scientist concerned has the capacity for sustained, self-motivated, creative research.

Awarding of the Lasker, Wolf, and MacArthur Prizes to Barbara McClintock

Barbara McClintock was already a quiet legend when I first met her here at Cold Spring Harbor over the summer of 1948. Her mastery of cytogenetics and the clever, determined way she analyzed the mutations of corn, coupled with her self-effacing, principled, Yankee behavior, made her unlike any scientist I had or would ever see. Only at the rare moment would she reveal her thoughts, but when she did you knew exactly where she stood and hoped that you would pass her muster. But then you would never know, at least not by direct communication. By 1951 she began to talk of her experiments that told her that genetic elements need not have fixed locations but might jump from one chromosomal locus to another. In doing so, gene function could cease or suddenly be restored. Soon there was a steady procession of the world's best geneticists to CSH to hear Barbara tell her story, and afterwards they would excitedly speak of what they had heard.

Already a member of the National Academy of Sciences in 1944, Barbara rapidly began to receive the peer recognition owed to her, culminating in the award of National Medal of Science in 1970, the first woman to receive our country's highest recognition for a scientist. And without our ever noticing her momentary absences, she quietly collected Honorary Degrees from a number of our leading academic institutions (e.g., Harvard and Rockefeller Universities).

In the midst of this last decade her concept of movable genes has become extended to all forms of living organisms, and the magnitude of her achievement in reaching these ideas some 20 years ahead of others became even more widely appreciated. The visible expressions that go along with anointment as one of the greats now flow to her, and within the past six months she has received the prestigious Albert Lasker Award for Medical Science, the Wolf Prize of Israel ($100,000) for Biology jointly with Stanley Cohen, and most recently the first prize fellowship of the John T. and Catherine MacArthur Foundation, which chose her for their first such yearly tax-free award of $60,000. This latter award, the most munificent prize so far given to any individual for academic achievement was given expressly to let Barbara continue to live exactly as she has in the past. She thus need have no concern for the monetary problems that so beset today's academic world. Everyone here is thrilled by Barbara's most deserved good fortune, but in characteristic fashion she has permitted no formal celebrations and continues her daily walks along Bungtown as if nothing has happened.

The Robertson Research Fund Strongly Provides Our Capacity for the Novel

As the director, each year I enjoy the freedom given to me through the existence of the Robertson Research Fund, the income from which is available to support projects too new yet to have support from conventional funding sources. Particularly important has been the availability of funds for the starting expenses of new members of our staff. This year the Robertson Fund allowed us to give John Fiddes, Fred Heffron, and Maggie So equipment and supply monies that let them commence their work here at full speed. These funds also provided support for two exceptionally talented Robertson Research postdoctoral fellows, Kim Nasmyth and Kenji Shimizu, whose foreign nationalities precluded them from federal fellowship support. In addition, we have the means to supplement the now more than tight Federal grants in support of our summer meetings and courses as well as help cover the cost of a new DEC computer for handling our DNA sequence information.

We Need Soon to Add to Our Demerec and James Facilities

That we have been so much at the moving edge of modern biology owes much to our past decisions as to when to seriously upgrade our facilities.

Now we see the need to make modest additions to both Demerec and James Labs that will prepare us for a concerted assault on the structures and functions of the proteins coded by the cancer genes that recombinant DNA tricks are allowing us to isolate. To do so, we must soon have the newest equipment for the microsequencing of proteins as well as a capability for in vitro site-specific mutagenesis. For the latter work we need a modern organic chemistry laboratory in which to synthesize needed polynucleotides and polypeptides. These new facilities are best obtained through the building of a 5000 sq.ft. addition to Demerec Lab. It will extend to the south, be two stories in height, and connect to the lower two floors of Demerec. It should be effectively invisible to visitors entering the lab grounds through either the upper or lower entrances off 25A.

We must also give ourselves better and more permanent facilities for the making of monoclonal antibodies. The cell lab in McClintock Lab already devoted to this purpose is totally committed to work on the cytoskeletal proteins. Our other hybridoma facility, located on the upper floor of James Lab, must close down during the summer months when we give there several advanced training courses. Clearly, James Lab must possess year-round hybridoma facilities. We are planning an approximately 5500 sq.ft. addition that will extend to the north and have three floors connected by elevator. The basement floor shall give to James its first adequate kitchen and media-making facilities.

The total cost of building and equipping these two new additions will be in the neighborhood of two million dollars. Toward this, we hope to apply a major construction grant that was awarded to us last May by the Pew Trust of Philadelphia. They pledged to give us $400,000 if we can obtain the remaining $1,600,000. Already we can apply toward the James addition the some $300,000 that still remains in the Harris Building NCI construction grant and which we have received permission to use towards our hybridoma facilities. This leaves us effectively $1,300,000 still to raise to qualify for full receipt of the Pew Grant. Of this sum, we have an effective pledge of $400,000 and now are actively pursuing the remaining sums. Common sense tells us not to try to start both additions simultaneously, and we propose to start, first, with the Demerec addition, whose working drawings are already more than half done. Now we would like its construction initiated by mid-year.

We Continue to Have a Super-effective and Cooperative Buildings and Grounds Staff

In many, if not most, academic institutions, even the simplest replacement of a lab bench is not a simple task, with a wide gulf separating the scientists from those support staff whose job it is to see that the facilities exist for the flourishing of science. Just the opposite is true here, with our Buildings and Grounds staff not only alert to the needs of our experiments, but responsible in seeing that our scientific careers are not held up by bureaucratic infighting over prestige and privilege. That we live under such remarkably good care owes very much to Jack Richards who heads our Buildings and Grounds Department. As a former contractor in the Huntington area, Jack knows how buildings (labs) should be built and maintained, and his high standards and good taste are visibly expressed throughout our grounds. With the steady increase in the level of our research, Jack's tasks become more demanding each year, but he handles them well, always politely listening to each request for still another renovation (maintenance) job.

Jack Richards

We Benefit from a Superbly Run Library

While we learn many new key facts through the scientific grapevine, our continued long-term existence demands that we maintain a first-rate library filled with the latest ways to disseminate the overpowering crush of new research facts. We are most fortunate that we not only possess a superb library, whose journals and books date back to the start of the century, but that it is also most professionally run by our super Librarian Susan Gensel, who has just marked her tenth anniversary with us.

The display and housing in stacks of our ever-increasing numbers of books and journals has become increasingly difficult, so we are now systematically remodeling the library. The basement floor is already sparkingly redone, and handsome new paneling now graces our main reading rooms

Susan Gensel

on the ground floor. By the end of 1982, the Library will have a completely new look.

Our Nichols Building Can No Longer Accommodate Our Administrative Needs

Nichols Memorial Building, the center of our administrative and publishing activities, now is bursting at its seams, having so many people and file cabinets that its functioning existence is badly threatened. This crowding does not arise from a needless proliferation of administrative support personnel. Compared to other research institutions, we are badly under-administered, with given individuals working beyond the call of civilized duty to keep us going. The most obvious way to relieve this situation is to move our Publications Department elsewhere, and when the funds become available we hope to move Publications into a totally new Urey Cottage, rebuilt from the foundation up to give them the space their growing activities demand. Meanwhile, we are most fortunate that our Administrative Director William Udry has not only assembled, but kept together, a first-rate administrative staff that includes our Comptroller Bill Keen, our Personnel Manager Pat Hickey, our Grants Manager Steve Kron, and our Purchasing Agent John Maroney.

LIBA Through Its Role in Mobilizing Community Support Remains a Vital Feature for Our Well Being

Located as we are remote from any major academic center, our more than 90 years existence defies the usual odds against the small institution whose inevitable ups and downs are bound to provide moments when the level of competence falls below the critical mass. Clearly helping us is our exceptionally beautiful site. Few institutions provide its occupants our "picture book" atmosphere. Equally important are our neighbors, most of whom, like us, know what a marvelous place Cold Spring Harbor is to live, and who have so long fought to maintain the uniqueness of our environment. Almost 60 years ago they banded together to form the Long Island Biological Association as a means of providing the financial aid and wise counsel that must lie behind every institution that aspires to carry out first-rate science. We are exceptionally fortunate that Edward Pulling, LIBA's Chairman for now almost 15 years, continues to mobilize so well our community behind us. As a distinguished educator and founder of the Millbrook School, Ed knows how high-quality institutions should be run and sees that we do not deviate from this course.

Creation of the Cell Biology Corporation

This lab's major involvement with recombinant DNA and hybridoma methodologies opens up the very real possibility of one or more of our research programs opening up commercial applications that should not be ignored. To help respond to any such opportunities if they arise, we have founded the "Cell Biology Corporation." To manage its operation, last July Angus McIntyre was appointed as its President. Angus is a long-time local resident and strong backer of our activities. His past experience includes membership on our Board of Trustees and from 1974 to 1977 acting as our Treasurer. At present, Cell Biology is 100% owned by the Lab, but given the right opportunity, it will amost surely need to raise outside capital and dilute our ownership. Now our mood is essentially one of caution. The financial community is no longer wild about biotechnology, and we see great danger in starting a new venture without the capitalization needed to give it a realistic chance of success. The opportunities nonetheless remain very real, and we should not miss them because of lack of a focused overview of what our science can lead to.

A Very Active Year for Our Board of Trustees

The need to assess critical possible relations between industry and us has necessitated many more meetings of our Board of Trustees and its Executive Committee than have been necessary in the past. With our staff totally the product of academic experience, the advice of our Trustees, both those with business backgrounds and those from institutions that have prior experience with industry, has proved exceedingly helpful in providing us the long-term outlook that rises above

the crises of the minute. We are particularly fortunate to have at our helm Walter Page, whose vast business experience and friendships give us entre to the highest levels of American industry.

Retiring from our Board at the conclusion of their statutory six-year terms were Arnold Levine, whose presence on our Executive Committee served us so well, and Norris Darrell, Jr., whose acute legal talents have been invaluable to our cause. And we accepted the resignation of Abe Worcel as an Institutional Trustee upon his move from Princeton to Rochester. Newly elected as an Individual Trustee was John Humes, a resident of neighboring Mill Neck, formerly of the law firm of Humes, Andrew and Botzow, and recently our Ambassador to Austria. Elected as the new Institutional Trustee representing Stony Book was Tom Shenk.

We Have Ended This Year Stronger Than at Its Start

This year commenced with our worrying much about the consequence of the darkening national economic pictures and the contractions in federal money for science that were bound to come. So we have spent much time considering alternative funding sources that might supplement dwindling support from Washington. Simultaneously, we have tried to run an even tighter ship and not take on new employees who we might soon have to disengage because of failure to gain new grants. Now that 1981 is over and our accounts properly audited, I can happily report that we are still in excellent financial health. More monies were spent on new buildings, renovations, and equipment than required to cover depreciation of previously acquired plant and equipment. In addition, we set aside in plant fund reserve modest sums to give us head starts for building projects of the near future.

We thus continue to pursue the highest of intellectual objectives and note with satisfaction our ongoing ability to attract here highly talented younger scientists. We realize, however, that good fortune can be very ephemeral and that we shall need the continued strong support from both our local community and our scientific peers elsewhere. Knowing that our many friends are still behind us should let us surmount the many moments of future anxiety that are bound to come. Cold Spring Harbor should thus remain an institution where the doing of science is an open, exciting experience that we shall share, to the very best of our abilities, with the outside world.

April 23, 1982 *James D. Watson*

With much pleasure I can now report the signing of an agreement between the Cold Spring Harbor Laboratory and the Exxon Research and Engineering Company (ER&E) to do collaborative fundamental research in molecular biology. Under the terms of the agreement, ER&E will provide 1.5 million dollars per year for the next five years to allow us to set up joint projects that will include new programs in the genetics of anaerobic bacteria and site-specific mutagenesis. We shall also be able to strengthen several preexisting research programs, in particular our efforts in protein chemistry. This agreement, coming at a time when the future of federal funding for science is filled with dark doubts, should allow us to maintain the intellectual vigor that has let us play a major, worldwide role in molecular biology over the past decade. Before concluding this agreement its exact terms were thoroughly gone over by our scientific staff, and by our Board of Trustees, both of which bodies enthusiastically and unanimously gave their support. The detailed legal papers were negotiated most effectively by our Administrative Director, Bill Udry, and by Norris Darrell, Jr., our counsel, a member of the New York law firm, Sullivan and Cromwell.

May 26, 1982 *J.D.W.*

YEAR-ROUND RESEARCH

TUMOR VIRUSES

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

MOVABLE GENETIC ELEMENTS

Insertion Elements and Plasmids
Yeast and Plant Genetics
Molecular Approaches to Microbial
Pathogenicity and Virulence
Mechanism of Transposition

VERTEBRATE GENE STRUCTURE AND EXPRESSION

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

CELL BIOLOGY

Cell Motility
Cell Biochemistry
Quest 2-D Gel Laboratory

NEUROBIOLOGY

Neurobiology Laboratories
Neurobiology Workshops:
Single-Channel Recording
Electrophysiology of the Mammalian Brain Slice

First row: *Y. Gluzman; J. Engler, R. Guilfoyle; B. Stillman; T. Kost; G.P. Thomas*
Second row: *J. Fiddes, W. Boorstein; M. Wigler, K. Shimizu, D. Levy, M. Goldfarb, C. Fraser; J. Lewis*
Third row: *T. Kamata; S. Silberstein, G. Penzi, C. Monaghan, M. Goradia, B. Vogel; L. Cascio; J. Garrels, J. Leibold*
Fourth row: *D. Kurtz; J. Lin, S. Blose, F. Matsumura; M. Mathews*
Fifth row: *M. Rossini; A. Klar, P. Creatura, J. Wood, J. Abraham, J.B. Hicks, R. Malmberg, J. Strathern, D. Prudente, S. Dellaporta, C. McGill, J. McIndoo, J. Ivy; L. Chow*

TUMOR VIRUSES

Although the work of the Tumor Virus Group remains almost totally concerned with the adenoviruses and the papovaviruses, the last year has seen some major changes of emphasis. There have been no less than three separate approaches to developing adenoviruses as vectors: Terri Grodzicker, in collaboration with colleagues at Berkeley, has concentrated on the analysis of recombinants between SV40 and adenoviruses generated chiefly by in vivo recombination; David Solnick has used almost exclusively in vitro recombinant DNA techniques to construct specific hybrid viruses; and Yakov Gluzman and his colleagues have taken an entirely different approach, concentrating on developing helper-free, non-defective adenoviral vectors. In all three cases the aim has been to provide cloning vehicles that can be used not just to propagate foreign DNA sequences, but also to synthesize large quantities of foreign gene products in eucaryotic cells. There is now good reason to believe that such gene products will be processed, modified, and transported through the cell in an authentic manner. In collaboration with Mary-Jane Gething (ICRF, London), Joe Sambrook has shown that influenza hemagglutinin, expressed in copious quantities from a cloned copy of the gene inserted into SV40 vectors, appears on the cell surface in a glycosylated form that is indistinguishable from hemagglutinin encoded by influenza virus itself. This result opens the way to study the signals governing the traffic patterns of protein within cells (Janet Brandsma).

Finally, a considerable fraction of the group's efforts remains focused on the central problem of tumor viruses—the mechanism of transformation. Jim Stringer has continued studies of the mechanism by which tumor virus genes become installed into and excised from the cellular genome; Ron McKay has further developed his antigenic and functional analysis of SV40 T antigen; and a large group of people (Bill Topp, Marilyn Anderson, Dick Frisque, Bruce Anderson, Margaret Hightower, and their associates) have continued to follow a line of work aimed at defining the structure and function of transforming genes of adenoviruses and papovaviruses and the contribution of particular viral gene products to the phenotype of the malignant cell.

TUMOR VIRUSES

J. Sambrook, T. Grodzicker, Y. Gluzman, R. McKay, W. Topp, J. Stringer, *B. Anderson, M. Anderson, J. Brandsma, R. Frisque, M. Hightower, S.-L. Hu, H. Reichl, D. Solnick, O. Sundin, K. Willison, M. Yamada, C. Adler, B. Ahrens, P. Barkley, J. Bier, R. Chisum, M. Costa, L. Garbarini, M. Goodwin, R. Greene, C. Grzywacz, D. Hanahan, D. Holtzman, R. Kelch, L. Kleina, R. McGuirk, M. Ramundo, P. Rosman, D. Smith, C. Stephens, L. Vander Wahl, K. VanDoren, J. Wiggins*

Viral Vectors

Adenovirus-SV40 Hybrids

T. Grodzicker, M. Yamada, D. Solnick, M. Costa, C. Stevens

Last year we reported the construction of adeno-SV40 hybrid viruses that express SV40 T antigens under the control of adenoviral promoters. These and new hybrid viruses are being studied to (1) produce large amounts of SV40 T antigens; (2) analyze genetic controlling elements, such as promoters, splice sites, and ribosome binding sites; and (3) test viral genomes that are suitable for use as cloning vectors.

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

To place the SV40 early region under adenoviral transcriptional control, the structural sequences that code for SV40 large and small T antigens were separated from the viral early promoter, purified, and amplified in *E. coli.* As recipients for the SV40 early region, we used two Ad2/Ad5 hybrid viruses that each contain only two of the three *Bam* sites present in the Ad2 genome. These interserotypic viruses, designated 1x51i and 4x225b, were formed in vivo as wild-type recombinants from crosses between temperature-sensitive mutants of Ad5 and the nondefective Ad2-SV40 hybrid virus Ad2$^+$ND1. Neither of these viruses contains SV40 DNA. The SV40 early region was inserted at the *Bam* sites of these adenoviruses to generate a population of defective recombinant viruses that contain SV40 insertion(s) in place of essential internal adenoviral genes. The recombinants were propagated in monkey cells in the presence of helper adenovirus. Expression of SV40 large T antigen by the recombinants provides a helper function for the growth of adenovirus in monkey cells, and this in turn provides a selection for recombinants that express SV40 T antigen. Analysis of four independent hybrids indicates that they contain the SV40 early region inserted in different positions and orientations within the adenoviral genome. (1) Ad-SVR5 has a tandem insertion of SV40 inserted between the *Bam* sites at positions 42 and 60. The SV40 DNA is oriented with its 5′ end at position 42 and is transcribed from the same strand as the adenoviral major late transcripts. (2) Ad-SVR15 has one copy of the SV40 early region inserted between positions 29 and 60 and oriented so that the *A* gene is transcribed in the same direction as the *E2* and *E4* that lie upstream from it. (3) Ad-SVR1 and R6, which were made by insertion of SV40 into an Ad2/5 recombinant virus known as 1x51i, have grossly rearranged genomes, although the SV40 coding region is intact. We have cloned the segment of R6 containing the SV40 insertion into plasmid vectors. Mapping experiments using these clones, as well as analyses of heteroduplexes of Ad-SVR6 and adenoviral helper DNAs (in collaboration with Louise Chow, Electron Microscopy Section) show that the SV40 early region is inserted into the early region 1b transcription unit at the left end of the adenoviral genome. The DNA is inserted at least 130 bp past the site that codes for the nucleotides corresponding to the 5′ cap site of the 1b mRNAs. Downstream from the SV40 insertion is a segment (~3 kb) of calf thymus DNA that was present in the original transfection mixture. We do not know the mechanism of integration of this DNA, but it has been carried for many passages of the hybrid virus.

Using a different approach, David Solnick has also constructed a new adeno-SV40 hybrid virus that holds promise as a vehicle for the expression of cloned DNAs. This hybrid carries two copies of the adenoviral late promoter. To construct this virus, a plasmid containing the SV40 segment coding for T antigen was inserted next to a segment of adenoviral DNA containing the late promoter. The late-promoter–SV40 unit was then inserted in the late region of the adenoviral genome.

Regardless of the arrangement and orientation of the SV40 insertions in the different hybrids, all produce wild-type SV40 early proteins. However, the hybrid viruses produce different amounts of large and small T antigens with different kinetics of synthesis. Ad-SVR15-infected cells yield low amounts of T antigen. Ad-SVR5-infected cells produce high amounts of T antigen as a late viral protein, as do cells infected with Ad-SVR1 or R6, which make three to five times more T antigen than is found in SV40-infected cells. Large and small T antigens produced by all viruses have the same mobility as the wild-type SV40 proteins, as judged by immunoprecipitation with various T antisera and analysis of antigens on SDS-polyacrylamide gels. The Ad-SVR6 T antigen has been shown to be identical to SV40 T antigen by partial proteolysis with *Staphylococcus aureus* V8 protease and analysis of tryptic peptides. Using Ad-SVR6-infected cells, it has been possible to purify about 2 mg of T antigen from 8 liters of infected cells. The large amounts of T antigen produced at late

times after infection of R6-infected cells are striking because the SV40 early region is inserted into one of the adenoviral early transforming regions. The SV40-containing RNAs made by the different recombinants are transcribed from different positions on the genome, although in all cases they contain intact SV40 early sequences and display normal splice patterns. However, the ratio of small-T-antigen mRNA to large-T-antigen mRNA in cells infected with hybrid viruses is reduced relative to the ratio found in mRNA extracted from lytically infected cells. Some hybrid mRNAs appear to be transcribed from the adenoviral late promoter and to contain the tripartite leader sequence (Ad-SVR5). Others (Ad-SVR15) are probably transcribed from *l*-strand early promoters. The SV40-containing mRNA in R6 is transcribed from the early adenoviral 1b promoter. This result is striking in several respects. The adenoviral 1b region codes for several RNAs that share the same 5′ and 3′ ends but have different internal amounts of RNA removed by splicing. Some of these early RNAs are produced in very low amounts, whereas one RNA (that codes for the 15K polypeptide; see Protein Synthesis Section) is made in larger amounts and at late as well as at early times after infection. SV40 T antigen is made in large amounts at late times after infection from R6-infected cells. Thus, transcription from the early adenoviral 1b region is very efficient at early and late times and some posttranscriptional event must set the levels of the mRNAs. In addition, the SV40 early region in R6 is inserted over 100 bp downstream from the site that codes for the ATG of the 1b 15K protein, although wild-type SV40 T antigen is produced. Thus, initiation of translation of T antigen can start at the T antigen AUG codon, although the mRNA carries the 1B 15K initiation codon and ribosome-binding site at its 5′ end. This may be similar to the pattern found with the 1B 15K and 57K polypeptides (see Protein Chemistry and Protein Synthesis Sections).

Because SV40 T antigen can be expressed from different adenoviral promoters, we extended our studies to use the SV40 early region as an insertional mutagen and attempted to place it in regions of interest in the adenoviral genome. The first new hybrid we made, Ad-SVR26, contains SV40 DNA close to (~150 bp) but downstream from the major late promoter and the first segment of the tripartite leader. Because we did not have an adenoviral vector that contained restriction enzyme sites that could be used to insert the SV40 segment directly into the desired position in the genome, we used a combination of in vitro and in vivo recombination to create the desired hybrid virus (see Fig. 1). We hope this method will be useful for the construction of a variety of viruses carrying different insertions.

Although the SV40 sequences transcribed in R26 are located in a region of adenoviral primary transcripts that are normally removed by splicing, substantial amounts of SV40 large T antigen are in fact produced at late times in infected cells (50–100-fold higher than in SV40-infected cells). Two T antigen species, as judged by immunoprecipitation with monoclonal antibodies, are detected. One is slightly larger than SV40 T antigen, whereas the other has the same mobility as T antigen; however, these proteins have not yet been analyzed to determine their exact relationship to wild-type T antigen.

Extremely high amounts of SV40-containing mRNAs are produced in R26-infected cells. Analysis of these transcripts by nuclease-S1 analysis, sandwich hybridization, and analysis of glycosylated RNAs gives us a picture of how they are produced. Two predominant T-antigen mRNAs with different 5′ ends are found in infected cells. The larger species of T-antigen mRNAs contains all 190 bases of adenoviral sequences between the adenoviral late 5′ cap site and the beginning of the SV40 *A* gene. Because there are two ATG codons for the adenoviral intervening region in frame with T-coding sequences, translation of this mRNA would give rise to the large species of T antigen. Thus, although all adenoviral late mRNAs carry the tripartite leader, large amounts of R26 message in which the first donor splice site is not used are produced. In the second species of R26 T-antigen mRNA, the first leader segment is present but is spliced to an acceptor present either in the adenoviral intervening region downstream from it or at the very beginning of the SV40 portion of the transcript. In this case cryptic splice acceptor sites that normally are not used are very efficient points of processing.

We are continuing these studies in several ways. Shiu-Lok Hu has constructed a set of plasmids in which the sequences coding for T antigen are joined to a segment of adenoviral DNA containing the major late promoter and extending downstream to various positions between the three leader segments and the block of late genes. These Ad2-SV40 segments are being excised from the plasmids and inserted into adenoviral vectors to yield viruses containing SV40 DNA in different positions of interest in adenovirus. By studying transcription of SV40 DNA in these hybrid viruses, we hope to learn more about control of transcription and processing.

We are also attempting to insert early adenoviral genes that code for products involved in replication or control of transcription into new positions on the adenoviral genome in an effort to overproduce the proteins of interest. Examples include the Bellett protein and the immediate early 52K polypeptide (in collaboration with Bruce Stillman and Mike Mathews, Protein Synthesis Section). We are also doing experiments to see whether human genes can be inserted and expressed in adenovirus.

As we have reported previously, we (in collaboration with Dan Klessig, University of Utah)

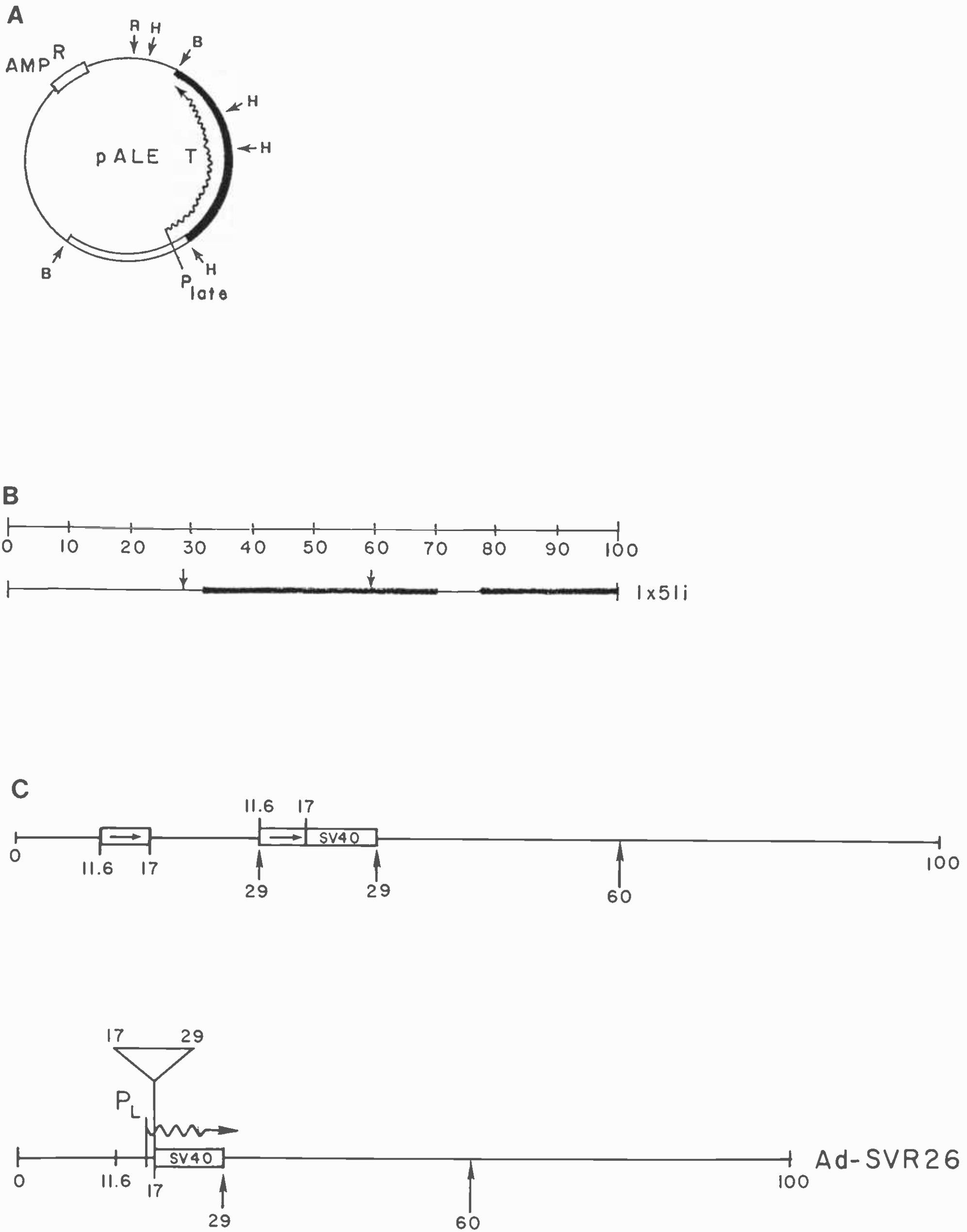


Figure 1

Construction of Ad-SVR26 which contains the SV40 early region downstream from the adenoviral major late promoter and the first segment of the tripartite leader. (*A*) pALE is a derivative of pBR322 that contains adenoviral and SV40 early sequences linked together. The thin white bar represents Ad2 DNA and the black bar represents SV40 DNA. The Ad2 sequences extend in a counterclockwise direction from a *Bam* site (B) at 11 map units to a *Hin*dIII (H) site at position 17 over 200 bp from the major late promoter (P_{late}). The SV40 DNA, containing coding sequences for large and small T antigens (T), is joined to Ad2 sequences at a site (H) 10 bp upstream from the initiation codon for T antigen and is transcribed (~) in a counterclockwise direction. The Ad2-SV40 insert is excised with *Bam* and inserted into Ad2/Ad5 1x5li:R:*Eco*RI. (*B*) The map of 1x5li showing its two *Bam* restriction enzyme cleavage sites (↓), Ad2 sequences (thin line), and Ad5 sequences (thick line). (*C*) The genome structure of Ad-SVR26. The upper diagram represents the deduced precursor to the final R26 genome structure depicted in the lower diagram. The two boxed arrows emphasize the duplicated adenoviral sequences from map position 11.6 to 17 present in both the vector and insert DNA of the precursor. The other arrows show the *Bam* sites in the recombinant genome. In the R6 map, the late promoter and direction of transcription are indicated.

have obtained human cell lines that express the left-hand early adenoviral genes and can be used as permissive hosts for the propagation of conditional mutants (Grodzicker and Klessig, *Cell 21:* 453 [1980]). We have used the procedure of cotransformation to introduce unselected Ad2 genes, along with the HSV-1 *tk* gene, into permissive thymidine kinase (TK)-deficient human 143 cells. We shall use the cotransformation procedure to introduce some of the late-promoter–early-gene constructs, described above, into 143 cells to see whether the early genes can be expressed. If so, we shall use them as permissive hosts for propagating viral mutants.

Construction of Helper-free Adenovirus-5 Vectors

Y. Gluzman, B. Ahrens, K. Van Doren, H. Reichl, D. Solnick

The use of defective adenovirus as vectors has proven to be a powerful tool for overproduction of SV40 T antigen and related molecules (Hassell et al., *J. Mol. Biol. 120:* 209 [1978]; see above). For some purposes, however, it is preferable to have a vector population that can grow in the absence of helper. By and large, DNA tumor viruses and vectors based on them have well-defined packaging constraints, and it is rare that a DNA molecule

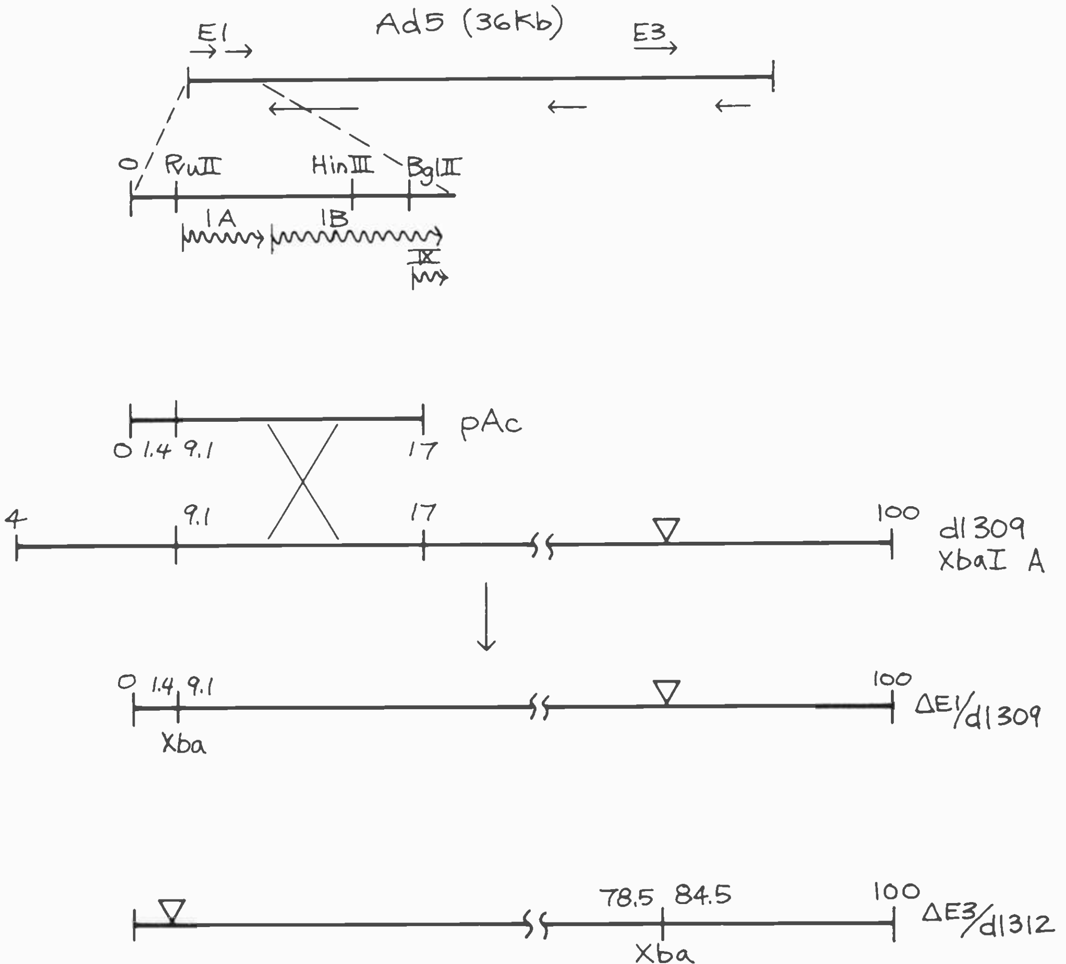


Figure 2
(*a*) Ad 5 map of early transcriptional regions. Early regions 1 and 3 are labeled E1 and E3. Region E1 is shown on larger scale and the following features of E1 are depicted: sites for *Pvu*II (1.4%), *Hin*dIII (8%), *Bgl*II (9.1%); transcriptional unit 1A and 1B; and mRNA for protein IX. (*b*) Construction of ΔE1/*dl*309. Adenoviral DNA insert in the pAC plasmid consists of (*left to right*): *Eco*RI linkers, 0→1.4% Ad5 DNA (*Pvu*II), Xba linker, 9.1→17% Ad5 DNA (*Bgl*II→*Hin*dIII). Adenoviral DNA in pAC retains sequences encoding the structural polypeptide IX (*Bgl*II site is located 250 bp upstream from the 5′ end of polypeptide-IX mRNA). In vivo recombination between pAC and *Xba*I A fragment of *dl*309 resulted in production of vector virus ΔE1/*dl*309. (c) Structure of ΔE3/*dl*312. This vector is missing *Xba*I fragment D (78.5–84.5%) from its right arm and contains the *dl*312 left arm with a deletion of ~2.5% in region 1A.

more than 5% longer than the wild-type genome can be packaged. For construction of helper-independent vectors, therefore, it is necessary to define regions that are nonessential for lytic growth, and that therefore can be eliminated from the virus, or regions that might be complemented by viral DNA integrated in a permissive host. From work with defective Ad2-SV40 viruses (Kelly and Lewis, *J. Virol. 12:* 643 [1973]) and deletion mutants of Ad5 (Jones and Shenk, *Cell 13:* 181 [1978]), it is known that region E3 (about 6% of the adenoviral genome) is not essential for lytic growth of the virus. Furthermore, it is possible to propagate deletion mutants in region E1 in a permissive line of human cells (293) (Graham et al., *J. Gen. Virol. 36:* 59 [1977]) that expresses an integrated E1 gene. We therefore set out to construct a viral genome that lacked both E3 and E1 and contained a single *Xba*I site in a suitable position for cloning.

The deletion of E1 sequences was carried out by manipulation of bacterial plasmids containing viral DNA fragments. Because of the presence of a covalently attached protein, the termini of the adenoviral genome are refractory to ligation and therefore to cloning. To overcome this problem, the protein was removed by treatment with piperidine and a left-terminal fragment was fitted with an *Eco*RI linker. We constructed a plasmid (pAC) with an insert of Ad5 DNA extending from the *Eco*RI linker at the left end of the genome to the *Hind*III site at map coordinate 17.0, with a deletion between the *Pvu*II site at 1.4 m.u. and the *Bgl*II site at 9.1 m.u. An *Xba* linker was inserted at the site of the deletion. pAC thus retains the leftmost 454 nucleotides of the viral genome, which harbor an origin of replication and probably the signal for packaging of DNA into virions. Also, pAC retains sequences encoding the structural polypeptide IX. This protein, although not essential for viral replication, is known to contribute to the termostability of the virion (Colby and Shenk, *J. Virol. 39:* 977 [1981]).

To construct a virus containing the E1 region altered in the plasmid, we took advantage of the efficiency with which adenoviral DNA recombines in vivo. Line 293 cells were transfected with a mixture of the insert from pAC and the large *Xba*I fragment from *dl*309, which extends from 3.7 to 100 m.u. and thus shares about 8 m.u. of homology with the pAC insert. The structure of recombinant virus is shown in Figure 2b. This virus, named E1, thus contains a deletion of 8.8 m.u., or about 3.3 kb.

Because of the presence of useful restriction sites, the deletion of region-3 sequences was performed directly using viral DNA. Briefly, we constructed a recombinant genome (*dl*455) with a left half from *dl*312, containing no *Xba*I sites, and a right half from wild-type Ad5, which contains two *Xba*I sites. *dl*455 DNA is therefore cleaved by *Xba*I at only two sites, both in E3. E3/*dl*312 was then constructed by ligation of the two terminal *Xba*I fragments of *dl*455. It contains a unique *Xba*I site in E3 and has a deletion of 2.5 m.u. from E1 and 6.8 m.u. from E3, a total deletion of 9.3% or about 3.4 kb (Fig. 2c).

As a preliminary test of the utility of these vectors, we have used E1 as a recipient for a fragment containing the adenoviral major late promoter harnessed to the SV40 early region devoid of its own promoter. This fragment was used previously in the construction of a helper-dependent recombinant producing SV40 T antigens (Solnick, *Cell 24:* 135 [1981]). For our purpose, it was fitted with *Xba*I linkers and ligated to the *Xba*I "arms" of E1, after treatment of the arms with alkaline phosphatase. Of the 20 plaques isolated after transfection of line 293 cells, 19 contained the insert in either of the two possible orientations. It was found that following lytic infection of line 293 cells, both recombinants produce large quantities of SV40 T antigen 20 hr postinfection. We also have cloned the rat β_2 globulin gene into the nondefective E1 vector and at the present time we are investigating if dexamethasone-dependent expression of this gene is preserved in the cloned state.

Foreign Proteins Expressed on Eucaryotic Cell Surfaces

J. Sambrook, J. Brandsma, in collaboration with M.-J. Gething (Imperial Cancer Research Fund Laboratories)

In recent years the hemagglutinin of influenza virus has become a model for the synthesis and structure of eucaryotic transmembrane proteins. Analysis of the amino acid sequence of hemagglutinin reveals three functional domains characteristic of membrane proteins. A short, N-terminal "signal" sequence probably directs the nascent polypeptide chain through the membrane of the endoplasmic reticulum (McCauley et al., *FEBS Lett. 108:* 422 [1979]). A hydrophobic C-terminal domain seems to anchor the completed protein in the lipid bilayer. Between these two is the long sequence that carries the hemagglutinating activity and major antigenic determinants (Hirst, *J. Exp. Med. 76:* 195 [1942]; Brand and Skehel, *Nature New Biol. 238:* 145 [1972]).

The aims of this project are to obtain the expression of the viral hemagglutinin on the outer membranes of mammalian cells using recombinant animal virus vectors to (1) study the rules that govern the functioning of the "signal" and "anchor" sequences and (2) develop a method whereby foreign proteins not normally found in membranes can be displayed on the surfaces of eucaryotic cells.

The cloned copy of the Japan HA gene (Gething, *Nature 287:*301 [1980]) contains no intervening sequences and is a convenient size for insertion into the late or early region of the SV40 genome. Because the HA gene contains no promoter of its own, the vectors were designed so that HA was expressed under the control of the SV40 early or late

promoters. (For details of the constructions and of the analysis of the expressed HA see Gething and Sambrook, *Nature 293*:620 [1981].)

Late Replacement Vector SVEHA3

To construct the late-replacement vector, the HA gene was inserted into the late region of SV40 between the *Hpa*II site at nucleotide 346 and the *Bam*HI site at nucleotide 2533. This recombinant viral genome was cloned into the *Bam*HI site of PAT153 and propagated in *E. coli*. For transfection into simian cells, the recombinant SV40-HA genome was excised from the plasmid by digestion with *Bam*HI and purified and ligated to yield the structure SVEHA3 shown in Figure 3. This recombinant viral genome contains the SV40 origin of DNA replication and an intact set of early genes. The HA gene is joined to the noncoding sequences that would normally be transcribed into the untranslated 5′ region of SV40 late mRNAs. Late in infection of permissive simian cells this recombinant should express a hybrid mRNA consisting of untranslated SV40 sequences at its 5′ end and an intact set of HA-coding sequences. Polyadenylation could occur either at a site that has been postulated to function during the synthesis of HA mRNA from its natural influenza viral RNA template (Robertson et al., *J. Virol. 38*:157 [1981]) or at the normal site for late SV40 mRNAs at nucleotide 2674. Translation of this hybrid mRNA should begin at the first AUG that marks the beginning of the HA-coding sequences. Because the vector SVEHA3 contains an intact copy of the gene coding for SV40 large T antigen, its DNA will replicate efficiently in permissive simian cells. However, production of infectious virions containing the recombinant genome requires complementation by a helper virus such as *dl*1055, an early deletion mutant of SV40 that can supply SV40 capsid proteins for the assembly of virions.

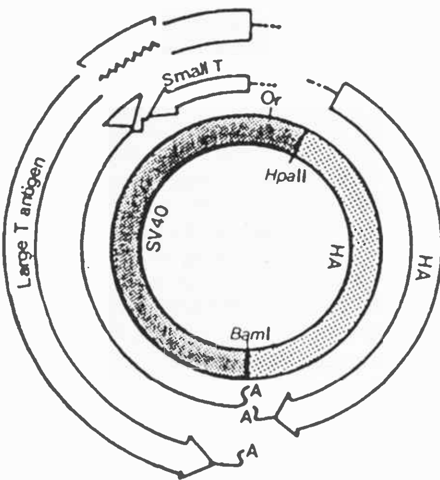


Figure 3
Late replacement vector SVEHA3.

SVEHA3 and *dl*1055 DNAs were introduced together into CV-1 cells using DEAE-dextran. The virus produced by these cells was serially passaged twice on fresh monolayers. At this stage a virus stock was obtained that induced a total cytopathic effect within 72 hr after infection of CV-1 cells.

Early Replacement Vectors SVLHA8 and SVLHA10

A segment of DNA was constructed that contained the intron of the small-T-antigen gene of SV40 linked directly to the poly(A)-addition site derived from the same gene. This segment was linked to the HA gene and the composite fragment inserted into the SV40 genome between the *Hin*dIII site at nucleotide 5171 and the *Bam*HI site at nucleotide 2533. The recombinant viral genome was cloned into the *Bam*HI site of pAT153 and propagated in bacteria. For transfection into simian cells, the re-

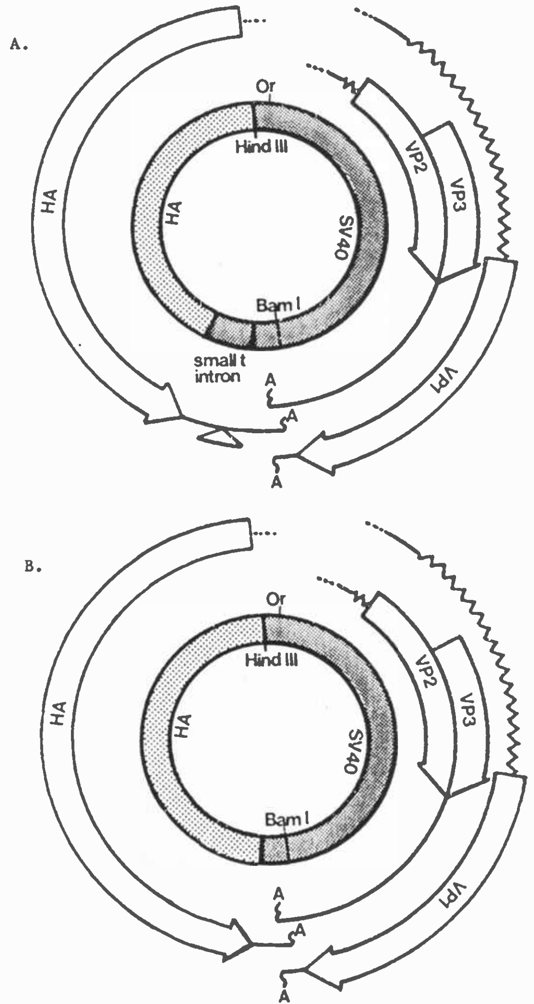


Figure 4
Early replacement vectors SVLHA8 and SVLHA10

combinant SV40-HA genome was excised from the plasmid by digestion with *Bam*HI and purified and ligated to yield the structure SVLHA8 shown in Figure 4. This recombinant viral genome contains the SV40 origin of DNA replication and an intact set of late genes. The HA gene lies downstream from the early promoter, within the untranslated sequences that normally precede the sequences coding for SV40 early gene products. Transcription from this promoter should therefore generate hybrid mRNAs that begin at the usual major sites in SV40 DNA (nucleotides 5320 and 5326) and consist of 70 nucleotides of untranslated SV40 sequences linked to the entire coding sequence of HA. Polyadenylation could occur either at the postulated site within the 3′ untranslated sequences of the HA gene or at the normal site for early SV40 mRNAs at nucleotide 2587. In the latter case there is an opportunity for a spliced mRNA to be formed by removal of the SV40 small T intron that is inserted into the construct downstream of the HA sequences and upstream from the SV40 early poly (A)-addition site.

At the time when these vectors were constructed there were indications from other workers that foreign genes inserted into SV40 recombinant vectors were expressed more efficiently when splice donors and acceptors were provided (Mulligan, *Nature 277*: 108 [1979]; Hamer and Leder, *Cell 18*: 1299 [1979]; Gruss and Khoury, *Proc. Natl. Acad. Sci. 78*: 133 [1981]). To test whether the intron sequence that had been built into SVLHA8 was necessary for the efficient expression of HA, a derivative (SVLHA10) was constructed that was identical to SVLHA8 except that it lacked the intron.

Because the early replacement vectors lack the gene coding for large T antigen, they cannot replicate in simian cells unless functional T antigen is supplied. This can be done by using as a permissive host the COS-1 line of SV40-transformed monkey cells, which endogenously expresses large T antigen. SVLHA8 and SVLHA10 were introduced (Gluzman, *Cell 23*: 175 [1981]) without helper into COS-1 cells using DEAE-dextran. The viruses produced by these cells were serially passaged twice on fresh monolayers to obtain stocks that induced total cytopathic effect in COS-1 cells within 72 hr after infection. The same stocks produced no visible cytopathic effect in CV-1 cells.

Radioimmunoassay of Vector-infected Cells

Following infection of CV-1 cells by the SVEHA3 (+*dl*1055) virus stock, or of COS-1 cells by the SVEHA8 or SVEHA10 virus stocks, cell extracts were assayed by radioimmunoassay for HA production. The results obtained 62 hr postinfection are given in Table 1.

Thus, the late replacement vector SVEHA3 expressed extremely high levels of HA in CV-1 cells. The amount is equivalent to 80 μg of HA per 90-mm dish of cells.

Similarly, the intron$^-$ early replacement vector SVLHA10 expressed high levels of HA in COS-1 cells. The lower level of expression in the intron$^+$ SVLHA8 was in part due to the low percentage of cells that became infected.

Analysis of the recombinant DNA molecules isolated by Hirt extraction of cells infected with sequential passages of the SVLHA8 stock showed a rapid increase in the proportion of rearranged (and presumably defective) molecules. By the fourth or fifth passage the stock became essentially noninfectious and contained a preponderance of deleted and rearranged molecules. Similar experiments with the SVEHA3 and the intron$^-$ SVLHA10 viruses showed no such alterations in the infectivity or in the structure of the recombinant genome.

These results show that the provision of splice junctions is not required for efficient expression of HA cloned within either SV40 early or late replacement vectors. It might be tempting to argue that the presence of the intron in the early replacement vector is actually detrimental to the expression of the HA gene. However, the rapid accumulation of rearrangements in the genome suggests that any deleterious effects are mediated at the level of DNA rather than RNA. Why genomic instability should be correlated with the presence of the intron remains to be elucidated.

Analysis of the HA Protein Product

To establish the structure and biological activity of the HA product expressed by either the early or late replacement vectors, the following assays were performed: immunoprecipitation, cytoplasmic and cell surface immunofluorescence, red cell binding and low-pH-induced cell-cell fusion.

Table 1

	ng HA/ 10^6 cells	*Av. no. moles HA/cell*	*percentage cells infected*[a]	*No. moles HA /infected cell*
SVEHA3:CV-1	6800	5.7×10^8	95	6.0×10^8
SVLHA8:COS-1	22	1.5×10^6	5	3×10^7
SVLHA10:COS-1	1700	1.4×10^8	95	1.5×10^8

[a] Estimated by red cell binding.

By all these tests the protein was indistinguishable from authentic HA produced in influenza-virus-infected cells.

Immunoprecipitation

The cell extracts from vector-infected cells contained a protein that was specifically precipitated by a monospecific anti-HA antiserum and that is indistinguishable in size (M_r =75,000) from authentic glycosylated HA precipitated from extracts of influenza-virus-infected cells.

Immunofluorescence

Vector-infected cells showed bright cytoplasmic immunofluorescence, with an area that is probably the Golgi aparatus staining particularly brightly. Surface staining showed more uniform but dimmer fluorescence over the entire cell surface. Influenza-infected cells showed a similar but less-bright pattern of fluorescence, and mock-infected cells were dark.

Red Cell Binding

Monolayers of cells infected with SVEHA3 or SVLHA10 bound erythrocytes in a dense carpet over the cells' surfaces. Approximately 5% of the cells infected with SVLHA8 were covered in a dense layer of erythrocytes. These results indicated that the vector-expressed HA is displayed at the cell surface in a biologically active form.

Transient Expression of HA in COS-1 Cells

The experiments described above were carried out using high-titer virus stocks containing the recombinant genome. Starting from transfection of DNA, preparation of these stocks normally takes 2-3 weeks. To screen the phenotypes of mutant HA genomes, a faster expression assay is clearly desirable. We have shown that it is possible to study the expression of HA in COS-1 cells only 48 hr after transfection of the SVHA vectors. Because the COS-1 cells contain endogenous SV40 large T antigen and are permissive for SV40 replication, plasmids containing SV40 origins of DNA synthesis are replicated to high copy number. A large pool of recombinant genomes is built up and transiently transcribed and translated. Transient expression of HA from both the early and late replacement vectors can be analyzed in COS-1 cells, and we have detected large amounts of HA in cell extracts 48 hr after transfection using DEAE-dextran of SVEHA3 or SVLHA10 (75μg DNA per 60-mm dish of COS-1 cells). Approximately 10-30% of the cells became transformed with DNA, and the yield of HA is 1-10% of that obtained after infection with a recombinant virus stock. This transient assay will provide a convenient method for screening the expression of mutant HA genomes.

Analysis of the Expression of Mutant HAs

As a first step in the analysis of the functions of the N-terminal and C-terminal hydrophobic regions of HA, deletion mutants were constructed that lack the DNA sequences coding for these peptides.

Preparation of the signal-deleted mutant utilized plasmid pJHB29 in which all the 5′ nucleotides coding for the signal had been removed using *Bal*31 exonuclease. This truncated HA gene replaced the wild-type HA gene in the late expression vector. It was ligated into the *Bal*I site immediately following the original HA AUG so that the HA initiation codon was fused in phase to the codon for the first amino acid of the HA1 polypeptide.

Virus stocks containing the signal$^-$ mutant are just now being prepared, and analysis of phenotype will be carried out in the near future.

Plasmid p2G10 contains a copy of the HA gene lacking sequences corresponding to the C-terminal membrane anchor. This truncated HA gene replaced the wild-type HA gene in the late expression vector, and virus stocks containing the mutant genome (SVEHA20-A$^-$) were prepared.

A most exciting result has been obtained with the C-terminal-deleted, anchor$^-$ mutant. Infection of CV-1 cells with this mutant recombinant leads to the accumulation of large amounts of HA in the tissue culture medium. The results of radioimmunoassay of cell extracts and the supernatant medium from one 10-cm culture dish at 60 hr postinfection is shown in Table 2.

To facilitate the purification of the secreted protein, it was determined that serum-containing medium was not necessary once the viral infection was established. Under serum-free conditions the secreted HA was essentially the only protein in the culture medium.

From these results we conclude that the C-terminal anchor is not required for translocation of HA to the cell surface. It now seems that the rules for transport (if not for gylcosylation) may be simple. Furthermore, the possibility is raised of obtaining and easily purifying large amounts of the

Table 2

	HA (μg) in cell extracts	*HA (μg) in culture medium*
Mock-infected CV-1 cells	0	0
SVEHA3-infected CV-1 cells	200	0
SVEHA20-A$^-$-infected CV-1 cells	28	300

tity of their members, and simple-sequence DNA may be of general importance in the gene conversion process. It may be that the TG repeat found in rat DNA is one of many such repeats in the cellular genome. It is also possible that integration of SV40 DNA is facilitated by enzymes that recognize simple-sequence DNA. These possibilities are currently under investigation.

Excision of SV40 DNA. SV40 DNA insertions are powerful probes with which to explore organizational plasticity of the cellular genome. Insertions of SV40 DNA provide physical and genetic markers in the cellular genome, and such insertions are physically accessible through nucleic acid hybridization techniques; furthermore, morphological transformation by SV40 is a well-defined, selectable genetic marker. More importantly, SV40 DNA insertions can be induced to excise from the integrated state. This activity appears to be unique to integrated papovavirus genomes and has great potential utility for the study of recombination and rearrangement of DNA in the chromosomal milieu. SV40 excision results from the interaction of four elements: (1) the origin of SV40 DNA replication, (2) the viral large-T antigen, (3) recombination between homologous DNA sequences, and (4) a permissive cellular environment. The viral origin, T antigen, and the degree of DNA sequence homology are accessible to manipulation through in vitro recombinant DNA techniques. It should be possible to construct cell lines with DNA insertions that potentiate rearrangements of chromosomal DNA and to examine the influence of the elements listed above in any such process.

In addition, it should be possible to study other recombinational processes, such as sister chromatid exchange and gene conversion, by using SV40 along with other selectable genetic markers to construct lines of cultured mammalian cells that contain in their genomes exogeneous genes whose specific recombination will manifest itself in the biochemical phenotype of the cell. It is hoped that this approach will detect in mammalian cells the action of genetic processes known to be important in lower eucaryotic organisms.

Transformation and Viral Tumor Induction

W.C. Topp, M. Hightower, M. Ramundo, D. Smith

Our work this year continued a trend, begun several years ago, away from studies centered on SV40 and towards projects related to transformation and tumor induction by the human adenoviruses. Two broad project areas can be defined within the group: alterations in cellular nutritional requirements that accompany viral transformation and the mechanism of induction of breast fibroadenomas and sarcomas by the human adenovirus serotypes of group D.

It is well know that virus-transformed cells require a medium supplemented with significantly lower concentrations of whole serum than required by normal cells for the maintenance of optimal exponential growth. This past year, in collaboration with Don McClure (University of California, San Diego), we began a series of studies designed to characterize this alteration in the requirement for serum-supplied macromolecular "growth factors." We have chosen REF 52 cells as a parental line from which to isolate transformants. This line, isolated in our lab by serial passage of 15-day-gestation rat embryo cells in a regimen similar to that used for 3T3 cells, is characterized both by an unusually low saturation density (10^4 cells/cm^2) and a strong dependence on serum. It was determined that with a basal medium consisting of 75% DME and 25% Ham's F12, a rate and efficiency of clonal proliferation identical to that observed in 10% FCS-supplemented medium could be obtained for REF 52 cultures with a mixture consisting of insulin, transferrin, epidermal growth factor (EGF), vasopressin, dexamethasone, and the high-density lipoprotein complex (HDL). The responsiveness of REF 52 cells to HDL and vasopressin suggests that these cells may be derived from vascular endothelium.

Five independent SV40 transformants of REF 52 were isolated by selecting colonies with an altered morphology following serial dilution of virus-infected cells. These lines were cloned through microwells and characterized both as to nutritional requirements for optimal clonal growth and as to possession (in 10% FCS) of a variety of phenotypes commonly associated with viral transformation. All the transformants proliferate in low serum supplement, but only three of five show marked alterations in cytoplasmic actin networks. Of these three, two express plasminogen activator; and of these two, one grows well suspended in soft agar. All five transformants no longer require either EGF or vasopressin for optimal growth, although cellular morphology is still affected by these factors. This is similar to results from other laboratories. In addition, steroid hormones such as dexamethasone appear to be toxic for the transformants. Insulin and transferrin are still required to the same degree as for REF 52, but the HDL complex is no longer required by any of the transformants. Perhaps the most exciting result from this study is that although two of the transformants have obviated the need for any part of the HDL complex, three others still require the fatty acid component (supplied by bovine serum albumin binding aracadonic and oleic acids). These three lines are those expressing the more transformed phenotype. Whether this phenomenon is related to the altered lipid metabolism often observed in virus-infected cells or to some other effect such as cellular growth response to prostaglandins, for which these fatty acids serve as precursors, remains to be determined. Whatever the reason, it is intriguing that a phenomenon related to lipid metabolism appears to correlate with the degree

external domains of membrane proteins for analysis and perhaps vaccine production.

Finally, we have begun experiments in which the signal and anchor sequences of HA are attached upstream and downstream, respectively, of the coding sequences of another gene. In this way we hope to be able to cause foreign proteins to (1) display on the surfaces of cells or (2) be secreted from them. For example, we are attempting to alter the localization of SV40 T antigen by replacing the sequences normally used in the initiation of its transcription with the sequences coding for the signal peptide of hemagglutinin. This recombinant was able to replicate its DNA in COS-1 cells but not in CV-1 cells. When virus stocks obtained from COS-1 cells were used to infect CV-1 cells, expression of T antigen was not detectable either by immunofluorescence or immunoprecipitation, and dot blots of cytoplasmic RNA revealed only a very low level of transcription of the SV40 *A* gene. Sequencing studies are in progress to determine the precise structure of genetic region spanning the modified N-terminus of the T-antigen gene.

In another approach, cDNA copies of the hemagglutinin gene were put into a newly constructed helper-free adenoviral vector (Gluzman, see above). As control elements, the late promoter of adenovirus was placed upstream and the polyadenylation site for SV40 T antigen, downstream of the HA gene. Cotransfection of live 293 cells with the *Xba*I A fragment of adenovirus *dl*309 DNA yielded eight plaques. Three of these contain the hemagglutinin gene, as determined by Southern blotting of Hirt DNAs. Two of the three express hemagglutinin at 3–6 x 10^5 molecules per cell, as detected by radioimmunoassay. Subcloning of the hemagglutinin gene from these three recombinants is underway. In the near future we plan to replace the central portion of the hemagglutinin gene with *A* gene of SV40 and to study the expression of T antigen in this system.

Transformed Cells and Transforming Genes

Integration and Excision of SV40 DNA

J. Stringer

Integration of SV40 DNA. Integration of SV40 DNA probably reflects the action of a cellular recombination system that can incorporate any foreign DNA into the cellular genome, and analysis of SV40 DNA insertions may therefore reveal the action of cellular recombination enzymes. The structure of SV40 DNA insertions is different from those of retroviral proviruses and movable genetic elements. No single DNA sequence in either the cell or the SV40 genome serves as an obligatory site of SV40 integrative recombination, and SV40 DNA insertions are not bordered by the repeat structures characteristic of transposons and retroviral proviruses (Annual Report, 1980). Integration of SV40 could involve the matching of short stretches of homologous sequences present in the otherwise heterologous SV40 and cellular genomes. To explore this possibility, I have sequenced a rat DNA fragment that contains an unoccupied site of SV40 DNA integration. Comparison of this sequence with that of SV40 and that at the rat-SV40 recombinant junction (Fig. 5) showed that SV40 DNA became linked to rat DNA at a point where the two genomes shared 5 bp of DNA sequence homology.

This is the first direct demonstration that DNA sequence homology may mediate integrative recombination between SV40 DNA and the cellular chromosome. Although analysis of more unoccupied sites of SV40 DNA insertion will be needed to understand fully the role of base pairing in SV40 integration, it is now clear that cells are able to use a short sequence homology to integrate an otherwise heterologous SV40 DNA molecule. This example of homologous recombination suggests an additional difference between integration of SV40 DNA and integration of retroviruses and transposition of movable elements.

The DNA sequence of the unoccupied SV40 integration site shown in Figure 5 is striking in two ways. It is repetitive, containing 12 TG pairs in tandem, and it is composed almost entirely of G and T. Thus, this DNA chain is simple in both its sequence arrangement and its base composition. This TG repeat is remarkably similar to the "simple-sequence" DNA present in the second introns of human γ-globin genes. There is evidence to suggest (Slightom et al., *Cell 27*: 627 [1980]) that simple-sequence DNA may act as hot spots for the initiation of gene conversion between human γ-globin genes. Gene conversion is one mechanism by which multigene families may conserve the iden-

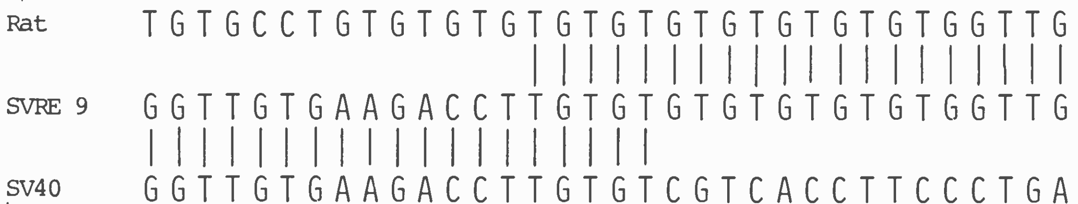


Figure 5
SV40 and rat DNA share 5 bp of homology at the point of their recombination. The three lines of DNA sequence are those of the parental molecules, SV40 and rat DNAs, and that of the recombinant, SVRE9. Vertical lines indicate sequence homologies between parent and progeny DNAs.

of transformation. We are currently extending these studies to the characterization of REF 52 transformed by mutants of SV40 that do not encode the viral small-T protein (as well as to REF 52 cultures transformed by human adenoviruses) in order to determine the individual roles of the two viral gene products.

Our third area of interest is the mechanism of induction in female rats of benign breast fibroadenomas by members of group-D of the human adenoviruses. We have confirmed the results first reported by Ankerst and Jonsson (*Int. J. Cancer 13:*286 [1974]) that human Ad9 induces multiple breast tumors within 3 to 4 months in virtually 100% of female Wistar/Furth rats injected at birth. Tumors do not develop in male rats. We expect that fibroadenoma induction may be a general feature of group-D viruses, and we know that at least one other member of group D, Ad10, is similarly tumorigenic. However, tumor growth is strongly dependent on the physiological environment provided by the host. Both Lewis and Wistar/Furth rats are susceptible but Sprague-Dawley, Fisher, Axl, Taconic Farms, and Buffalo rats are not, nor are Syrian hamsters or BALB/c, C57B6, or C3H mice. Moreover, tumor induction is strongly hormone-dependent: Ovariectomization at 4 weeks of age completely eliminates tumor appearance, and preliminary data suggest that ovariectomization after tumor appearance leads to regression.

Although the fibroadenomas possess both an epithelial and a mesynchymal component, we have shown by double immunofluorescent staining of cryogenic tumor sections with both anti-tumor serum and a serum directed against epithelial keratins, that viral antigens are expressed only in the mesynchymal cells. The tumors are quite similar in this regard to other adenovirus-induced tumors, which are almost universally of mesynchymal origin. This is consistent with the fact that when tumors are explanted to culture, only cells with a fibroblastoid morphology give a stain with anti-tumor sera. The pattern of staining is virtually identical to that observed in cells transformed in vitro by viruses of groups A and B.

Very little was known about the molecular biology of the group-D viruses when we began this project. Consequently, in collaboration with Louise Chow and Tom Broker (Electron Microscopy Section), we have recently completed an extensive comparative study relating these viruses to the better-characterized members of groups A, B, and C. The transcriptional map of Ad9 is virtually identical to that of Ad2. However there are some major differences in the patterns of viral gene expression. Most notably, Ad9 early region 1a is substantially underproduced as compared to the same region in Ad2, whereas Ad9 early region 3 is greatly overproduced, becoming the most common early message. E3 is also nearly twice as large as in Ad2, resulting in a greatly foreshortened fiber gene. By DNA/DNA heteroduplex analysis, the members of group D are closely related to the viruses of groups A, B, C, and E as are the members of these groups one to another. A striking exception to this is that the inverted terminal repeat sequences of the group-D viruses appears to be only distantly related to that of the other groups, a fact confirmed by Jeff Engler (Electron Microscopy Section) at the sequence level. Thus, although some differences exist, there is very little here to suggest the source of the unique oncogenic behavior of the group-D viruses.

Studies on this area are continuing and center about the isolation of viral variants with potentially altered oncongenicity. There are also in progress ongoing in vivo experiments to characterize further the involvement of the host endocrine system.

Transformation of Rat Cells by Mutants in Early Region 1 of Adenovirus 2

M.A. Anderson, R. Frisque, M. Cahn

Early regions 1A and 1B of Ad2 (encompassing map units 1.5 to 11.5) encode the genes required for virus-induced transformation of rat cells. We are interested in identifying among the several genes within this region those that are involved in the establishment and maintenance of the transformed state.

The problem has been approached using a series of plasmids constructed in this laboratory by Nigel Stow and Dick Frisque. These molecules contain 0–9% of the Ad2 genome inserted into pBR322, with small deletions generated within the E1A or E1B region by site-directed mutagenesis (see Fig. 6). These mutagenized fragments have been reestablished in viral genomes by recombination with *dl*309, a viable deletion mutant of Ad5 (see N. Stow and P. Rosman, Annual Report 1980).

Both the viruses and the plasmids have been used in transformation studies with baby rat kidney cells. One of the two deletion mutants at the *Xba* site in E1A and one of two mutants at the *Sst* site in E1B fail to transform. Cell lines have been established using the wild-type viruses and plasmids and the mutants with deletions at the other sites. These cell lines differ from each other in morphology, number of actin cables, fibronectin production, and distribution of the 1B 57K protein. We are examining whether these variations are correlated with changes in viral DNA or protein.

Hr 440 an Ad5 host-range mutant isolated by D. Solnick (*Nature 291*: 508 [1981]) has also been used in transformation studies with baby rat kidney cells. Hr 440 is a 1A splicing mutant that produces no 12S mRNA and a truncated protein from the 13S message yet is wild type in expression from the 1B region. This mutant transforms at greatly decreased efficiency as compared with wild type, suggesting that the 1B products alone are not sufficient to induce transformation in this system.

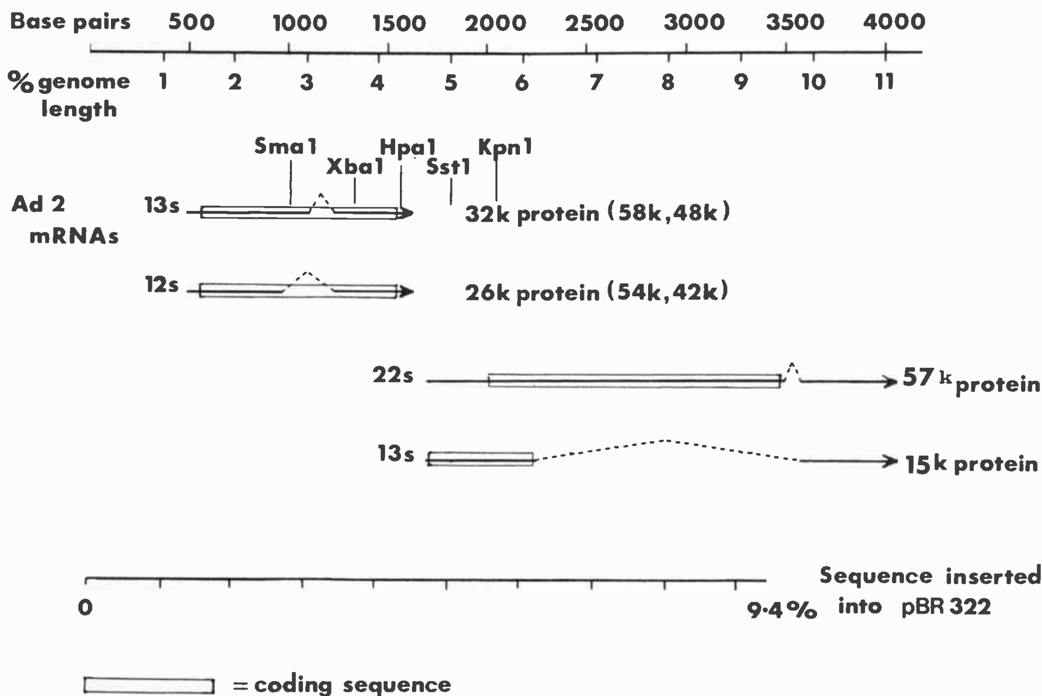


Figure 6
Position of the deletions generated within early region 1 of Ad2 relative to the splice sites (----) and coding regions (▭) on the 1A and 1B mRNAs.

Proteins of Transformed Cells: Plasminogen Activator

B. Anderson, J. Sambrook, in collaboration with D. Rifkin (New York University Medical School)

Many lines of virus-transformed and tumor cells synthesize and secrete large quantities of proteases or protease activators, and it has been suggested that in some cases, at least, the secretion of these enzymes leads to increased proteolysis of the cell's surface, which in turn induces several of the phenotypic changes that characterize malignancy.

Among the proteases secreted from transformed cells are two that are capable of activating plasminogen to plasmin. The more common of these, urokinase, appears to be a fairly broad-spectrum protease; by contrast, the so-called tissue activator that is synthesized by a relatively small number of cell types is highly specific for plasminogen. The enzyme may be similar to one that is synthesized by cells of the vascular endothelium and that may be responsible for initiating the dissolution of fibrin clots. Little is known about plasminogen activator, and the aims of this project are to analyze the enzyme in detail at the molecular level and to clone and study the structure of its gene.

During the last year we have raised polyclonal sera in both rabbits and mice against denatured and native forms of the activator purified from human melanoma cells. These sera immunoprecipitate a family of highly glycosylated molecules (~70,000 m.w.) from a variety of transformed cell and tumor lines of human origin. The secreted form of the activator is of slightly lower molecular weight than the intracellular form. This result is consistent with pulse-chase experiments that show that the protein is synthesized as a precursor that is cleaved as it is secreted from the cell.

A solid-phase radioimmunoassay for plasminogen activator has been developed to screen sera for anti-plasminogen-activator binding activity. The assay has already been used to follow the progress of the various animal immunization programs and is currently being used to identify murine hybridoma cell lines that secrete monoclonal antibodies directed against plasminogen activator.

These sera also neutralize the activity of purified plasminogen activator and appear to recognize molecules synthesized in in vitro protein-synthesizing systems using mRNA extracted from human melanoma cells.

Monoclonal Antibodies to the Early Proteins of SV40 and Adenovirus

R.D. McKay

A large number of monoclonal antibodies to SV40 T antigen have been generated both here and at other laboratories. We have mapped the sites at which ours bind to T antigen by using a set of adeno-SV40 hybrid viruses that express different extents of T antigen stretching from the C terminus. We have anti-T antibodies that bind to the amino

terminal sequence shared by small T antigen and to each of the regions of large T defined by the hybrid viruses Ad2D2, Ad2ND2, and Ad2ND1dp2. These antibodies have been used to probe two functions of SV40 large T antigen: (1) its ability to bind specifically to DNA and (2) its ability to act as a target for virus-specific cytotoxic T-cell killing (in collaboration with Dr. L.R. Gooding, Emory University).

DNA Binding

Using a simple modification of the standard *Staphylococcus* immunoabsorbtion procedure, it is possible to sensitively and quantitatively measure the affinity of the interaction between SV40 T antigen and DNA (McKay, *J. Mol. Biol* 145:471 [1981]). This technology has now been extended in several ways. First, DNase protection and "footprinting" assays on the specific binding of T antigen in a crude cell lysate have been developed. Second, the immunobinding assay was shown to be sufficiently sensitive to detect the binding behavior of T antigen from transformed cell lines. Third, SV40 T antigen has been shown to bind specifically to sites in polyoma and JC viral DNAs.

This assay has been used to study the consequences of mutations both in the regulatory DNA sequnces of SV40 (McKay and DiMaio, *Nature 289:*810 [1981]) and in the structural gene of large T antigen (see section above by J.R. Stringer).

Virus-specific Cytotoxic T-cell Killing

Animals immunized with purified T antigen reject syngeneic SV40-transformed cells. It is presumed that T antigen exposed on the transformed cells acts as a target for the immune response. Although immunoreactive material can be found on the surfaces of transformed cells, there is no direct proof that it is either the target for the immune system or specifically oriented in the cell membrane.

We have tried to block cytotoxic killing and failed, even though a monoclonal anti H-2 antibody specifically and effectively blocks cell killing.

To pursue this question further, we have used quantitative microfluorimetry to ask whether certain antigenic sites on T antigen are inaccessible on the cell surface and whether we can specifically remove T antigen from the cell surface and leave H-2 intact. Our current data indicate that there are antibodies that recognize nuclear T antigen and do not recognize cell-surface T antigen, suggesting that T antigen is specifically oriented on the cell surface. It is also possible enzymatically to remove T antigen from the surfaces of cells and leave H-2 antigenically intact. This observation suggests a route to show directly that SV40 T antigen is itself the target for virus-specific cell lysis.

Antibodies to Adenoviral Early Proteins

Although we now know a great deal about the number and structure of adenoviral early proteins, we know little of their functions. As a first step toward the purification and study of these proteins, Bruce Stillman (Protein Synthesis Section) and I have immunized mice with either the cytoplasm or a nuclear extract of HeLa cells infected with adenovirus. Hybridomas derived from these mice have been screened by immunofluorescence against (1) hydroxyurea-blocked adenovirus-infected HeLa cells and (2) adenovirus-transformed cells. Antibodies that directly inhibit adenoviral DNA replication in vitro are also being sought (see B. Stillman). In a recent fusion we screened 300 hybridoma cell lines in this way, several of which stain only adenovirus-infected HeLa cells. In two cases antibodies have been cloned in soft agar and shown by several criteria to recognize a gene product of adenoviral early region 1b. A second class of specific monoclonal antibody binds to particular regions of the cytoplasm of infected HeLa cells. A third class is characterized by diffuse cytoplasmic fluorescence of infected cells. We have discarded any antibodies that recognize determinants in uninfected HeLa cells. We have shown, however, that certain antibodies with a high affinity for particular sites in adenovirus-infected cells immunoprecipitate the same proteins from infected and uninfected HeLa cells. A possible explanation for this finding is that adenovirus infection induces a relocation of normal cellular proteins.

Analysis of Regulatory Sequences of SV40

Y. Gluzman, R. Frisque

The region of the SV40 genome around the unique *BglI* restriction site at .67 m.u. contains several regulatory signals. Replication-deficient mutants of SV40 were constructed by cloning SV40 DNA in a plasmid vector and altering the sequences near the *BglI* site by site-specific mutagenesis techniques (Gluzman et al., *Cold Spring Harbor Symp. Quant. Biol. 44:* 293 [1980]). Many of these mutants have small deletions (4–241 nucleotides), and some induce early viral proteins in permissive and nonpermissive cells, complement early temperature-sensitive mutants, and transform cells as well as wild-type SV40 (Gluzman, 1980 [*ibid*]; Gluzman, *Cell 23:* 175 [1981]). Analysis of viral mRNAs isolated from these transformants showed that, irrespective of the size or location of the deletions, the 5′ ends of the viral mRNAs were located approximately the same distance from the putative early promoter (i.e., the TATA box), rather than at a specific site in the viral genome.

There are indications that a second region (a tandem repeat of 72 bp) 90 nucleotides upstream from the TATA box may be part of the SV40 early promoter (Benoist and Chambon, *Nature 290:* 304

[1981]; Gruss et al., *Proc. Natl. Acad. Sci. 78:* 943 [1981]). We have made deletion mutants within this region using the restriction enzyme *Sph*I, which cleaves once within each 72-bp repeat. The resulting virus remains viable after removal of the sequences between the *Sph*I sites (one complete 72-bp repeat), which is in agreement with earlier studies. In contrast to recent reports, however, deletion of additional sequences by limited nuclease-S1 or *Bal*31 treatment still yields viable (though impaired) virus. We are presently determining what effect these deletions have on early and late transcription of the SV40 genome and on transformation.

In a parallel approach, two clones have been constructed that contain the entire early coding region of SV40 but lack the sequences just upstream from the initiation codon for the early viral proteins large and small T. The pT6 clone (Y. Gluzman, S.-L. Hu, and J. Sambrook, unpubl.) was constructed by positioning the Ad2 major late promoter 60 nucleotides upstream from the initiating codon. The pBD1 clone (L. Ling and Y. Gluzman, unpubl.) was derived from the adeno-SV40 hybrid D1. This clone contains the adenovirus early region 2 promoter, with the cap site of the mRNA located 200 nucleotides upstream from the AUG codon of SV40 T antigen. The transformation efficiencies of these DNAs were compared to that of a clone containing wild-type SV40. Although the SV40 clone transformed rat cells at a frequency of 300–600 foci/μg DNA/10^6 cells, the clones containing the adenoviral promoters were a hundredfold less efficient. Furthermore, pT6 and pBD1 transformants took longer to appear than wild-type transformants, were less transformed morphologically, and contained fewer viral early proteins. Expression of large-T and small-T proteins could be enhanced fivefold by superinfecting cells transformed with pBD1 with Ad2. This result might be due to the ability of early region 1A to turn on other adenoviral transcription units, including the E2 promoter of pBD1, which is now integrated into the cellular genome.

Transformation of Permissive Cells with Origin-defective Mutants of SV40

Y. Gluzman, O. Sundin

Isolation of simian cell lines transformed by ori⁻ SV40. Lines of simian cells transformed by origin-defective SV40 have been isolated (COS-1, COS-7) (Gluzman, *Cell 23:* 175 [1981]). The integrated copy of SV40 in these cells directs the synthesis of a wild-type T antigen in sufficient quantity to drive the replication of sequences of foreign DNA that are attached to the SV40 origin of DNA replication. Several foreign gene sequences have been replicated to high copy number in this way. Some of these genes are transcribed into mRNA, and in one case (influenza HA) large quantities of the foreign protein are synthesized. Thus, this system provides an opportunity to use the techniques site-directed mutagenesis and recombinant DNA technology to analyze the roles of various control sequences in the expression of particular genes. Experiments of this type are being undertaken, in collaboration with Pamela Mellon and Tom Maniatis, using the human α-globin gene (see Mellon et al., *Cell 27:* 279 [1981]). Although this general approach has been quite informative, the limitation of the COS system lies in the fact that once viral DNA replication begins, it continues for 2–3 days until the cells die. To avoid this problem and to gain more control over the replication of SV40 recombinants and their transcription, temperature-sensitive and origin-defective SV40 mutants were used in an attempt to isolate temperature-sensitive COS cells. At the present time, several T-antigen-positive colonies of CV-1 cells transformed by either tsA58-ori$^-$ or tsA209-ori$^-$ mutants have been isolated. We are currently investigating to see whether SV40-specific DNA replication in these cells is temperature-sensitive.

Human cells (in collaboration with Harvey Ozer, Hunter College). Establishment of human lines in culture is a very difficult process. Primary human cells, if they grow at all in vitro, have a very short life time. When human cells are transformed by SV40, however, they can be passaged for longer times, and lines can be established with greater ease. Unfortunately, transformation of human cells by SV40 virus or viral DNA is an inefficient process. The resulting transformed colonies very often contain free SV40 DNA and produce infectious virus at low levels (Girardi et al. *J. Cell Comp. Physiol. 65:* 69 [1965]). To bypass this problem, origin-defective mutants of SV40 were used. The DNAs of such mutants were three to ten times more efficient in transforming human fibroblasts than wild-type SV40 DNA extracted either from virions or cloned into the plasmids. The further investigation of this problem involves establishment of permanent human lines transformed by origin-defective mutants, as well as attempts to transform human cells with origin-defective *tsA* mutants of SV40. The latter project might be useful if the transformed phenotype of human cells is extinguished at 40°C and the properties specific for differentiated cells prior to transformation are reestablished.

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NUCLEIC ACID CHEMISTRY

R.J. Roberts, T.R. Gingeras, *R.M. Blumenthal, J.E. Brooks, N. D'Anna, G. Freyer, B.-D. Jiang, Y. Katoh, P.M. Lin, P.A. Myers, D. O'Loane, K. O'Neill, B. Parsons, P. Rice, M. Wallace, C. Yen*

During the past year the Nucleic Acid Chemistry group has continued to accumulate data toward the elucidation of the Ad2 DNA sequence. Many computer programs have been written to assist both in the assembly and in the analysis of the growing body of sequence data now available. Our long-standing interest in restriction endonucleases and methylases has been maintained, and we have been successful in cloning a type-II restriction system into *E. coli.*

Adenovirus-2 Genomic Sequence

T.R. Gingeras, C. Yen, Y. Katoh, B.-D. Jiang, R.J. Roberts

At the present time two main blocks of sequence have been collected. The first segment consists of 15,000 nucleotides located between 0% and 41% on the genome, of which 0–33% is in final form and two short stretches of sequence are missing between 33% and 41%. The second main block of sequence is located at the right end of the genome and consists of 4000 nucleotides of completed sequence between genome coordinates 89% and 100%. As a result of the information available from this laboratory and from others, most notably the groups of F. Galibert (Hôpital St.-Louis, Paris) and U. Pettersson (University of Uppsala, Sweden) a total of 22kb of sequence from the two ends of the genome are now available in final form. In addition, several large stretches of contiguous sequence exist between coordinates 41% and 70%.

During the course of the last year the strategy employed for sequencing the Ad2 genome has focused on the collection of data using the M13 cloning-sequencing system. Not only does this approach provide a simplified means of obtaining second-strand sequence information within segments already sequenced, but it has also allowed us to establish an M13 clone bank which consists of single-stranded segments from both the *l* and *r* strands of the genome. These clones have already proven useful for the purification and characterization of specific Ad2 mRNAs and will provide a valuable resource for the future.

Analysis of the sequence presently available and its correlation with data gathered elsewhere now gives a reasonably complete picture of the organization of information at the left end of the genome between coordinates 0% and 33%. By comparison of our sequence data with information obtained by J. Smart (Protein Chemistry Section) and B. Stillman (Protein Synthesis Section) concerning the tryptic peptides of the Ad2 terminal protein, the gene for that polypeptide has been localized between coordinates 23.5% and 29% and is transcribed from the viral *l* strand. Of considerable interest is the finding of an unexpected large open reading frame lying between coordinates 23% and 14.2% on the *l* strand. This open reading frame has a total capacity in excess of 120K daltons. The N group mutants of Ad2, which are defective in replication, map within this gene. Recently, J. Hurwitz (Albert Einstein College of Medicine) and his collaborators have described a polypeptide of 140K that is associated with the Ad2 terminal protein during purification and that appears to be a DNA polymerase. It is possible that this protein is the translation product of this new open reading frame.

In addition to these two large open reading frames in early region 2B, there are more than ten other open reading frames within the left-end sequence, each of which has a coding capacity in excess of 10K and which presently cannot be assigned to any known polypeptides. Some of these show extensive overlap with other reading frames. It is becoming increasingly apparent that the organization of information within the Ad2 genome is highly economical.

Computer Program Development

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

From the start of the Ad2 sequencing project we have been developing software designed to aid both in the DNA sequencing and the subsequent analysis of the data. Such programs have elicited considerable interest due to the huge volume of sequence data being generated worldwide and because they are written in a language (FORTRAN) that is relatively transportable to other computers. Copies of these programs have been widely distributed.

Among the new programs written during the course of the last year, there have been two designed specifically to handle data associated with the M13 cloning-sequencing system. One of these, called M13, provides a master data base in which information about the various M13 clones is stored. A second program, SEQ, provides complementary information about the sequencing reactions that have been carried out on the various M13 clones. Both data bases can be searched by a third program, called FIND, which allows the ready retrieval of sequence information obtained from individual clones. The programs M13 and SEQ, which are responsible for generating the data bases, require a minimum of manual input and efforts are underway to reduce this input even further. During the next year we hope to consolidate these programs into a single package that will simplify both input and retrieval of data.

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A number of small utility programs have also been written that assist in the analysis of sequence data and that include routines for finding repeated sequences, some specialized searching routines, and a program that provides annotated sequence information. This latter program, called COMBINE, was specifically designed to provide a means of annotating sequence files that are not yet in completed form. Often a situation is encountered when some specific feature of a sequence is known to be associated with a particular biological property and yet its absolute coordinate within the sequence is not known because of uncertainties elsewhere in the sequence. Thus, it is not possible to associate an absolute nucleotide number with a particular feature. This problem has ben overcome in a simple fashion by using an information file that contains a unique identifying sequence, which is associated with a comment to be annotated. By combining a main sequence file with this information file, it is possible to produce an annotated output on which all known significant features of a particular sequence are indicated. The system is quite versatile and time-saving.

Restriction Endonucleases

J.E. Brooks, P.M. Lin, P.A. Myers

The list of restriction endonucleases has grown dramatically during the last year, with 97 new entries having been added, including 17 new specificities. We have been involved in a joint project with D. Comb and I. Schildkraut (New England Biolabs), and 87 new strains have been examined during the year, yielding a total of 32 type-II restriction enzymes. Among those enzymes identified, eight have been shown to possess new specificities, although in several cases the specific sequences recognized have not yet been determined. These results are summarized in Table 1. Much of our work has focused on species from two genera, *Nocardia* and *Bacillus,* both of which have proved to be valuable sources of type-II restriction enzymes.

Early Region 4 of Adenovirus 2

G. Freyer

Early region 4 of Ad2 maps between coordinates 91.3% and 100% on the viral genome and is ex-

Table 1

Strain	*Enzyme*	*Recognition sequence*
Bacillus stearothermophilus C1	*Bst*CI (*Hae*III)	GGCC
Bacillus stearothermophilus C11	*Bss*CI (*Hae*III)	GGCC
Bacillus stearothermophilus G3	*Bst*GI (*Bcl*I)	TGATCA
	*Bst*GII (*Eco*RII)	CC (A/T) GG
Bacillus stearothermophilus G6	*Bss*GI (*Bst*XI)	?
	*Bss*GII (*Mbo*I)	GATC
Bacillus stearothermophilus H1	*Bst*HI (*Xho*I)	CTCGAG
Bacillus stearothermophilus H3	*Bss*HI (*Xho*I)	CTCGAG
	*Bss*HII (*Bse*PI)	?
Bacillus stearothermophilus H4	*Bsr*HI (*Bse*PI)	?
Bacillus stearothermophilus P1	*Bss*PI	?
Bacillus stearothermophilus P5	*Bsr*PI	?
	*Bsr*PII	?
Bacillus stearothermophilus P6	*Bse*PI	?
Bacillus stearothermophilus P8	*Bsa*PI	?
Bacillus stearothermophilus P9	*Bso*PI (*Bse*PI)	?
Bacillus stearothermophilus T12	*Bst*TI (*Bst*XI)	?
Bacillus stearothermophilus X1	*Bst*XI	?
	*Bst*XII (*Mbo*I)	GATC
Microbacterium thermosphactum	*Mth*I (*Mbo*I)	GATC
Nocardia aerocologenes	*Nae*I	GCC↓GGC
Nocardia amarae	*Nam*I	GGCGCC
Nocardia argentinensis	*Nar*I	GG↓CGCC
Nocardia blackwellii	*Nbl*I (*Pvu*I)	CGAT↓CG
Nocardia corallina	*Nco*I	CCATGG
Nocardia erythropolis	*Ner*I	?
Nocardia opaca	*Nop*I (*Sal*I)	G↓TCGAC
	*Nop*II (*Sal*II)	?
Nocardia otitidis-caviarum	*Not*I	?
Nocardia rubra	*Nru*I	TCG↓CGA
Nocardia uniformis	*Nun*I	?
	*Nun*II (*Nar*I)	GG↓CGCC
Staphylococcus saprophyticus	*Ssa*I	?
Streptomyces endus	*Sen*I	?
Streptoverticillium flavopersicum	*Sfl*I (*Pst*I)	CTGCA↓G
Thermoactinomyces species	*Tsp*I	?

pressed early in infection. Although the transcription of this region has been studied extensively and some of the polypeptides encoded by it have been partially characterized, its role in the Ad2 life cycle is not well understood. With the recent availability of the complete genomic sequence of this region, an interesting observation has been made. A large open reading frame has been found located between coordinates 92.3% and 94.8% and capable of coding for a protein of 34K. This sequence is wholly contained within two E4 mRNAs. However, both of these mRNAs have complete open reading frames available upstream of this large one. Unless these mRNAs are polycistronic or subject to true internal initiation, they would not be expected to be used for the translation of the 34K polypeptide. All other mRNAs from E4 are missing these sequences because of a splicing event that removes sequences from coordinates 92.2% to 94.2%. However, one mRNA does exist that contains a short exon located between coordinates 94.8% and 94.2% and that is then spliced a second time to coordinate 92.2%. If a precursor to this RNA that is lacking the second splice exists, then this precursor would be an ideal mRNA from which to translate the 34K polypeptide. Such a situation might be analogous to the *box* locus of yeast mitochondria (Lazowska et al., *Cell 22*: 333. [1980].)

We are examining this system in several ways. A cDNA library of early Ad2 mRNAs has been constructed and is being screened for sequences from E4. We hope that the putative mRNA from which the 34K protein is made will be present. The library will also allow us to determine the splice points for the other E4 mRNAs, since in only one case is there sequence information presently available. We are also exploring an immunological approach to identify the 34K polypeptide. Based upon the DNA sequence, we have had two short oligopeptides synthesized, containing 10 and 17 residues from the C terminus of the 34K polypeptide. These will be used to prepare antisera which we hope will cross-react with the intact protein and will allow us to detect it both in vivo and in vitro. This approach has proved successful in several other systems. We are also pursuing a genetic approach to examine the effects of specific deletions within this reading frame.

Restriction and Modification Genes

J.E. Brooks, T.R. Gingeras

We have been interested in the study of bacterial restriction-modification (r-m) systems for several years. Although restriction enzymes are widely used as research tools, few have been carefully studied. As yet, little is known about the control, organization, and expression of these systems in their bacterial hosts. Although a few of the r-m systems have been shown to "restrict" the entry of foreign DNA into bacterial cells, in most cases there is no defined function for these r-m systems. Since most restriction enzymes have been isolated from obscure bacteria with little formal genetics, we have begun to transfer the genes coding for the r-m into *E. coli*.

Our work in this area initially began with the isolation and characterization of one of the better understood bacterial methylases, the *E. coli dam* gene (Brooks et al., *J. Mol. Biol.* [1982b] in press). Concomitant with this work were efforts to identify and isolate several bacterial type-II r-m genes. A plasmid-borne Type-II r-m system (Jacoby and Sutton, *Plasmid 1*: 115 [1977]) is present in *Pseudomonas aeruginosa*. This *Pae*R7 system was originally shown to be a type-II system by Hinkle and Miller (*Plasmid 2*: 387 [1979]) and was later determined to be an isoschizomer of *Xho*I (P. Myers, pers. commun.) that recognized the sequence CTCGAG. *Bam*HI fragments of this plasmid (pMG7) were cloned into pBR322, and individual clones were tested for the presence of the *Pae*R7 r-m system. A list of clones that express the PAO r-m system is detailed in Table 2 and includes recombinant plasmids that overexpress both the endonuclease and methylase genes. Of interest is the fact that although the *Pseudomonas* plasmid pMG7 carries four antibiotic resistance genes, none are expressed in any of the new recombinant plasmids, not even in PAO 9 (see Table 2) which contains pMG7 in its entirety.

Our studies of the products derived from these clones have proved somewhat interesting. The *Pae*R7 endonuclease cleaves at the same position within the recognition sequence as *Xho*I (C↓TCGAG); however, it does not cleave all legitimate *Xho*I sites. One such site mapping at 26% on the Ad2 genome is entirely refractory to *Pae*R7 cleavage, whether present in native Ad2 DNA or present in cloned copies of this segment of the genome. Although it has been observed for a long time that the kinetics of DNA cleavage varies considerably depending on the position of the recognition site, this is the only known example of a totally refractory site. We do not know whether this is a failure in recognition or cleavage or both. However, it is possible that failure is only in cleavage, in which case this will prove a useful system in which to study recognition. Towards this goal, reconstructed versions of this Ad2 site are being cloned and tested for inhibition of cleavage. The methylase produced by these clones modifies the adenine residue in the recognition sequence (CTCGȦG). Although the *Pae*R7 methylase is encoded by a separate gene from the endonuclease, the *Pae*R7 methylase modifies the Ad2 site at 26% at a very low rate. Again the reasons are unclear at present. These features of the *Pae*R7 system should prove useful for studying how these two separate enzymes interact with the same site.

Aided by a clone that contains a 4-kb insert and that overproduces both the methylase and en-

Table 2

Properties of the PAO Clones

Clone name	Insert		Size (kb)	R-M phenotype in vivo R[a]	M[b]	in vitro R[c]	M[d]
PAO 4[e]	*Bam*	*Bam*	39	no phage growth		+	+
PAO 9	*Bam*	*Bam* (partial)	43	+	+	+	+
PAO 10	*Bam*	*Bam*	39	+	+	+	+
PAO H4	*Bam*	*HindIII*	6.5	+	+	++[f]	++[f]
PAO N4	*Bam*	*Nru*	4	+	+	++[f]	++[f]

[a] *The clones were scored + for in vivo restriction if they produced 10^{-5} less φ80 phage plaques than a host bacterium containing pBR322.*

[b] *The clones were scored + for in vivo modification if, after one cycle of growth on the host clone, φ80 phage were able to grow equally well on restricting and control bacteria.*

[c] *In vitro restriction activity was determined using a crude lysate of the bacterial clone to cleave Ad2 DNA.*

[d] *In vitro methylation activity was determined using a crude lysate of the bacterial clone to modify Ad2 DNA and demonstrating the prevention of subsequent cleavage by the* PaeR7 *endonuclease.*

[e] *PAO 4 clones express a function that prevents the adsorption of both φ80 and λ phage.*

[f] *Both the restriction and methylation assays showed these extracts to be more than twice as active as their PAO 4 parental clone.*

donuclease, isolation and purification of these enzymes is in progress. The availability of purified products from these clones will aid in the identification and mapping of these genes.

There is mounting evidence that eucaryotic DNA methylation is important for gene regulation. This has prompted us to explore the possibility of transferring prokaryotic methylase genes into mammalian tissue culture cells. In collaboration with Dave Kurtz (Hormonal Control of Gene Expression Section), initial transformation experiments have been carried out with the *E. coli dam* gene and the results are now being analyzed.

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ELECTRON MICROSCOPY

T.R. Broker, L.T. Chow, J.A. Engler, M. Rossini, *R.A. Guilfoyle, B. Urso, M. van Bree, M. Hoppe, M. Hallaran*

We have used electron microscopy heteroduplex techniques to study the genetic organization and expression of a variety of systems such as adeno-SV40 hybrids, human and bovine papilloma viruses, and human α- and β-tubulin gene families, as well as of the adenoviruses on which we have concentrated for six years. With the near completion of the electron microscopic mapping of cytoplasmic RNAs specified by human Ad2, we have now also applied a complementing group of additional techniques—cloning, site-directed mutagenesis, DNA sequencing, and microinjection of genes isolated on plasmids—to investigate questions of adenoviral gene regulation and the fine structure of genes and their transcriptional signals. We have also compiled a vast amount of technical data on the human and animal adenoviruses and adeno-SV40 hybrids—maps of RNA transcripts, protein-coding regions, point mutants and deletions, insertions and substitutions, and DNA restriction cleavage sites; DNA, RNA, and protein sequences; and alignments and comparisons of homologous sequences—which are published as Appendixes in *DNA Tumor Viruses* and in *Genetic Maps.* (Broker 1981, 1982).

Adenoviral Early Gene Regulation

M. Rossini, R. Guilfoyle, J. Engler, L. Chow, B. Urso, M. Hoppe

The sequential appearance of cytoplasmic viral mRNAs at early times during infection (Neuwald et al., *J. Virol. 21:* 1019 [1977]; Chow et al., *J. Mol. Biol. 134:* 265 [1979]; Nevins et al., *J. Virol. 32:* 727 [1979]) suggested the existence of regulatory interactions among the different early genes. Transcriptional analyses using viruses mutated in defined early regions have provided evidence for such interactions (Jones and Shenk, *Proc. Natl. Acad. Sci. 76:* 3665 [1979]; Berk et al., *Cell 17:* 935 [1979]; Carlock and Jones, *J. Virol. 40:* 657 [1981]; Montell et al., *Nature 295:* 380 [1982]). Recently, the stimulation of early region 2A expression by the products of early region 1A has been recreated in a microinjection system (Rossini et al., *J. Virol. 38:* 982 [1981]). In our research, cloned segments of the viral genome containing genes of interest have been introduced into mammalian cell nuclei in a variety of combinations and analyzed for the expression of encoded proteins by an immunofluorescence assay. This method allows us to address detailed questions concerning the expression and functions of early regions 1A and 2A: (1) What are the effects with regard to the activation of early region 2A caused various region E1A mutations located in different parts of the gene and affecting different spliced RNAs and their products? (2) How do the wild-type and mutant E1A plasmids affect expression of the late promoter for E2A? (3) Will other adenoviral promoters lead to transcription of region 2A if they are substituted for the natural promoters, and how will they respond to co-injection of E1A plasmids? (4) What are the roles of E1A and E2A gene products in the activation of other early genes?

To investigate whether (and how) region E1A products interact with the different E2A promoters, three E2A clones in the vector pBR322 have been tested in the microinjection system. The first (constructed by D. Klessig, University of Utah) contains a DNA segment between coordinates 59.5 and 76.0 that includes the DNA (DBP)-coding sequence and both the early and late promoters located at coordinates 75 and 72, respectively. The second plasmid (constructed by S.-L. Hu, Tumor Virus Section) contains an Ad2 *Hin*dIII A fragment (50.1–73.2) in which is present the DBP gene and only the late promoter. The third, control clone (constructed by J. Engler) is an Ad2 *Bam*HI-*Eco*RI fragment (59.5–71.2) that includes only the DBP gene but none of its promoters.

The E2A clone containing the DBP gene with early and late promoters is poorly expressed after microinjection into hamster or human cell nuclei, but the presence of E1A in the microinjection solution leads to a full expression of the DBP, as determined on the basis of indirect immunofluorescent tagging of an antibody to DBP (Table 1). Surprisingly, the E2A clone containing the DBP-coding sequence and only the late promoter was able to produce a detectable amount of 72-kD protein after microinjection in the absence of E1A. But when the E1A plasmid was coinjected with the late DBP gene, expression was dramatically reduced, suggesting that an E1A product acts as a repressor of transcription initiated at the late promoter (Table 1). The E2A clone containing only the gene without early or late promoters was, as expected, unable to produce the 72-kD protein either in the absence or presence of E1A products.

Various cloned E1A mutants (Stow, *J. Virol. 37:* 171 [1981]) containing small deletions were tested for their abilities to activate or repress E2A expression. By DNA sequence analysis, we have learned that two of the E1A mutants have deletions of 4 bp and 7 bp around the *Xba*I restriction site. These deletions shift the translation frame and introduce stop codons that truncate the carboxyterminal portions of the gene products specified in common by all E1A mRNAs. Phenotypically they eliminate the activation of the E2A clone containing both the early and the late promoters

TABLE 1

Expression of Early Region E2A after Microinjection into Hamster Cell Nuclei

DBP plasmid:			*pBR730*	*pAd15.1*	*pAd207*
DBP promoters:			*early (+ late)*	late	none
Coordinates:			59.5-76.0	50.1-73.2	59.5-71.2
E1A plasmid	Coord.	Mutation	percentage nuclei fluorescent		
—			15	55	0
BE5	0-9.1	wt	58	15	0
HE4	0-4.3	*	60	nd	0
M6	0-9.1	Sma^R (2.8)	66	8	nd
M14	0-9.1	Sma^R (2.8)	48	18	nd
Sx1	0-4.3	Sma^R (2.8)	47	18	nd
BE5.1	0-9.1	Sma^R (2.8)	2	nd	nd
X4	0-9.1	Xba^R (3.7)	17	53	nd
X18	0-9.1	Xba^R (3.7)	16	52	nd
H22	0-9.1	Hpa^R (4.3)	41	15	nd
*hr*1	0.7-5.7	(2.9)	15	nd	
*hr*440	0.7-5.7	(2.7)	17	nd	

* *Sequence deleted beyond the* HpaI *restriction site.*

In each experiment, thymidine-kinase-deficient BHK cells were grown for 3 days on 22 × 30-mm glass cover slips in DME containing 1% calf serum and thymidine. (tk⁻ cells have larger nuclei, facilitating the microinjection.) Fresh medium containing 10% calf serum was added to the cultures 5-6 hr before injection.

About 10-14 liters of the buffer solution containing plasmids was injected into each of 100-200 cell nuclei in up to ten replicate experiments. Cells were fixed for 15 min with methanol at −20°C 20-22 hr after microinjection.

The 72-kD DBP was visualized in microinjected cells by indirect immunofluorescence using hamster anti-DBP serum as the first antibody and fluorescein isothiocyanate-labeled goat anti-hamster glubulin as the second antibody. Numbers in parenthesis are adenoviral map coordinates where the mutations are located.

and the repression of the E2A clone with just the late promoter. The Ad5 mutant *hr*1 has a single base deletion which results in the premature termination of proteins encoded by the 13S mRNA, but those encoded by the 12S RNA are not affected (Esche et al., *J. Mol. Biol. 142:* 399 [1980]; Ricciardi et al., *Proc. Natl. Acad. Sci. 78:* 6121 [1981]). The two-base change observed in the Ad5 mutant *hr*440 inactivates the donor splice site of the 12S RNA and also introduces a termination codon in the utilized translation frame of the 13S RNA, with the appearance of a truncated protein (D. Solnick and M. Anderson, *J. Virol.,* in press). E1A plasmids constructed by D. Solnick from H5*hr*1 and *hr*440 both gave negative responses when injected with the E2A clone that has the early and late promoters (Table 1). This is in agreement with in vivo results using whole viruses containing these mutations (Berk et al., *Cell 17:* 935 [1979]; Solnick, *Nature 291:* 508 [1981]). The *Sma*I mutant M14 was sequenced and found to have a six-base deletion. Although the resulting protein has a deletion of two amino acids, M14 acted like wild type in its effects on the E2A promoters. Nonetheless, it has a host-range defect when reintroduced into a whole viral chromosome and can only grow in adenovirus-transformed line 293 cells (which contain a wild-type copy of early region 1) (M. Anderson, Tumor Virus Section). Another *Sma* mutant, BE5.1 (constructed by J. Engler) has eight-base insertion in the restriction site. This E1A plasmid is not able to stimulate DBP synthesis from the early promoter (cf. Carlock and Jones, *J. Virol. 40:* 657 [1981]). The *Hpa*I-resistant clone H22 and the HE4 clone—completely deleted beyond the *Hpa*I cleavage site—also act like wild type. The *Hpa*I restriction site is beyond the E1A protein-coding region but before the site of RNA polyadenylation. Together these results suggest that both the carboxyl half of the E1A proteins and the internal domain unique to the proteins encoded by the 13S RNA are involved in the regulatory control of early region 2A. Our data do not rule out the involvement of the products of the 12S mRNA generated from early region 1.

The availability of an early region 1B plasmid, pXX6 (constructed by N. Stow, Institute of Virology, Glasgow), and various antisera to E1B proteins (from R. Frisque, R. McKay, Tumor Virus Section, and B. Stillman, Protein Synthesis Section) opened the possibility for testing the effect of E1A on their expression. E1B proteins were produced in cells microinjected with high concentrations of pXX6 by itself, a response that decreased linearly with the concentration of the plasmid. The fluorescence of the anti-E1B-protein serum was localized in a few perinuclear bodies. Coinjection of the E1A plasmid had no stimulatory effect. But when the early DBP gene was also coinjected, E1B expression increased dramatically. If the E1A plasmid was then omitted, no such stimulation was observed. This response suggested that the effect of E1A was to stimulate DBP synthesis, which in turn increased E1B expression. This conclusion was strengthened by the observation that the late promoter clone of DBP was able by itself

to stimulate E1B. But when E1A was also added, it depressed DBP synthesis from the late clone and E1B expression remained unstimulated. It appears, then, that the DBP plays a significant role in the expression of E1B proteins, and there emerges the concept of a regulatory cascade: E1A → E2A → E1B. Whether this is a direct effect on the E1B promoter or, instead, on mRNA splicing or stability is not yet known. Recently, Richardson and Westphal (*Cell 27:* 133 [1981]) demonstrated that the DBP also stimulates expression of early region 4 genes.

To learn about the spacial relations between the sequences necessary for transcription and those that interact with E1A products, Richard Guilfoyle is creating and mapping nested sets of deletions extending from the upstream (5') sides of both the early and late promoters for the DBP toward the gene. The deletions range from −135 to +18 around the early promoter and from −308 to +7 around the late promoter. (+1 is the capped 5' end of the RNA.) These will be tested by in vitro transcription to determine whether RNA is made and by microinjection to learn whether primary transcripts are properly processed to messages and to determine how RNA synthesis responds to the absence or the presence of early region 1A. As a necessary control, we have shown that the early promoter for DBP works in a cell-free transcription system.

Other preliminary experiments have demonstrated that the DNA sequence containing the major late *r*-strand promoter and encoding the complete first leader segment and donor splice site, when coupled in the proper orientation upstream of the DBP gene and its internal leader, can lead to the expression of the DBP gene. This suggests that splicing successfully joined the first late *r*-strand leader segment to the DBP gene in the hybrid transcript. Other promoters, including those for the IVa_2 gene and for early region 3, have also been placed before the DBP gene. Several other clones have been made that contain the early, but lack the late, promoter for DBP. All will be tested for expression in the presence and absence of various adenoviral gene products.

DNA Sequences of Adenovirus Types 7 and 12

J. Engler, M. van Bree, M. Hoppe

The DNA sequence of group-B Ad3 was determined between coordinates 8.9 and 11.0, as reported last year. The sequence of another group B-virus, Ad7, was determined between coordinates 11 and 31 (linking to the sequence of Ad7 between coordinates 0 and 11 determined by Dijkema, Dekker, and van Ormondt [see Broker 1981]). Over much of that same interval the group-A Ad12 was also sequenced. As described by the Nucleic Acid Chemistry Section, the group-C Ad2 was sequenced from coordinates 0 to 31. Thus, the data are available for detailed comparisons of transcriptional signals, open reading frames, inferred protein sequences, and conservation and divergence on an evolutionary scale. The IVa_2 genes of the several viruses are 80% homologous, with most nucleotide changes being transitions or transversions. There is a corresponding conservation of amino acid sequences, in as much as most base changes occur in the third position of the coding triplets. The Ad7 IVa_2 protein consists of 448 amino acids and has a molecular weight of 50,600. The positions of the 5' end and of the donor and acceptor splice sites of the Ad7 and Ad12 messages can be inferred by analogy to those of Ad5 (Fig. 1) (van Beveren et al., *Gene 16:* 179 [1981]).

Between coordinates 29 and 30, the genes for two virus-associated (VA) RNAs are evident. They have somewhat limited homology to the corresponding VA I and II RNAs of Ad2, but are rather more conserved at the 5' and 3' ends and the internal promoter region for polymerase III transcription, and also with respect to the extensive secondary structure. The T_1 oligonucleotides previously determined for Ad7 VA RNA (Ohe et al., *J. Biol. Chem. 244:* 5320 [1969]) match the complete sequence of VA I. Between coordinates 29 and 23, the leftward-transcribed strand has an open reading frame that, over most of its length, is closely related to those of Ad2 and, where completed, Ad12. Studies of the trypic peptides of the Ad2 DNA terminal protein (55 kD) and its 87 kD precursor have recently identified this open frame as the corresponding gene (J. Smart and B. Stillman, submitted; T. Gingeras et al., submitted; P. Alestrom et al., submitted). The sequences giving rise to the late RNA leader segments 1, 2, i, and 3 have also been identified by their close homology to those of Ad2 (Akusjarvi and Petterson, *Cell 16:* 841 [1979]; Zain et al., *Cell 16:* 851 [1979]; A. Virtanen et al., submitted). There is more homology than would have been expected from the failure to establish Ad7 RNA:Ad2 DNA heteroduplexes (Kilpatrick et al., *J. Virol. 30:* 899 [1979]). This raises the probability that the scattered mismatched bases lower the optimum annealing temperatures more than predicted by previous equations to the point where the propensity for intrastrand RNA secondary structures may, for all practical purposes, prevent efficient RNA:DNA cross-annealing. This has a great bearing on the general design of hybridization analyses using RNA:DNA probe combinations.

In Ad7 DNA, a long open reading frame starts to the right of coordinate 20 and overlaps the coding region for the amino-terminal portion of the IVa_2 protein, but it is in a different reading frame (Fig. 1). This second gene encodes the 105-kD protein specified by early region 2B (Stillman et al., *Cell 23:* 497 [1981]); cf. T. Gingeras et al., submitted; P. Alestrom et al., submitted). Within the overlapping genetic regions, the carboxyterminus of the 105-kD protein is substantial-

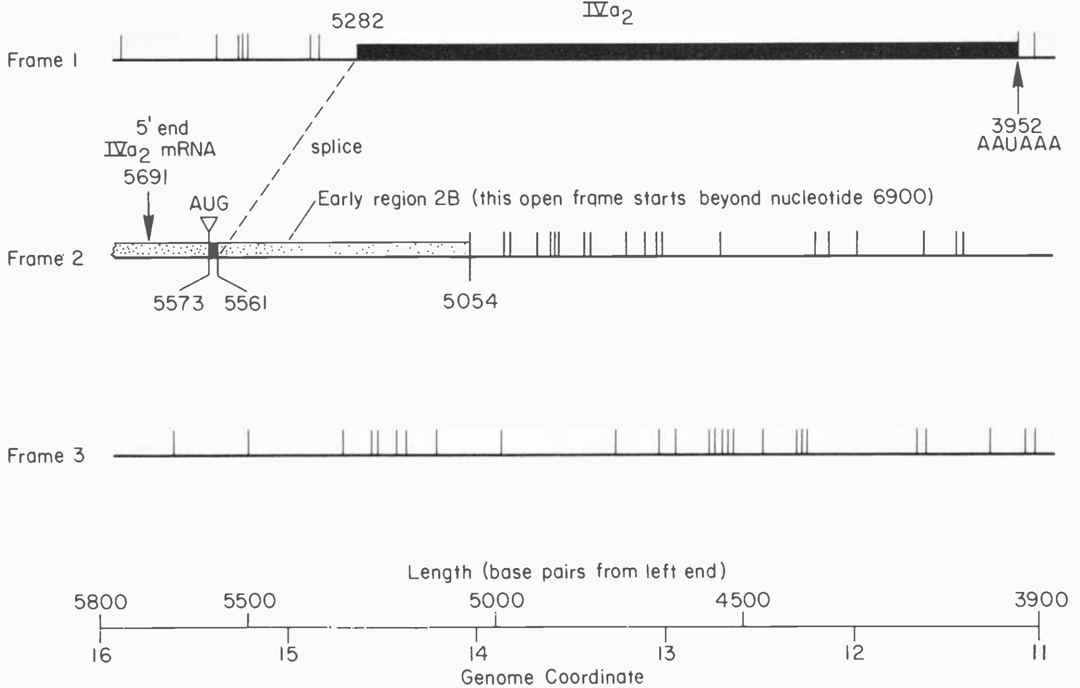

Figure 1
Potential open translation frames in the IVa_2 gene of Ad7. The scale length indicates map coordinate and base pairs from the left end of Ad7. Frame 1 begins at nucleotide 5769. Vertical lines show the positions of stop codons (UAG, UGA, UAA) in each potential translation frame. The arrow in frame 2 marks the most abundant 5′ end of the IVa_2 mRNA at nucleotide 5691, determined analogy to Ad2 (Baker and Ziff, *J. Mol. Biol. 149:* 189 [1981]). The dotted line marks the positions of the donor (nucleotide 5561) and acceptor (nucleotide 5282) RNA splice sites determined by analogy to Ad5 (van Beveren et al., *Gene 16:* 179 [1981]). The coding frame for the IVa_2 polypeptide is shown by the solid bar; the position of the first ATG (nucleotide 5573) downstream from the presumed 5′ end of the IVa_2 mRNA of Ad7 is marked by the open triangle. This position is probably also used in Ad5 and Ad12. The stippled bar represents the early region 2B protein-coding frame, which overlaps the 5′ end of the IVa_2 gene.

ly more conserved than the amino terminus of the Iva protein, suggesting that the primary evolutionary pressure was exerted on the 105-kD protein. On the basis of its position in a transcriptional unit that encodes other DNA replication functions (DBP and the precursor to the DNA terminal protein), the 105-kD protein can be inferred to play a role in replication.

Sequences of the Inverted Terminal Duplications of Adenovirus Types 4, 9, 10, and 31

J. Engler

Ad4, 9, 10, and 31 DNAs were isolated by Diane Smith and Bill Topp. The DNA terminal proteins covalently linked to the 5′ ends were removed by exhaustive digestion with pronase. The 3′ ends were labeled by [^{32}P]dGMP incorporation using the large fragment of *E. coli* DNA polymerase I. The terminal fragments were clipped off with restriction nuclease *Rsa*I, and the fragments were isolated by digestion with *Rsa*I followed by polyacrylamide gel electrophoresis, and then sequenced by the Maxam-Gilbert method. As with all other adenoviruses, the ends are inverted duplications of one another. Those of Ad4 (a group-E virus) are 116 nucleotides long; those of Ad9 (a group-D virus), 158 nucleotides; and those of Ad31 (a group-A virus), 148 nucleotides. By way of comparison, the terminal duplications previously determined for Ad2 and Ad5 (group-C viruses) are 103 nucleotides long, and those of Ad12 (another group-A virus) are 165 nucleotides long. All the human serotypes share some sequence homology, particularly over the first 22 nucleotides. This undoubtedly reflects conservation of recognition sequences for the linking of the (highly conserved) terminal proteins, which serve as primers for DNA replication, and for the binding of other proteins involved in the initiation of replication at the terminal origins for DNA synthesis. Nucleotide-by-nucleotide alignments and comparisons of the inverted terminal duplications of these and several other human and animal adenoviruses indicate those sequence elements that are most highly conserved, those that are more free to diverge, and the extent to which the various viruses are in-

terrelated evolutionarily (see Broker 1981; T. Broker and J. Engler, in prep.).

Electron Microscopic Studies of Adenovirus Type 9

L. Chow, T. Broker

Human Ad9 is a representative of the group-D adenoviruses. Although they have not been studied extensively at the molecular level, but have a number of unusual biological properties, including the specific induction of breast tumors in female Wistar/Furth rats, as described in more detail by Bill Topp (Annual Report, 1981). In collaboration with Bill Topp and Diane Smith, we have been analyzing the DNA homology relationships among the group-D viruses Ad9, 10, and 26 and with representatives of group-A (Ad12), group-B (Ad7) and group-C (Ad2). The primary differences among the group-D viruses reside in early region 3 and the fiber genes. Those differences between Ad9 and viruses from other groups are in early regions 1a, 1b, 3, and 4, as well as in the fiber gene (Fig. 2). Early region 2a, encoding the DBP, is somewhat diverged, and E2B, encoding the DNA terminal protein and the 105-kD protein, is quite highly conserved, as are the genes for many of the late proteins.

Studies of Ad9 RNA in heteroduplexes with Ad9 DNA showed rather typical spliced adenoviral RNA structures, reflecting a conservation of genome organization, except in early region 3, which was nearly twice as long in Ad9 (coordinates 74–88) as in Ad2 (coordinates 78–86). The Ad9 fiber gene is shorter than its analog in Ad2. Early region 3 RNAs exhibited a multitude of alternative splicing patterns, with one or two intervening sequences and two possible 3′ ends. Furthermore, E3 is notably overexpressed relative to transcripts from other early regions, and E1A RNAs are significantly underrepresented. The possible significance of these qualitative and quantitative differences with regard to the unique behavior of Ad9 are not presently known.

Studies of Adeno-SVR6 Hybrid Viruses

L. Chow, T. Grodzicker

The genomic structure of the hybrid virus adeno-SVR6 constructed by C. Thummel, R. Tjian, and T. Grodzicker, *Cell 23:* 825 (1981) and the SV40 T-antigen RNA transcript derived from it have been studied by electron microscopy heteroduplex analysis (Fig. 3). The SV40 insertion was shown to be at adenovirus map coordinate 5.0, within early region 1B, instead of at the *Bam*HI restriction site at coordinate 29.2, as originally constructed, indicating that a major rearrangement took place. The SV40 sequences are in the

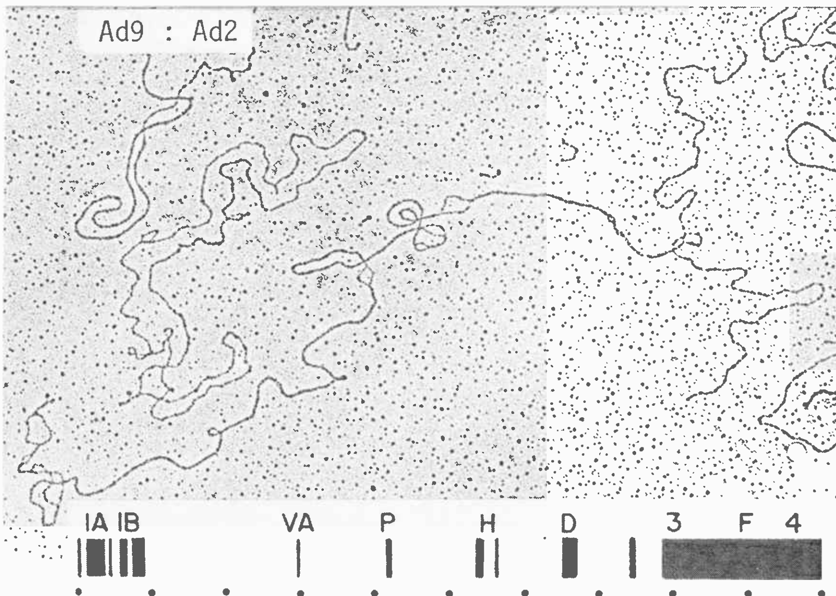

Figure 2
Heteroduplex between Ad9 and Ad2. Unpaired regions in the heteroduplex are shown by solid bars in the interpretive graphic. Early regions 1A, 1B, 3, and 4; the DBP gene (D, part of early region 2A), and the positions of the VA RNAs, penton (P), and hexon (H) are labeled. The single-stranded circular molecule in the micrograph is adenoviral DNA with the inverted terminal duplication paired together.

correct orientation to be transcribed from the adenoviral early region 1B promoter. A variable length of DNA that does not share any homology with either adenoviral or SV40 DNA is located transcriptionally downstream from the SV40 specific-sequences. The adenoviral DNA between coordinates 5.0 and 59.5 is entirely deleted. The SV40 T-antigen mRNA is a hybrid transcript that originates from the E1B promoter and is processed using the polyadenylation signal of the SV40 insertion. It contains a 490-nucleotide leader separated from the main body of 2660 nucleotides by a splice deletion of 365 nucleotides. The hybrid RNA leader therefore consists of 160 nucleotides of adenoviral E1B sequence linked to 330 nucleotides of SV40 sequence. Correlation of this mRNA structure with the adenoviral DNA sequence shows that the adenoviral DNA contains a potential AUG initiation codon. This AUG is very likely out of phase with the SV40 T-antigen coding frame and, in any case, is apparently not utilized during translation. This conclusion may be drawn because the T antigen of the hybrid virus is identical to the genuine T antigen encoded by wild-type SV40 virus (Thummel et al., *Cell 23:* 825 [1981]). This presents the unusual situation of an internal AUG, rather than the 5'-proximal AUG, initiating the synthesis of a eucaryotic protein. Interestingly, E1B proteins of wild-type Ad5 can be translated from either the 5'-proximal or an internal AUG (Bos et al., *Cell 27:* 121 [1981]).

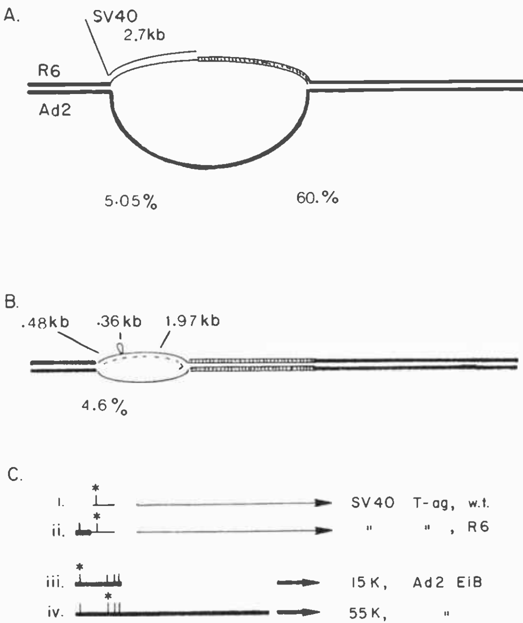

Figure 3
Structure of pSVR6 and its RNA transcript. (*A*) Schematic representation of a heteroduplex formed from Ad2 DNA (solid thick strands), SV40 DNA (thin strands), and pSVR6 hybrid virus (containing adenoviral sequences from coordinates 0-5.05 and 60-100 m.u., the SV40 early region, and a variable length of other DNA (hatched line) (which turns out to be carrier calf thymus DNA used during transfection (T. Grodzicker, Tumor Virus Section). (*B*) Schematic representation of an R loop formed with pSVR6 and hybrid RNA recovered from infected cells. The 5' end of the RNA starts at the early region 1B promoter (coordinate 4.6), the 3' end is at the SV40 early region polyadenylation site, and the splice corresponds to that found in the RNA for the SV40 large T antigen. (C) Comparison of RNAs encoding: (i) SV40 large T antigen, (ii) pSVR6 large T antigens, (iii) the 15kD protein of Ad2 E1B, the 55-kD protein of Ad2 E1B. As above, thick lines refer to adenoviral sequences and thin lines to SV40 sequences. AUG initiation codons are indicated by tick marks, and those utilized in the four RNAs are shown with an asterisk(*). The 55K RNA species from both pSVR6 and adenoviruses are translated starting at the second AUG initiation codon.

Characterization of Human α and β-Tubulin Genes

L. Chow (in collaboration with N.J. Cowan, D.C. Wilde, and F.C. Wefald, Princeton University)

Several lambda recombinant phage made from a human genomic library have been identified to contain either α- or β-tubulin-specific sequences by Cowan, Wilde, and Wefald using cloned chicken tubulin cDNAs as probes. They have shown that there are multiple copies of each of the genes and that genes in either family are not linked closely. The structures of one β-tubulin and two α-tubulin genes have been studied by electron microscopy after heteroduplex formation with chicken cDNAs (Figs. 4 and 5). All three genes are spliced, and their 5' and 3' ends as deciphered from electron microscopy studies are consistent with those determined by biochemical methods. The sum of the lengths of human sequences that hybridize to the chicken cDNAs accounts for essentially the entire length of the cDNAs. Furthermore, the human and chicken sequences form stable heteroduplexes under rather stringent conditions, suggesting a high degree of homology in sequences expressed as mRNAs. The β-tubulin gene in clone 5-beta and the α-tubulin gene in clone 2-alpha both contain three intervening sequences (IVS). The α-tubulin gene in clone 19-alpha has only two IVS, corresponding in position to the first two in clone 2-alpha. However, the intervening sequences in 2-alpha and 19-alpha differ in both size and sequence. In addition, the 5' and 3' ends of these two genes are somewhat diverged. Several pairs of short inverted duplications, presumably of the *Alu* family of middle-repetitive sequences, are found in the intervening and flanking sequences in clones 5-beta and 2-alpha. Whether the sequences play any role in the regulation of these genes is not yet clear.

Homology Relationships of Human and Bovine Papilloma Viruses

L. Chow, T. Broker

Papilloma viruses cause numerous types of warts

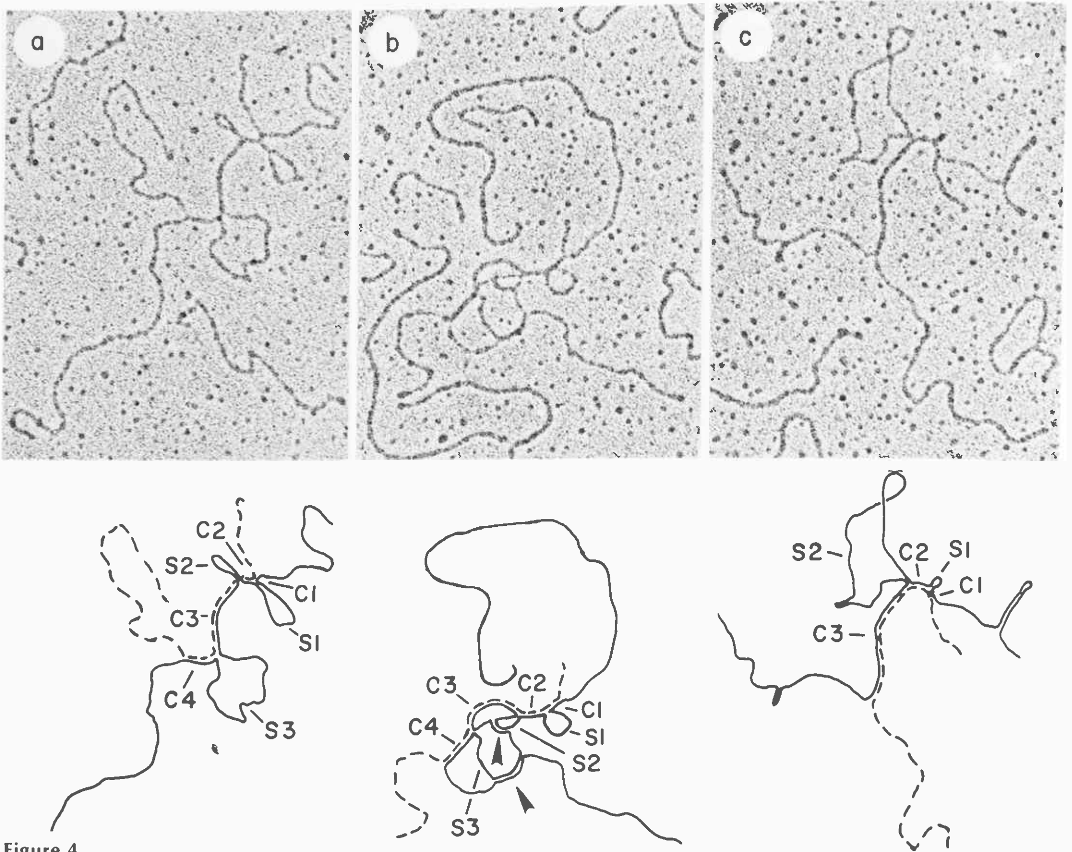

Figure 4
Structures of α-tubulin-specific sequences in clones 2-alpha and 19-alpha. (*a*) Heteroduplex between a *Sac*I fragment of 2-alpha and *Eco*RI-linearized chicken α-tubulin cDNA, pT1 DNA. The inverted duplications in 2-alpha did not pair and gave a well-laid-out heteroduplex. (*b*) Like *A*, except that the two pairs of inverted duplications in 2-alpha had annealed. (c) Heteroduplex between *Eco*RI-linearized pT1 DNA and a 19-alpha in a fragment generated by digestion with *Eco*RI/*Sma*I. (——) 2-alpha or 19-alpha restriction fragment; (— — —) pT1 DNA; (**) inverted duplications. C1–C4 are regions of hybridization; S1–S3 are intervening sequences. (*d*) Schematic representations of α-tubulin-specific sequences. Raised bars are sequences specific for α-tubulin; equivalent regions of 2-alpha and 19-alpha are indicated by dotted lines; the scale indicates kilobase pairs.

in humans and other mammals and birds. Most are benign epithelial cell proliferations, but a few types have been correlated with the eventual development of invasive carcinomas. Thus the papilloma viruses appear to be bona fide tumor viruses. They have been little studied because they cannot be propagated in cell culture systems. They replicate slowly in infected basal epithelial cells sufficient to maintain a low copy number of free HPV plasmids. Productive replication, late protein synthesis, and the assembly of new virion particles take place in situ in differentiated keratinocytes. But such cells do not divide in situ or in culture, and therefore large quantities sufficient for experimental work with the viruses cannot be prepared conveniently. At best, it has been necessary to recover virus particles or viral DNA and proteins from wart specimens surgically isolated from afflicted individuals, which has made the standardization of viral materials most difficult. Nevertheless, with the advent of DNA cloning technologies, it became possible to produce large amounts of homogeneous papilloma virus DNA. One early finding was the variety of human and bovine papilloma viral types, each characteristically associated with specific epidermal sites and tissues and with different clinical manifestations. The types were categorized on the basis of extent of DNA homology, as scored by mass hybridization (cf. Orth et al., *Proc. Natl. Acad. Sci. 75:* 1537 [1978]; Heilman et al., *J. Virol. 36:* 395 [1980]; Ostrow et al., *Virology 108:* 21 [1981]; Gross et al., *J. Invest. Dermatol. 78:* 160 [1982]). Human papilloma virus type 1 (HPV-1) and HPV-4 are primarily associated with deep plantar warts; HPV-2, with common warts of the hands and face; HPV-7, with warts of meat handlers (although there is no defined relation with animal wart viruses); HPV-3, HPV-5, HPV-8, HPV-9, and HPV-10 with flat warts and the pityriasislike lesions of rare individuals afflicted with epidermodysplasia verruciformis, a most serious infec-

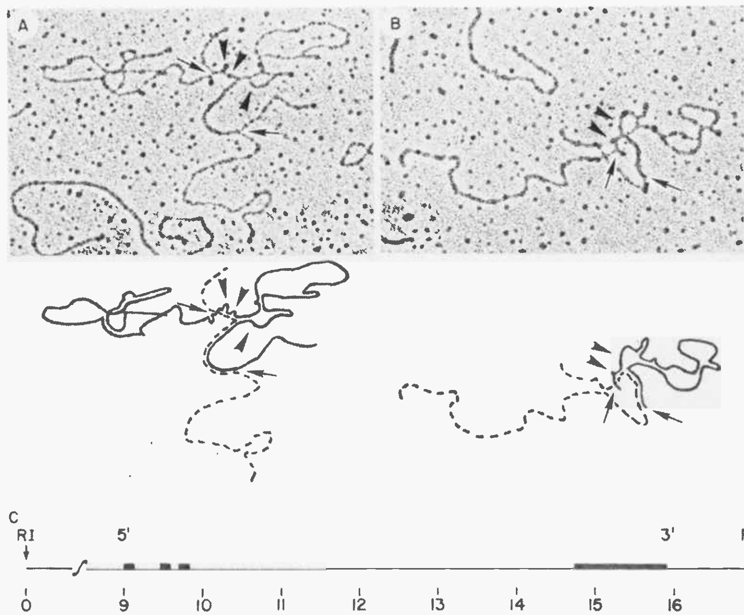

Figure 5

Structure of the human β-tubulin gene in clone 5-beta. (*A*) Heteroduplex formed between an *Eco*RI fragment of 5-beta DNA and a cloned chicken β-tubulin cDNA, T2. (*B*) Heteroduplex formed between a *Sma*I fragment of 5-beta DNA and a linearized T2 plasmid. Arrowheads point to intervening sequences; arrows indicate the ends of the heteroduplexes. In both cases, the amino-terminal encoding regions of the genes are located at the left of the heteroduplexes. (——) Human DNA in 5-beta; (— — —) pT2 DNA. (C) Diagram of the human β-tubulin gene in the 5-beta genomic DNA clone. Raised bars indicate sequences homologous to those in T2 DNA. The length of the DNA in kilobase pairs is indicated below the line.

tion that shows distinct familial tendancies and a propensity to develop into invasive carcinomas; HPV-6 causes condyloma acuminata, a highly infectious veneral disease that accounts now for about 10% of all new VD cases and is incurable. Some cervical dysplasias also seem to be due to HPV-11. The various genital warts may, rather infrequently, progress to invasive carcinomas. There also appears to be a significant correlation between venereal warts and the infection of newborns with (presumably) HPV-6 that, within several years of birth, gives rise to juvenile laryngeal papillomatosis. Left untreated, this can cause hoarseness, loss of speech, then asphyxiation. It was formerly treated with X-irradiation, with the consequent induction of throat cancers; now the lesions are burned back with lasers, though the process must be repeated at regular intervals. Adult laryngeal papillomatosis is on the rise, an apparent consequence of oral-genital sex.

Last year, Maurice Green approached us with HPV-1, 2, 3, and 4 DNAs he and Karl Brackmann had cloned at St. Louis University using viral DNAs isolated in Gerard Orth's laboratory (Pasteur Institute); with an unknown pair of cloned *Bam* restriction fragments—presumably of HPV origin—from a patient having vulval cancer and epidermodysplasia verruciformis that they worked up in collaboration with Magdalena Eisinger (Memorial Sloan-Kettering); and with HPV-5 DNA cloned by Dina Kremsdorf and Moshe Yaniv (Pasteur Institute). We prepared and analyzed all possible pairwise heteroduplex combinations of the viral DNAs (excised from the pBR322 vector sequences). The results were, in several respects, unexpected. HPV-1 and HPV-4 form only very limited heteroduplexes, with short, widely separated, but reproducible pairing, in spite of the fact they cause nearly identical lesions (Fig. 6a). Each of these establishes similar limited heteroduplexes with HPV-5, an epidermodysplasia verruciformis virus. HPV-1, 4, and 5 have not yet been found to form heteroduplexes with HPV-2 or 3. But HPV-2 and 3, implicated in lesions of a quite different nature and seriousness, establish heteroduplexes over much of their length, as shown in the electron micrograph of Figure 6b and as summarized in the histogram in Figure 7. When the stringency of pairing is raised by increasing the solvent concentration of formamide, the pairing is diminished to about 10% to 15% of their length. These results indicate that the HPV-2 and HPV-3 DNAs are approximately 45-62% homologous over most of their length and more so in the regions with the most persistent pairing. When the two *Bam* restriction fragments of the unknown DNAs were mixed with either HPV-2 or HPV-3 DNA, they formed extensive heteroduplexes and lined up end to end to span the full length of the complementary HPV-3 or HPV-2 DNA strand. Thus, together, the two fragments represent an entire papilloma virus chromosome. They are rather

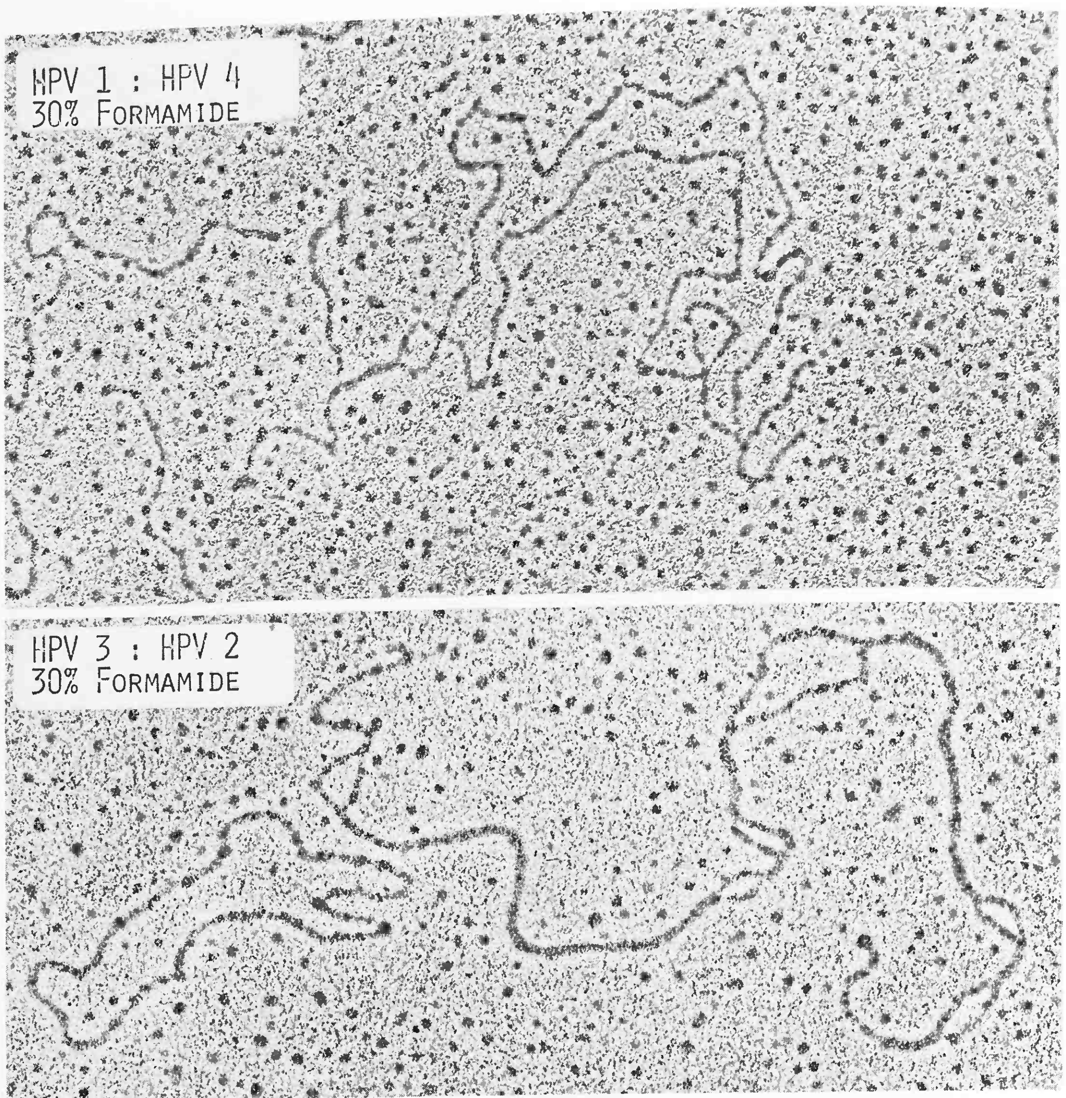

Figure 6
Electron micrographs of human papilloma virus DNA heteroduplexes. (*Top*) HPV-1 and HPV-4 DNAs were both linearized at their single *Bam*HI restriction site. Only limited (though reproducible) pairing occurs under nonstringent conditions of 30% formamide, 22°C; nonetheless, both viruses are associated with deep plantar warts. (*Bottom*) HPV-2 (opened at its single *Eco*RI site) formed extensive heteroduplexes with HPV-3 (opened at its single *Bam* site) in the presence of 30% formamide, as shown; pairing in certain regions persisted in 50% formamide, indicating substantial homology. Despite this similarity, HPV-2 is associated with common warts and HPV-3 with the rare and serious disease epidermodysplasia verruciformis.

Figure 7
Graphic summary of heteroduplexes found between papilloma virus DNAs (excised from their plasmid vector sequences). (*a*) HPV-2 was opened at the single *Eco*RI restriction site and HPV-3 at its single *Bam*HI site. Heteroduplexes were found in 30% formamide and prepared for electron microscopy in 50% formamide (spread over a hypophase with 20% formamide). (*b*) The same heteroduplexes prepared for electron microscopy under less stringent conditions of 30% formamide spread over 5%. (c) HPV-2 in heteroduplex with an HPV-3 variant that was cloned as two separate but contiguous *Bam*HI restriction fragments. Samples were prepared by spreading from 30% over 5% formamide at 20°C. An inverted duplication near its midpoint is shown in the line representation of the HPV-2 strand. (*d*) HPV-3 in heteroduplex with the HPV-3 variant used in c. Samples were spread from 60% over 30% formamide at 27°C. (These samples spread at 22°C had substantially more duplex.) In all cases, the DNAs were opened with restriction endonucleases at nonhomologous sites, hence the staggered ends. The partial homology patterns have not been oriented with respect to the restriction maps.

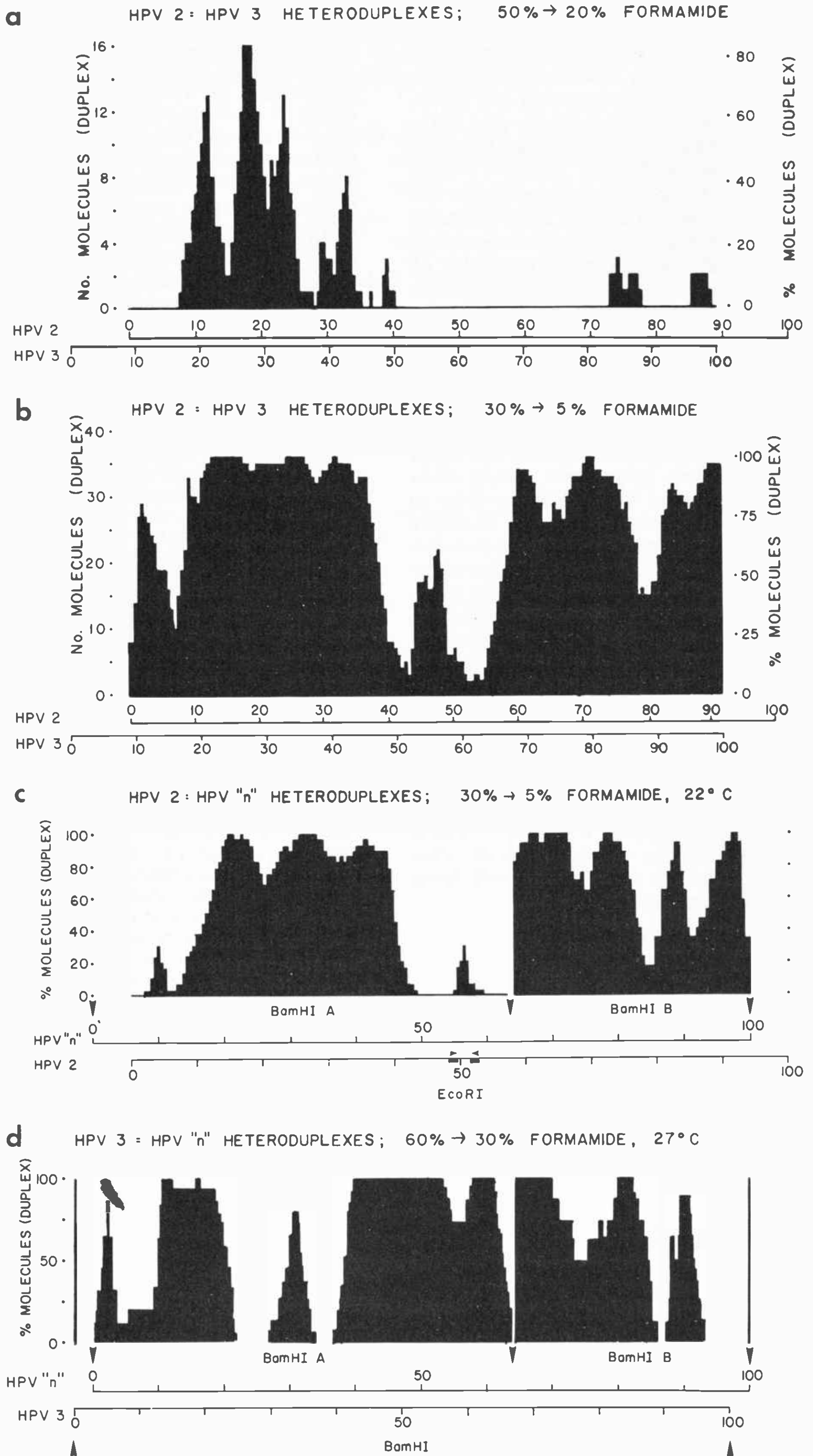

Figure 7

closely related to HPV-3, which is not too surprising since the patient had epidermodysplasia verruciformis, but the prominant differences from the group type HPV-3 in several regions of the chromosomes establish this new virus as a significant variant. All the human papilloma viral DNAs measure close to 7800 nucleotide pairs, based on comparisons with ϕX174 and colicin E1 DNA length standards. Future heteroduplex studies will test independent isolates of HPV types to search for other variants and to define a protoypte collection. We shall concentrate in particular on cloning and comparing viruses associated with epidermodysplasia verruciformis, condyloma acuminata, and laryngeal papillomas.

Law et al. *J. Virol. 32:* 199 ([1979]) had reported that certain restriction fragments of bovine papilloma virus 1 (BPV-1) and HPV-1 shared a limited amount of homology. We formed BPV-1:HPV-1 heteroduplexes and mapped the partial homology region precisely. This information should allow mapping data achieved with the two systems to be cross-correlated rather accurately, assuming that the rest of the genomes are organized similarly. Studies are in progress now to determine the RNA transcription map of HPV-1 so that the heteroduplex patterns and the DNA sequence can be interpreted with respect to genetic expression. To that end, we have cloned HPV-1 isolated from a plantar wart in a shuttle vector (provided by R. Tjian [University of California, Berkeley]) that contains the pBR322 replication origin and ampicillin resistance gene and the SV40 replication origin. Following transfection of SV40-transformed monkey COS-7 cells (from Y. Gluzman, Tumor Virus Section), RNAs were recovered. Several spliced mRNA species were observed after heteroduplex formation and are presently being mapped by electron microscopy.

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PROTEIN SYNTHESIS

M.B. Mathews, B.W. Stillman, F. Tamanoi, *F.A.M. Asselbergs, L.L. Cascio, E. Cilibrasi, S. Danheiser, A.M. Francoeur, S. Lemaster, P.A. Reichel, G.P. Thomas, L. Woodruff*

The expression of adenovirus genes in virus-infected and virus-transformed cells remains a focus of the research interests in the Protein Synthesis Section. In addition to continued progress in investigating the functions of virus-coded proteins, particularly those concerned with DNA replication, this year has seen the rapid development of new research projects. One of the new areas concerns ribonucleoprotein-particle formation and represents an outgrowth from other adenovirus-related studies that we have been pursuing. A second topic, the investigation of the stress response or "heat shock" phenomenon in human cells, now shows few traces of its origins (as far as our studies are concerned) in adenovirus work.

Control of the Productive Cycle

S. Lemaster, M.B. Mathews

In previous annual reports we have discussed the sequential expression of classes of adenovirus genes. The immediate early genes are autonomously active and do not require protein synthesis for their expression; the E1A region early gene,

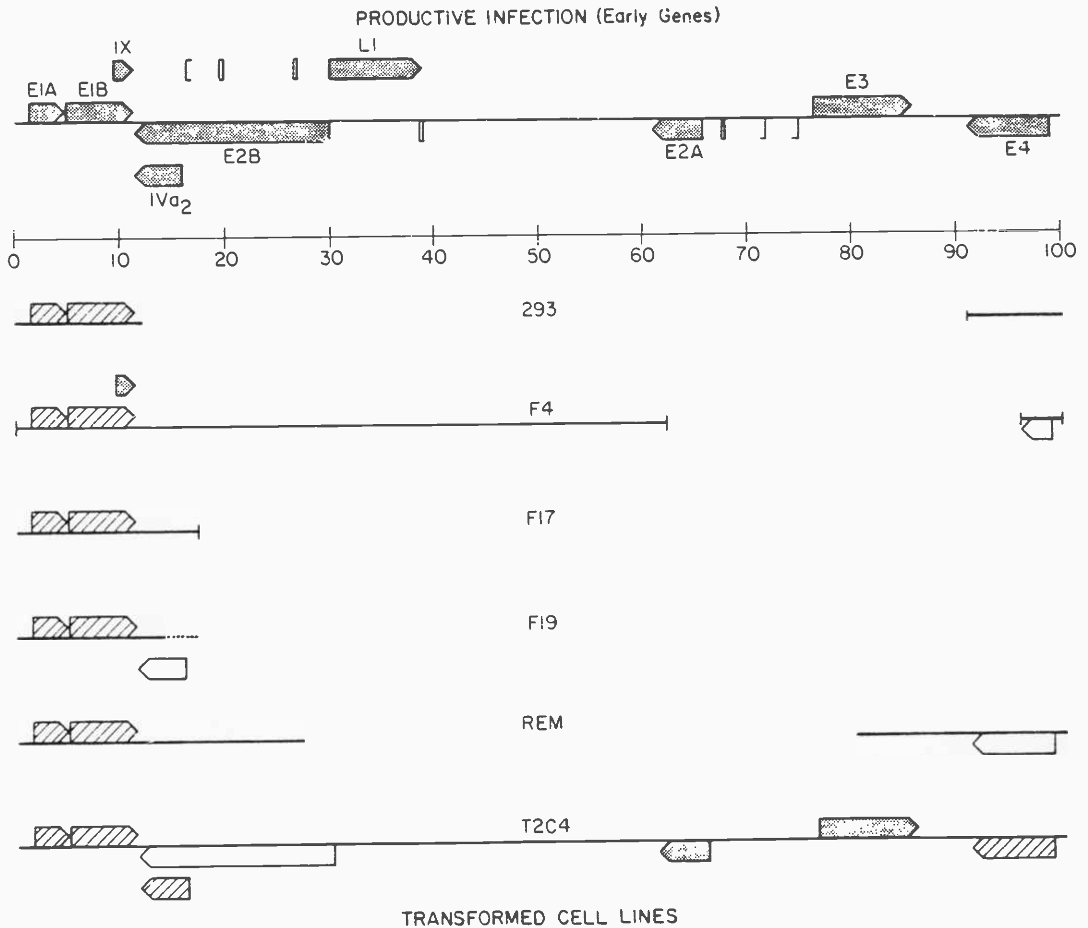

Figure 1
Expression of the adenovirus genome integrated into transformed cell DNA. The full set of early regions of the virus expressed in productive infection is illustrated in the top part of the figure. The portions of the viral genome that have been identified in six transformed lines are shown in the lower part of the figure. Arrows indicate the early genes expressed. The extent to which the products of a given early gene in transformed cells approximate those found in productive infection is suggested by the shading: stippled, full expression; hatched, intermediate; unshaded, poor correspondence. (Reprinted, with permission, from Lewis and Mathews 1981.)

itself sensitive to a rigorous blockade of protein synthesis, is responsible for activating most, if not all, of the other early genes—E1B, E2A, E2B, E3, and E4; the intermediate genes are transcribed, at least to some extent, before viral DNA replication commences; and finally, the true late genes are only expressed following viral DNA replication, an event that involves at least two of the early genes.

The distribution of these genes along the adenovirus chromosome is shown on the top line of Figure 1. We have taken several approaches to explore aspects of this complex set of regulatory interactions. Some of these investigations are well advanced and are described in their individual sections below. A novel approach that we have recently adopted involves the construction of chimeric genes that place the expression of the herpes simplex virus enzyme, thymidine kinase (TK), under the control of adenoviral DNA sequences thought to control transcription of early regions E4 and E1A and the late gene block. These chimeric genes will be introduced into the genomes of cultured mammalian cells by transfection and selection will be for stable TK-positive lines. If we succeed in isolating these lines, they will be used to examine the effects on TK expression of normal and mutant viral gene products introduced by infection. Manipulation of the adenoviral sequences that abut the *tk* gene will add an extra dimension to this analysis. With these complementary approaches we hope to examine the functions and interactions of viral gene products independently of the progression of an infection.

As a continuation of our work on the early-to-late switch in gene expression, we are also investigating the abortive growth of human adenovirus in Chinese hamster ovary (CHO) cells. In a productive infection of human cells, the activation of the late genes depends principally on the replication of the viral DNA that is to serve as template for transcription. Therefore, it was intriguing to learn that abortively infected hamster cells are competent for DNA synthesis but are deficient in late protein synthesis (Longiaru and Horwitz, *Mol. Cell. Biol. 1*: 208 [1981]), implying that replication and transcription are not necessarily linked. Closer examination of this point has revealed that some late mRNAs are present, albeit in low concentration and in relative proportions that differ from those observed in lytic infection of human cells. Since the early genes seem to be fully expressed, this implies that factors other than viral proteins are involved in the production of viral late gene products and that viral DNA replication per se is not sufficient to allow synthesis of all viral genes expressed late after infection. In short, the hamster cell situation may resemble, in a more extreme form, the well-known restriction of human adenovirus multiplication that occurs in monkey cells. We are currently investigating whether the block is at the level of RNA transcription, processing, or translation and whether it can be overcome, as in monkey cells, by SV40 T antigen or changes in the gene encoding the adenoviral DNA binding protein (DBP).

Adenovirus Genes in Transformed Cells

M.B. Mathews

The study of the activity of adenovirus genes integrated into the host genome in transformed cells offers insights into the regulation of the viral genes as well as into their oncogenic role. A survey of six of the more common adenovirus-transformed cell lines was carried out in collaboration with Dr. Jim Lewis while he was a member of this laboratory and completed at long range after his departure for the Fred Hutchinson Cancer Research Center in Seattle. In Figure 1, the thin lines represent the viral DNA segments, usually comprising less than the full genome, present in each transformed cell type. Those portions that are expressed as mRNA are indicated by thick arrows. As expected from previous work, none of the mRNAs present only at late times in productive infection was found in any line of transformed cells. Furthermore, of those mRNAs that are present in small amounts at early times and much greater amounts at late times, such as those for the intermediate products IVa_2 and IX, only IX was observed at all (in F4 cells), and then only in the small amounts characteristic of early times. We were unable to find in any transformed cell line the L1 52,55K protein mRNA, which, unlike the other late mRNAs, is present at early times during infection. This implies that the major late promoter is inactive, but it remains to be shown that its sequences are fully intact in the transformed cell genome. The absence of this and of other immediate early gene products implies that these products are not needed for the expression of other viral genes, at least in the transformed cell.

This exception apart, all of the early genes that are present are generally expressed as mRNA. In most cases, however, less than the full set of mRNA species (known from productively infected cells) is seen in the transformed lines. (This fact is illustrated by the shading of the arrows in Fig. 1.) As expected, the transforming genes E1A and E1B are expressed in all of the cell lines examined, and these are the only genes that are invariably expressed. As in other cases, these genes are not expressed in their entirety. The E1A species that are seen at early times in a productive infection, the 58K (58,000 dalton), 54K, 48K, and 42K species, are present in all cases, but the additional 28K species, which is characteristically found at late times of infection, is absent. The major E1B mRNAs, encoding the 15K and 57K proteins, are present in all the lines, with the notable exception that no 57K could be detected in F17 cells. This result, which has been confirmed with a monoclonal antibody (see below), implies that the E1B 57K

protein does not play any important role in maintaining the transformed phenotype.

What this study does not reveal is the basis for the varying levels of tumorigenicity exhibited by these lines of transformed cells. The differing degrees of tumorigenicity show no obvious correlation with the presence or absence of additional E1 gene products (detected in two of the lines), nor with the amounts of E1A and E1B gene products. On the other hand, the more tumorigenic lines generally contain and express more viral genes outside of region E1 than do the less tumorigenic lines. However, this variation does not correlate with the expression of any small number of viral genes but rather with a wide variety of early genes. Furthermore, several cell lines that we have not yet studied are as tumorigenic as T2C4 but have only the left 14% of the viral genome. Thus, it seems that tumorigenicity is not related in any simple way to the expression of integrated viral genes.

Monoclonal Antibodies

B. Stillman

In collaboration with Ron McKay (Tumor Virus Section), we have embarked on a search for monoclonal antibodies directed against proteins synthesized during the early stage of adenovirus infection of HeLa cells. Nuclear extracts from Ad2-infected cells were injected into mice, and after various periods of time spleen cells were removed and fused with myeloma cell lines. Hybridoma cells secreting antibodies of interest were selected by screening the supernatant medium for its ability to stain adenovirus-infected cells but not uninfected cells. In the approximately 25 monoclonal-antibody-producing cell lines that have been obtained in this way, two types of immunofluorescent staining patterns have appeared a number of times (Fig. 2), and we have preliminary descriptions of these.

Monoclonal antibodies of the first type are directed against the adenoviral E1B 57K protein, a component of the adenovirus T antigen. Two independently derived hybridomas produce antibody that immunoprecipitate the E1B 57K protein from infected HeLa cells, from transformed rat cells, and from the products of cell-free translation of E1B mRNA. Neither antibody recognizes any of the smaller E1B proteins (described in the 1980 Annual Report). By both immunofluorescence and immunoprecipitation tests, the E1B 57K protein is present in the transformed cell lines 293, F4, F19, REM, and T2C4 but not in F17 — which is in accord with the analysis of E1B 57K mRNA in these cell lines discussed above. By immunofluorescence, the E1B 57K protein is located predominantly in the nuclei of infected cells, although in some transformed cells it exhibits a perinuclear distribution with a concentration at one site (Fig. 2A).

The second type of antibody also appears to stain virus-infected cells but not uninfected cells. The immunofluorescence can be detected in infected cells as soon as 1 hr postinfection and by 4 hr is localized at the cell extremities (Fig. 2B). In addition, nuclear fluorescence is seen late during virus infection. Surprisingly, a set of proteins, 40,000–43,000 daltons, is immunoprecipitated from both infected and uninfected cells. The relationship between these proteins and the immunofluorescent antigen remains to be clarified.

We expect that these and other hybridoma specificities that we have detected will be useful probes in determining functions of viral and cellular proteins, and further characterization of the antibodies is in progress.

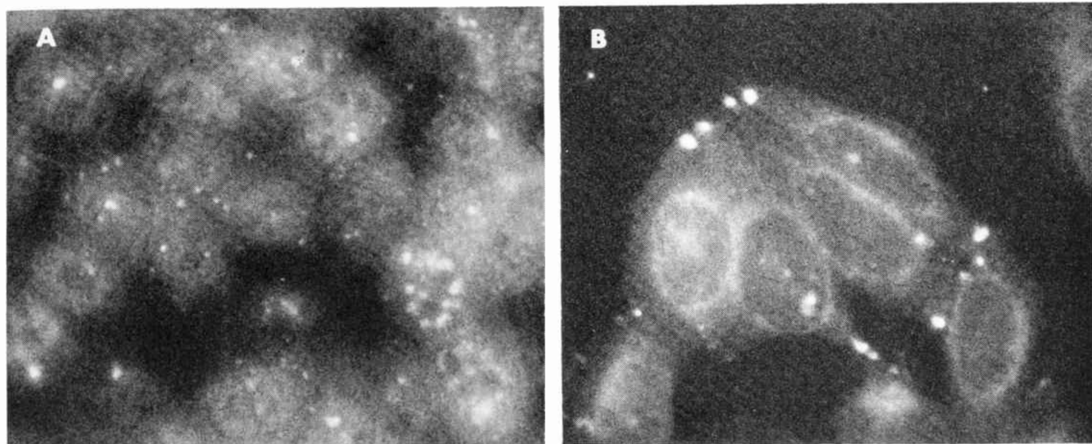

Figure 2
Immunofluorescence of Ad2-infected HeLa cells with monoclonal antibody to (*A*) the E1B 57K and (*B*) induced antigen. Cells were infected with Ad2, and hydroxyurea was added to prevent DNA synthesis. Cells were fixed at 21 hr postinfection.

The Role of Adenovirus Terminal Protein Precursor in DNA Replication

B. Stillman, F. Tamanoi

The widely accepted model for adenovirus DNA replication (see Annual Report 1980) entails at least three virus-coded proteins in different stages of the process: the terminal protein precursor in initiation, the DBP in elongation, and a protease (the *ts*1 protease) in terminal protein maturation. Briefly, the 87,000-dalton terminal protein precursor binds to the end of the template DNA molecule and initiates replication. This occurs by a strand-displacement mechanism starting at either terminus and requires the presence of DBP. Synthesis of the second strand follows a similar course, and maturation of the terminal protein involves a later proteolytic cleavage. Other enzymes of viral and host origin are probably also involved but these are less clearly defined.

The role of the terminal protein precursor (pTP) in the initiation of DNA synthesis is now quite well established. It is present in extracts of nuclei from adenovirus-infected cells, and during DNA replication in vitro it becomes covalently linked to the dCMP residue at the 5′ end of the nascent DNA chain. The initiation step can be studied in isolation by omitting three of the deoxynucleotide triphosphates from the reaction. In the presence of dCTP, a pTP-dCMP complex is formed in the presence of a suitable template (see below). No analogous complex is formed when dCTP is replaced with any of the other three deoxynucleoside triphosphates. This finding, which has also been reported by Lichy et al. (*Proc. Natl. Acad. Sci. 76:*2678 [1980]), provides further support for the protein-priming model of adenovirus DNA replication (Robinson et al., *Virology 56:* 54 [1973]; Rekosh et al., *Cell 11:* 283 [1977]) and affords an assay for initiation that underlies several of the experiments described in the next section.

Last year we reported that the gene for pTP is located between genome coordinates 29 and 11, in a newly recognized leftwards-reading E2B region. Together with John Smart (Protein Chemistry Section), we have now carried out partial sequence analysis of the protein using automatic sequential Edman degradation of tryptic peptides separated by high-performance liquid chromatography. Data obtained by analysis of pTP labeled with [^{35}S]methionine by cell-free translation or with ^{125}I by iodination of protein from Ad2 *ts*1 virions give the position of the labeled amino acids relative to arginine or lysine residues (trypsin cleavage points). Comparison with predictions derived from the DNA sequence of this region, recently completed by the Nucleic Acid Chemistry Section, makes it clear that the pTP maps between coordinates 23.4 and 28.9 on the viral genome, although it leaves open a strong possibility that some coding sequences come from the leader originating at 39 m.u. that is spliced onto the body of E2B mRNAs. Analysis of peptides from the mature 55K terminal protein shows that it derives from the C-terminal portion of the precursor, and a potential *ts*1 protease cleavage site has been recognized at the appropriate location in the predicted sequence (corresponding to coordinate 26.0). The site at which the initiating dCMP residue, and subsequently the daughter DNA chain, is covalently attached to the protein (corresponding to coordinate 24.2) has also been identified: This marks the first definition of a functional site within the protein.

A remarkable fact that emerged from this work is that the coding region for pTP does not overlap the region containing mutations of the class-N complementation group (Galos et al., *Cell 17:* 945 [1979]). Mutants in this group exhibit a temperature-sensitive defect in the initiation of DNA replication and map within an open reading frame that is capable of encoding a protein of 120,000 daltons. Thus, it is likely that an additional protein encoded by region E2B, perhaps the 105K protein described last year, functions in viral DNA replication. This possibility is currently under investigation.

DNA Sequence Requirements for Initiation

F. Tamanoi, B. Stillman

The development of a cell-free system for replication of adenovirus DNA (Challberg and Kelly, *Proc. Natl. Acad. Sci. 76:* 655 [1979]) made it theoretically possible to examine the DNA sequences required for initiation by introducing mutations into the DNA of the viral termini and studying their effects on replication in vitro. In practice, this approach was debarred by the apparently absolute requirement for parental terminal protein on the template DNA, rendering the functional origin unsuitable for genetic manipulation in bacteria. Until this difficulty was circumvented, we adopted a different approach, exploiting the diversity of DNA sequence available in nature.

In collaboration with Bill Topp (Tumor Virus Section) and Jeff Engler (Electron Microscopy Section), DNA-protein complexes were isolated from the virions of Ad4, 7, 9, and 31, and their abilities to function as templates for initiation (assayed by pTP-dCMP complex formation) and for chain elongation were compared to the template activity of Ad2 DNA-protein complex. In the replication system derived from Ad2-infected cells, the heterologous DNAs all functioned as template for both initiation and elongation of DNA chains but to varying degrees were less active than homologous Ad2 template DNA. All heterologous DNAs catalyzed the formation of pTP-dCMP complex, but no complex was observed when alternative deoxynucleotide triphosphates were used. DNA sequence studies showed that all the serotypes have a 5′-terminal dC residue and also revealed extended regions of

conserved sequence within the first 50 bases of each DNA, particularly between bases 9 and 18. Since all DNAs supported replication to some extent, this suggests that conserved DNA sequences play an important role as recognition signals for proteins involved in the initiation of DNA replication.

The key to a systematic study of the sequence requirements at the origin of replication came from experiments using plasmid DNA as template. With partially purified enzymes, we found that the initiation reaction can occur on the plasmid pLA1, which contains the left terminus of the viral DNA, provided that the origin is located at the end of the DNA molecule. When the origin is located internally in the linear DNA or the plasmid DNA is circular, initiation is not observed. This finding came as a surprise, since previous work, using pronase-treated viral DNA, suggested that the presence of terminal protein on the template DNA is necessary for initiation. To resolve this apparent contradiction, piperidine was used to remove the protein residue completely from pronase-treated viral DNA, with the result that activity was restored. Thus, the terminal protein on the template DNA is dispensable for initiation, and a specific DNA sequence is required. This sequence must be present at or very near the terminus: If as few as 20 nucleotides (or a short peptide) are interposed, as in the linear form of the plasmid *8, template activity is abolished.

Further surprise came when the linearized pLA1 DNA was heat-denatured and then used as a template. The single-stranded DNA still supported the initiation, again provided that the origin is located at the end of the DNA molecule. The fact that single-stranded DNA can act as a template suggests that initiation might comprise a two-step process; first, the unwinding of duplex DNA at its ends, and second, complex formation using single-stranded DNA as a template.

We are currently attempting to define the minimum length of wild-type sequence that retains template activity. To this end, we have created derivatives of pLA1 that contain decreasing stretches of adenovirus terminal sequence, and these will be tested for their ability to support the initiation of replication.

The DNA Binding Protein

F.A.M. Asselbergs, M.B. Mathews

The adenovirus DBP is a 72,000-dalton phosphoprotein, encoded by the E2A gene, with an important role in the elongation reactions of DNA replication. It has also been implicated in several other events in the virus life cycle related to mRNA regulation. Last year we reported that the DBP can be synthesized in *Xenopus* oocytes injected with adenovirus DNA, or subclones therefrom, and that some shorter products from the same gene are also made. We have now established that some of these shorter proteins are also present in extracts of virus-infected human cells and are the authentic products of identifiable

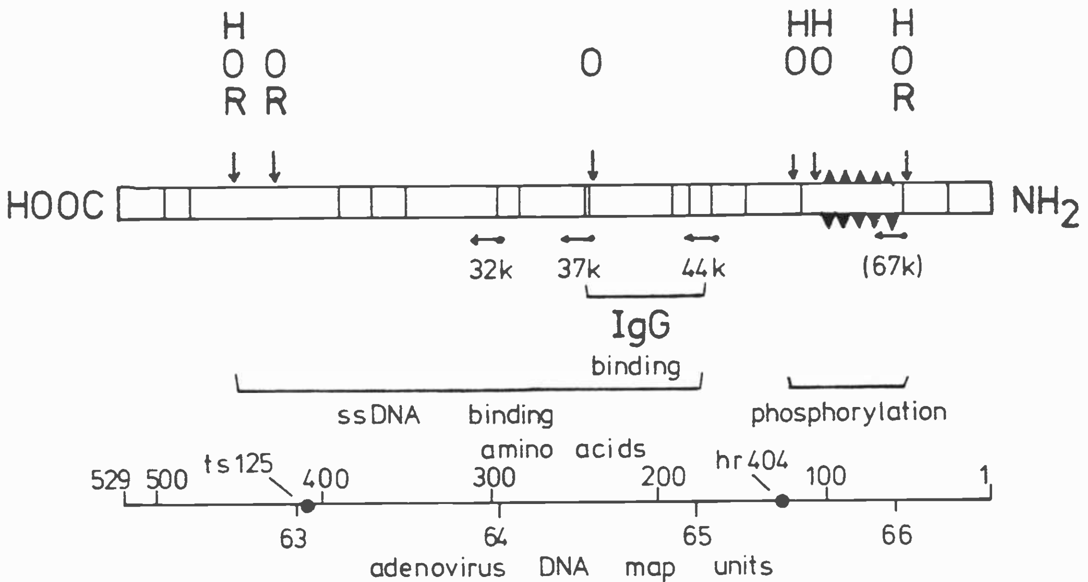

Figure 3
Functional map of the adenovirus DBP. The open bar depicts the 72K protein, and the vertical bars within it mark the positions of methionine residues. Brackets indicate regions involved in DNA binding and antibody (IgG) binding, as well as phosphorylation sites (black triangles). Proteolytic cleavage sites in HeLa cells (H), *Xenopus* ocytes (O), and reticulocyte lysate (R) are shown. Short horizontal arrows correspond to the N termini of related minor proteins. The bottom scale shows the positions to which two mutants have been mapped. (From F. Asselbergs et al., submitted.)

mRNA species that can be translated in the reticulocyte cell-free system. From a comprehensive study of the full-size DBP and the shorter polypeptides, carried out together with John Smart (Protein Chemistry Section), we have been able to define the location of several important features within the protein sequence. As described before with reference to the pTP, proteins labeled with [^{35}S] methionine were digested with trypsin, and the resultant peptides were separated by HPLC and subjected to sequential degradation from their N termini. Through a comparison of these data with the nucleic acid sequence published by Kruijer et al. (*Nucleic Acids Res. 9:* 4709 [1981]), individual peptides were identified, the sequence of DBP was confirmed along much of its length, and the structures of the shorter DBP-related proteins were defined. To identify functional domains within the molecule, these results were correlated with experimental determinations of DNA binding, antibody reactivity, and phosphorylation properties. The map in Fig. 3 depicts the DBP as a bar, with the protein's N terminus on the right, and summarizes the functional topography of the molecule as presently understood. This information will serve as a basis for future detailed investigations of the biochemistry of this key protein.

Adenovirus Genes in Frog Oocytes

F.A.M. Asselbergs, M.B. Mathews

Foreign genes can readily be introduced into the nuclei of immature eggs (oocytes) of the frog *Xenopus laevis* by microinjection, and in most cases they are expressed as mRNA and protein. In the case of adenovirus DNA, only three of the many viral genes are so expressed, and these are in several ways an unrepresentative subset. One is the DBP gene discussed above and in the 1980 Annual Report; the second is the gene for virion polypeptide IX, translated from an mRNA that (unusually for adenovirus) is unspliced; the third is the gene pair encoding the virus-associated (VA) RNAs, dealt with more fully in the next section. We cannot at present do more than speculate upon the reasons why the other adenovirus genes remain silent in the oocyte, but this has not deterred us from exploiting those genes that are active. In one series of experiments we have examined the sequence requirements for polyadenylation of mRNA transcripts. The sequence AAUAAA is usually found a short distance upstream from the polyadenylation point, and deletion of this signal from a cloned polypeptide-IX gene abolishes protein synthesis. On the other hand, replacement of adenovirus sequences with plasmid sequences at the site of poly(A) addition and beyond has no measurable effect on polyadenylation and polypeptide IX synthesis. Although we have not proved that the poly(A) tail is still added onto the same nucleotide in the mutants as in the wild type, our results indicate that the functional downstream boundary of the gene does not extend beyond its polyadenylation site.

Ribonucleoprotein Complex Formation

A.M. Francoeur, M.B. Mathews

We have continued our investigations into the structure and function of the two small, noncoding, adenovirus VA RNAs. The VA RNAs have been implicated in processing and/or transport of adenovirus mRNA and were recently shown by Lerner et al. (*Science 211:* 400 [1981]) to form ribonucleoprotein complexes (RNPs) with cellular protein. These RNPs can be specifically precipitated with a class of sera, called anti-La, from patients with the autoimmune disease systemic lupus erythematosus. We have determined that only about 2% of VA RNA labeled in vivo is found in precipitable RNPs and that VA RNA associated with RNPs appears to be very similar to, if not identical with, free VA RNA. Comparable results have been obtained with VA RNA synthesized in *Xenopus* oocytes injected with adenoviral DNA, showing that the La antigen is phylogenetically widespread.

As a first step toward analyzing the VA RNP particle, we have demonstrated that VA RNA synthesized in vitro, using an extract of HeLa cells as source of RNA polymerase III, is precipitable by anti-La serum. Interestingly, approximately 50% of the VA RNA_I synthesized is found in precipitable form, and 70% of the VA RNA_{II} synthesized is precipitable. Since deproteinized VA RNA does not immunoprecipitate, this means that a large fraction of the RNA combines with the antigen in vitro to form a VA RNP complex. The complex is stable and is formed during transcription or very soon after. To examine the RNA requirements for RNP formation, we have programmed the reaction with cloned VA RNA_I mutants that have inter-

Figure 4
The stress proteins of HeLa cells displayed on 2-dimensional gels. Polypeptides synthesized in normal HeLa cells (*lower panel*) and cells in which the stress proteins had been induced by exposure to AzC (*upper panel*) were pulse-labeled with [^{35}S] methionine. The proteins were separated according to their isoelectric points (pI, horizontal dimension) and sizes ("k" value, vertical dimension). The major stress proteins have been indicated with letters in both panels to illustrate the degree to which they are induced under these circumstances. The stress proteins are designated A, 110K/pI ~5.4; B, 100K/pI ~5.0; C, 90K/pI ~5.2; D, 80K/pI ~5.2; E, 73K/pI ~5.5; F, 73K/pI ~6.4; and G, 72K/pI ~5.6. Synthesis of the 73K protein cannot be detected in normal HeLa cells, and lower-molecular-weight protein (H) is not induced in HeLa cells.

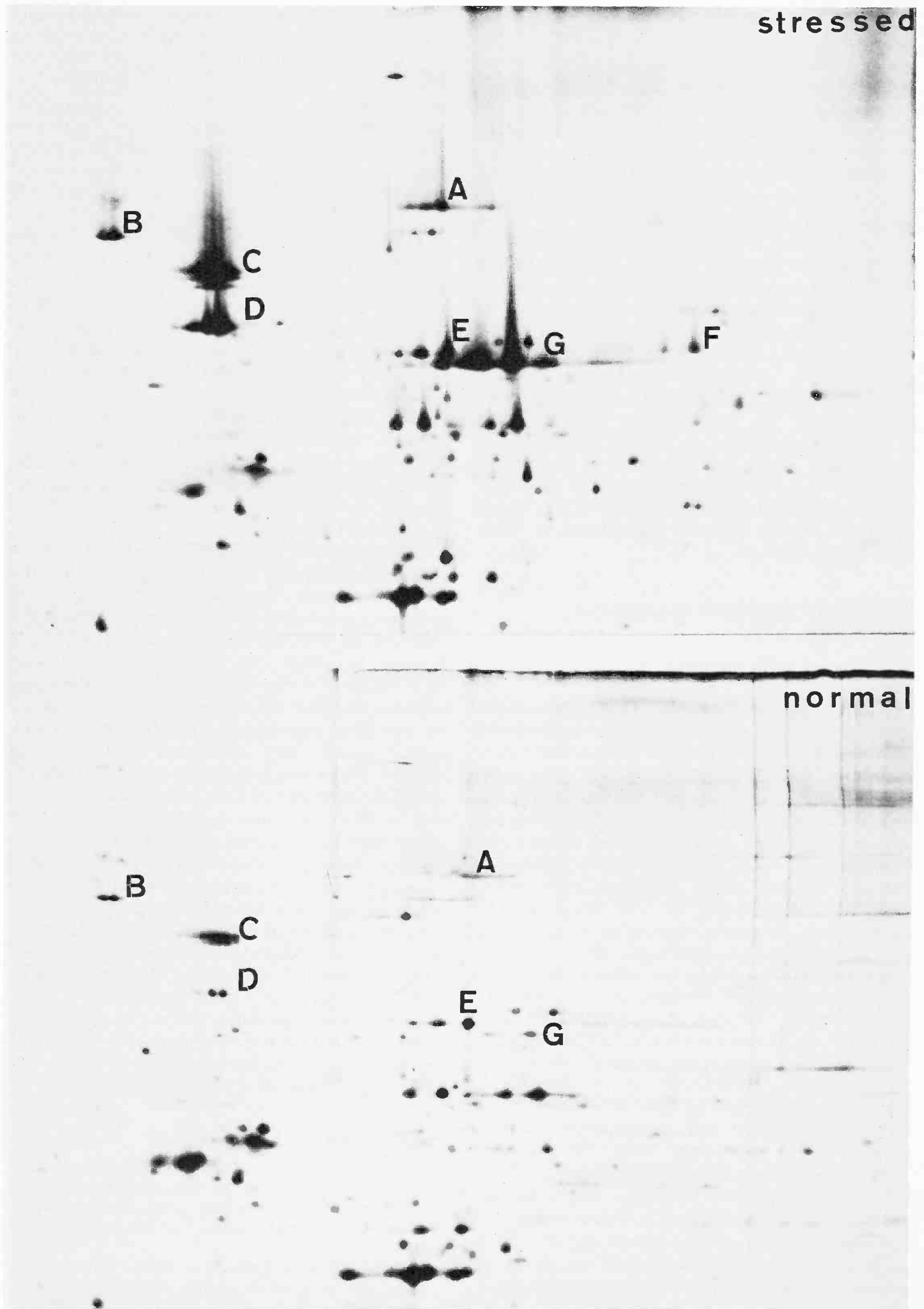

Figure 4

nal deletions or sequences replaced with those of pBR322. All the mutant RNAs were found competent to form precipitable RNPs in vitro, suggesting that the La antigen is recognizing a feature common to all the VA RNAs—the RNA termini and/or the intragenic control region sequences. These results hinted that the antigen might be involved in the transcription process itself, but this idea is not supported by experiments demonstrating that the transcription system depleted of La antigen by immunoabsorption retains full synthetic activity.

Further definition of the RNA sequences involved in RNP formation required establishment of the means to reconstruct the complex from naked RNA and factors present in the HeLa cell extract. Using partially digested VA RNA_I in such a reconstitution experiment, we found that only a small subset of the RNA fragments are able to bind to the La antigen, and these are located at the two termini of the RNA molecule. It is tempting to speculate that the antigen recognizes the stem structure containing the two base-paired ends of the molecule. We are presently examining this possibility and turning our attention increasingly towards the nature and properties of the protein antigen. The La antigen appears to be a cellular protein of molecular weight about 45,000 and is distinct from the antigens recognized by other classes of lupus sera.

Analysis of the Stress Response of Mammalian Cells

G.P. Thomas, M.B. Mathews

The term "stress response" covers the various forms of a highly conserved response displayed by many, if not all, organisms when subjected to metabolic insults of diverse nature. The most familiar and best characterized example is the heat shock response displayed by *Drosophila* larvae and cultured cells subjected to elevated temperature. Similar responses can be induced in bacteria, plants, yeasts, protozoa, birds, mammals, and invertebrates by heat shock and by other means. It seems likely that in each case similar target functions, perhaps with central roles in metabolism as a whole, are affected, leading to generalized metabolic imbalances of greater or lesser severity. The response is characterized by the rapid and extensive induction of a small suite of genes. Their polypeptide products soon dominate preexisting cell proteins in synthesis (Fig. 4) and in accumulation as a result of controls at both transcriptional and translational levels. A growing list of agents can induce the response. They range from chelators and ionophores to thiol ligands and respiratory inhibitors, and no unitary explanation comes to mind. It is noteworthy that some agents induce only a subset of the eight or so proteins that represent the full set, so the response is a linked but not fully coordinated induction.

Most of our work has employed an amino acid analog, L-azetidine-2-carboxylic acid (AzC), a particularly potent inducer. The proteins induced in HeLa cells by AzC have been characterized by 2-dimensional gel electrophoresis, limited proteolysis, and tryptic peptide analysis. Similar proteins are found in stressed hamster and chick cells, and they are all also present in (theoretically unstressed) normal cells. Each protein occurs in several forms differing in charge, and in at least one case the variation appears to reside in a single tryptic peptide. These studies have been carried out in collaboration with Jim Garrels (Quest 2-D Gel Laboratory) and John Smart (Protein Chemistry Section). Together with Bill Welch and Jim Feramisco (Cell Biochemistry Section), we have undertaken to purify the stress proteins and to raise antibodies against them. So far, the 100K, 90K, 80K, and 72–73K proteins have been extensively purified and in some cases are close to homogeneity. Based on their fractionation during the initial stages of purification, it would seem that the 100K and 80K stress proteins are associated with membraneous material, whereas the others are largely cytosolic. Instead, the 100K stress protein has been localized to the Golgi apparatus, as described more fully in the Cell Biochemistry Section.

A large body of experiments with the AzC/HeLa cell system supports the following model for the induction process. The analog is incorporated into proteins, presumably resulting in the formation of aberrant or inactive proteins, and leads to a decrease in overall RNA synthesis but an increased production of the mRNAs coding for the stress proteins. Simultaneously, regulation at the translational level augments the response by discriminating against the utilization of preexisting mRNAs. These conclusions are based on data obtained by treatment of cells with inhibitors of protein or RNA synthesis and by examination of mRNA levels by cell-free translation as well as by physical means. A remarkable feature is that translational control seems to be exerted at the level of protein chain elongation.

Further analysis of the mechanism will be facilitated by cloned copies of the genes and of their cDNAs. Bacteriophage clones carrying at least parts of the gene for the 90K protein are in hand, as are plasmid subclones, and we are exploiting these to make direct measurements of mRNA levels and synthesis. An immediate goal is to obtain clones of the other stress-protein genes.

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PROTEIN CHEMISTRY

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

During its second year of existence the Protein Chemistry Section has continued to focus on two main areas: (1) studies on the structure and function of the transforming proteins of tumor viruses: adenovirus type 2 (Ad2), avian sarcoma viruses, and simian virus 40 (SV40) and (2) structural and functional work on receptors for glycoprotein endocytosis.

Ad2 Early Proteins

J.E. Smart

Early during lytic infection by Ad2, four regions of the viral genome are transcribed into mRNA. These mRNAs encode proteins known to be involved in the initiation and maintenance of transformation, as well as other proteins that directly or indirectly affect DNA replication and the regulatory processes necessary for the switch from early- to late-gene replication. The main approach that we have used to analyze the Ad2 early proteins has involved the characterization of peptides produced by selective proteolysis and/or chemical cleavages of the polypeptides labeled both in vivo and in vitro with various amino acids ([^{35}S]methionine, [^{35}S]cysteine, [^{3}H]leucine, etc.). The resulting peptides are then separated by high-performance liquid chromatography (HPLC) on reverse-phase or cation-exchange columns and analyzed individually by sequential Edman degradation to locate the positions of the individual labeled amino acids. By correlating this partial amino acid sequence information with the DNA-sequence and mRNA-splicing data, the coding location of these polypeptides can be assigned unambiguously. This approach also permits deduction and confirmation of the complete amino acid sequence of these proteins.

During the past year our primary research efforts involving Ad2 have been directed towards a more detailed understanding of the structures and functions of early regions 2A and 2B. Both of these regions are involved in viral DNA replication. Studies initiated in 1980 allowed us to identify the gene and the mRNA encoding the adenoviral terminal protein (region 2B). These studies, which were published early last year, have been extended in collaboration with Bruce Stillman and Mike Mathews of the Protein Synthesis Section. Likewise, in collaboration with Fred Asselbergs and Mike Mathews (Protein Synthesis Section), an exhaustive study on region 2A (Ad2 DNA-binding protein) was completed.

Adenoviral Terminal Protein (Early Region 2B)

The adenoviral terminal protein functions as a primer for the initiation of viral DNA replication by covalently binding the first nucleotide in the viral DNA chain. It remains covalently attached to the 5′ ends of the viral DNA and is cleaved to the terminal protein during viron maturation. The gene encoding the terminal-protein precursor maps within a 7-kb region of the viral genome that specifies multiple mRNA and protein species. We have determined the location within this region of the coding sequences for the terminal protein by aligning partial amino acid sequence data with the amino acid sequence predicted from the DNA sequence (see Nucleic Acid Chemistry Section). The open translational reading frame between coordinates 23.4 and 28.9 on the genome contains the majority of the coding sequences for the precursor protein. The mature virion terminal protein derives from the C terminus of the precursor protein. Using this information, the site of covalent attachment of the terminal protein to the viral DNA has been established. This site also corresponds to the site that covalently binds dCMP. The coding region for terminal-protein precursor does not overlap with the region to which the N group of adenoviral mutants has been mapped. We conclude that these mutants define a protein, other than terminal protein, that also functions in the initiation of viral DNA replication. (See Protein Synthesis Section for a more detailed report.)

Adenoviral DNA-binding Protein (Early Region 2A)

The major early region 2A translation product is a 72K single-stranded DNA-binding protein. This protein is involved in the initiation and maintenance of viral DNA replication and appears to be involved in the downward modulation of the rate of synthesis of the gene products from the other early regions of the viral genome. We have analyzed the major DNA-binding proteins from Ad2 and Ad5 by peptide mapping and partial amino acid sequencing. The major E2A products, as well as several minor species, have been obtained from infected HeLa cells or from translation of E2A-specific mRNA in a reticulocyte lysate or in *Xenopus* oocytes. The results identify a continuous open reading frame of 1587 nucleotides between 62 and 65 m.u. on the *l* strand of the viral DNA as the region encoding the major 72K DNA-binding protein. Minor related proteins probably originate from partial proteolysis of the major protein as well as from independent translation of minor mRNA species that coterminate with the major species. (See Protein Synthesis Section for more details.) Detailed analysis of the minor region 2A products has permitted determination of those domains of the major protein that (1) are involved in DNA binding, (2) are the major an-

tigenic sites in the native molecule, and (3) are the major sites of serine and threonine phosphorylation in vivo.

Sites of Tyrosine and Serine Phosphorylation on the Transformation-specific Gene Products of Distinct Avian Sarcoma Viruses

J.E. Smart

Transformation of cells by Rous sarcoma virus (RSV) is initiated and maintained by the production of a virus-encoded 60-kD transforming protein, $pp60^{src}$. One of the functions of $pp60^{src}$ is to catalyze the transfer of phosphate from ATP to acceptor tyrosine(s) of various polypeptides. Furthermore, when ATP is added to the immunoprecipitated transformation-specific proteins of all three classes of avian sarcoma viruses, some of the phosphate is transferred to the transformation-specific proteins themselves. The transforming activity of these proteins implies that they may play an important role in metabolic regulation. In fact, by analogy with other protein kinases, the modification of a number of different enzymes and/or nonenzymic proteins via phosphorylation provides an attractive model for the pleotropic effects of the transformation event.

Classification of avian sarcoma viruses has been based on relatedness of their cell-derived sequences, which are apparently the source of their sarcomagenic properties. The viruses of class I are RSV and B77; of class II, Fujinama sarcoma virus (FSV), PRCII, and PRCIV (PRCIIp); and of class III, Y73 and Esh sarcoma virus (ESV). In the recently proposed system of nomenclature for the cell-derived sequences of RNA tumor viruses, classes I to II would contain *src*, *fps*, and *yes* sequences, respectively. Although avian sarcoma viruses of classes II and III contain transformation-specific sequences that by all criteria are apparently different from those of the class-I viruses, their immunoprecipitated transformation-specific gene products still show an associated tyrosine kinase activity. In earlier collaborative studies with J.C. Neil and P.K. Vogt (University of Southern California, Los Angeles), we established that within the class-I and class-III viruses the site of tyrosine phosphorylation was identical. Recently, we have extended these studies to show that a similar site of tyrosine phosphorylation occurs within the class-II viruses. These studies have employed structural analysis by sequential protease cleavage, a method of potential widespread application. These data suggest that the occurrence of acidic amino acids adjacent to the phosphoacceptor tyrosine may be a general property of the target sites for tyrosine-specific protein kinases. The observation of the apparent conservation of these target sites in the otherwise distinct class-I, -II, and -III avian sarcoma viruses supports the hypothesis that such sites may play a central role in regulating the function(s) of the viral transforming proteins as well as of their normal cellular homologs.

Although phosphorylation at the tyrosine residue of $pp60^{src}$ appears to be essential for its transforming activity, the phosphorylation of a serine residue also occurs at another site in the molecule and appears to exert some control on the rate of tyrosine phosphorylation, at least in vitro. Collaborative studies with A. Purchio and R. Erikson (University of Colorado, Denver) were undertaken to determine the site of serine phosphorylation in $pp60^{src}$. Employing the same methodology as that used to determine the site of tyrosine phosphorylation, we have established that the site of the cAMP-dependent serine phosphorylation in $pp60^{src}$ lies 17 amino acids from the N terminus of the molecule. Other workers have shown that in the case of most cAMP-dependent protein kinases, the amino acid sequences at the phosphorylation sites in physiologically significant substrates fall into two categories: (1) -Lys-Arg-X-X-Ser-X- and (2) -Arg-Arg-X-Ser-X-, in which X stands for any amino acid (except it should be noted that the amino acid residues immediately adjacent on either side of the serine to be phosphorylated usually have hydrophobic side chains). Interestingly, the site of serine phosphorylation on $pp60^{src}$ has the sequence -Arg-Arg-Arg-Ser-, which differs from the consensus sequence in that an arginine residue appears in the position immediately preceeding the serine that usually would be occupied by an amino acid with a hydrophobic side chain. Our studies have shown that the removal of the phosphate from the serine of $pp60^{src}$ occurs only at a very low rate with standard phosphatases, whereas phosphatases extracted from more specialized tissues exhibit a high level of activity against the phosphorylation serine of $pp60^{src}$ These combined data suggest that the level of phosphoserine in $pp60^{src}$ may be modulated by a discrete subset of cellular phosphatases.

Hepatic Lectins

K. Drickamer, G. Binns

Cell-surface receptors mediate the interaction of cells with their environment. Specific receptors are believed to make cells responsive to external signals such as hormones, to specify the uptake and degradation of certain extracellular components, and to facilitate cell-cell recognition. Aberrances in each of these receptor functions are responsible for several disease states, including malignancy. In an effort to understand the function and dysfunction of receptors, we have concentrated on one specific set of receptors, the hepatic lectins. These proteins are responsible for the recognition of partially deglycosylated serum proteins, which are taken up by hepatocytes. Previous work from this and other laboratories indicates that the cell biology of the uptake process has much in common with the uptake processes of other ligands such as low-density lipoprotein and insulin; it is now recognized as an example of

receptor-mediated endocytosis. The glycoprotein-recognition system provides a unique opportunity to study receptor function, because this is the only system in which the receptors can be readily isolated for detailed structural studies. The term hepatic lectins (HL) is used because these proteins have sugar-recognizing capacity; the receptor from chicken liver is designated CHL and the corresponding rat protein is designated RHL. Ongoing studies are concentrated in two areas: (1) understanding the previously determined primary structure of CHL and (2) comparison of this structure with that of RHL.

Further Characterization of CHL

As reported last year, the first stage in the investigation of CHL was determination of the complete amino acid sequence of CHL. Much of the current work is directed toward understanding and interpreting this sequence. The most outstanding questions involved are how CHL binds glycoprotein ligands and how it interacts with various hepatocyte membranes. Progress on the first question has been slow, but good data are now available to indicate that at least one of the seven sulfhydryl groups in CHL is located at or near the glycoprotein binding site. As all of the cysteine residues are found in the C-terminal two-thirds of the sequence, this already indicates that the binding site is located towards the C-terminus, as designated in Figure 1. Efforts are presently being directed at limiting the binding site more specifically.

The primary structure itself suggests how CHL may interact with the hepatocyte plasma membrane. As shown in Figure 1, a completely noncharged, hydrophobic stretch of amino acids (positions 25 through 48) is likely to be the site of interaction with the membrane lipids. This type of sequence is typical of that found in other membrane proteins and suggests that CHL may in fact span the membrane. Documentation of this fact would fulfill a long-standing prediction about the structure of membrane receptors. It was relatively easy to show that the C-terminal protein of CHL is extracellular, because this region of the polypeptide contains a site of glycosylation always found at the extracellular face of the membrane and because the ligand binding site must be at the cell surface and earlier evidence placed this site toward the C-terminal. This conclusion was further supported by direct labeling experiments in which cultured hepatocytes were labeled with the membrane-impermeant probe lactoperoxidase. The tyrosine residues that could be labeled with the reagent were all found in the C-terminal two-thirds of CHL.

These results clearly indicate that if CHL does in fact span the membrane, it must be oriented with the N-terminal end of the polypeptide in the cytoplasm. Evidence for this prediction was therefore sought. The clearest results to date have been obtained with phosphate-labeled cells. Cells grown in the presence of radioactive inorganic phosphate incorporate a small amount of the radiolabel into polypeptides by specific phosphorylation of certain serine, threonine, and tyrosine residues by cytoplasmically located protein kinases. Fortunately, it appears that CHL is a phosphate-acceptor protein. The acceptor site probably can be assumed to be in the cytoplasm. Although the incorporation of radioactivity is very small, it has been possible to localize the phosphorylation site toward the N-terminus of CHL, which is consistent with the postulate that this end of the molecule is probably facing the cytoplasm.

An alternative way of identifying cytoplasmically disposed portions of the CHL polypeptide is to label the exterior surface of vesicles that have the opposite orientation of the plasma membrane. The endocytosis process in which CHL functions requires that the receptor move into coated vesicles during the uptake process. CHL in these vesicles would have the reverse orientation to that of CHL in intact cells: The cytoplasmic domain would be located at the outside of the vesicle and should be accessible to impermeable probes such as lactoperoxidase. Coated vesicles were therefore isolated from chicken hepatocytes by modifications of available methods for other tissues. These preparations were shown to contain CHL by the immunological-gel-staining method of K. Burridge (Cell Biochemistry Section).

Sequence Work on RHL

The carbohydrate binding protein from rat livers has a binding specificity distinct from that of CHL; although the chicken protein recognized terminal *N*-acetylglucosamine residues, RHL binds terminal galactose. This difference reflects physiological differences between the carbohydrates of serum proteins in avian and mammalian species. In spite of the specificity differences, the receptors share many features in common, such as pH and ion requirements. The route of ligand uptake is also similar in both cases. It was therefore of interest to see whether the receptors from these two species were evolutionarily related to each other. As shown in Figure 1, the sequence of RHL has been partially established, and it already reveals that two portions of this protein are strikingly homologous (40% identity) to CHL. The presence of two homologous regions is apparently due to a large internal duplication that has been tentatively aligned as shown in Fig. 1. This suggests that RHL contains two binding domains, each homologous with the CHL binding domain. This would explain the larger size of RHL. The presence of two ligand binding sites would also explain some of the apparently anomalous binding results previously obtained by others working with this protein. The sequence work must now be pursued to completion

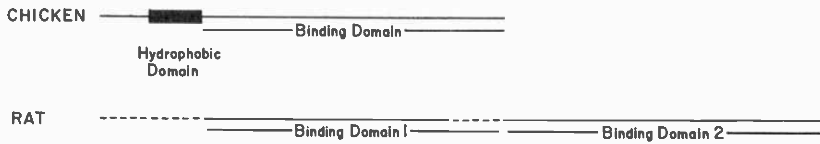

Figure 1
Summary of primary structures of chicken and rat hepatic lectins. Portions of RHL structure not yet determined are indicated as dashed lines. Binding domains 1 and 2 refer to large duplicated sequence homologous to ligand binding region of CHL.

to establish the full extent of the CHL-RHL homology. It will be particularly interesting to see whether the hydrophobic domain and cytoplasmic tail show structural similarities with the corresponding portions of CHL.

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Movable Genetic Elements

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

The groups in the Movable Genetic Elements Section are supported by a genetics program grant from the National Institutes of Health. This genetics program has led to an intellectual cohesion among the various groups and has allowed us to broaden our work. It is hoped that the work done by the various groups in this section will contribute to a general understanding of the principles underlying genetic flexibility.

INSERTION ELEMENTS AND PLASMIDS

A.I. Bukhari, R.M. Harshey, *G. Chaconas, L. Dalessandro, D. Evans, D. Foster, M. George (Goradia), G. McGuinness, K. Messina, C. Monaghan, G. Penzi, B. Vogel*

Transposable genetic elements require two things to function: (1) specific DNA sequences that define the ends of the element and (2) proteins that recognizes these sequence and thus bring about DNA transposition and all of its attendant DNA rearrangements. The sequences at the ends are repetitious and are generally present in an inverted order. For example, the ampicillin resistance transposon Tn*3* has 38 bp of perfect inverted repeats at the ends. The temperate bacteriophage Mu appears to be an exception to this rule. Mu is the most promiscuous transposon know, and yet its two ends do not show characteristic long inverted repeats. We know that the *A*-gene protein of Mu can recognize the two ends, since in the presence of *A* the Mu DNA can be inserted or excised precisely. The *A* protein in Mu is equivalent to transposases in other systems, with one striking difference: The *A* protein in Mu is required for excision, whereas in other procaryotic transposable elements, the transposases are not believed to play a role in excision. Although the *A* protein in Mu is interesting, there are other proteins, in particular the *B* protein, that play important roles in the processes by which Mu brings about DNA rearrangements.

We are interested in understanding the DNA features that are recognized by the *A* and *B* proteins and the manner in which they are recognized. To define more precisely the DNA sequences required for transposition, we have been deleting increasing amounts of Mu DNA near the ends. These experiments are being done by using the exonuclease *Bal*31 to degrade the Mu DNA from a point in Mu DNA close to the ends. By this technique, we have isolated a large number of deletions near the ends of Mu and are now systematically examining them for their biological properties and nucleotide sequences.

We have also been examining the expression and functioning of the *gin* protein, which is required for flip-flop of the invertible G segment of Mu, and of the *mom* protein, which causes an unusual modification of DNA. Both the *gin* and *mom* genes are located near the right end of Mu DNA. Thus, the deletions that we have generated in vitro also affect the functioning of these genes in various very interesting ways. We are now analyzing these effects.

DNA Intermediates in Transposition of Mu

R.M. Harshey

By a process that is closely coupled to its replication, Mu can transpose into DNA sites that may have no homology to it. Upon induction of Mu replication, "key" structures are generated. These structures are circles of variable size attached to tails of variable length. We had proposed that these molecules arise as a result of an intrachromosomal event of transposition that links replicating Mu DNA to different host sequences, thus accounting for the variability in size of these structures. We have now isolated these structures using the following technique.

We cloned the *lac* operator into mini-Mu DNA, which can undergo transposition when supplied with replication proteins in trans by means of a helper Mu prophage. This plasmid was allowed to undergo transposition. The transposing mini-Mu DNA was then trapped on nitrocellulose filters by using the *lac* operator-repressor interaction. This procedure enriched for key structures, which were eluted from the filters with IPTG. Mu sequences within these structures were localized in the electron microscope by visualizing the *lac* operator-repressor interaction after complexing the repressor with antibody and ferritin-conjugated anti-antibody. The location of the bound ferritin cores was measured with respect to the junction between the circle and the tail in the *key* structure. These studies have confirmed that key structures contain Mu DNA and that it is replicating. Thus, these structures are intermediates in transposition as hypothesized. The method we have used to find these intermediates can be applied to many different systems.

Figure 1 shows four molecules that were analyzed. The molecules in A, B, and C contain ferritin cores at positions equidistant from the junction of the circle and tail in the key structure. Thus, replication of Mu sequences has occurred, and the junction must be a replication fork. The sizes of the circles in A and B are 12 kb and 20 kb, respectively. Since the size of the mini-Mu is 16.5 kb and the *lac* operator sequence is located 5 kb from the left end, the molecules in A and B could have arisen only if replication had started at the left end of Mu. The molecule in D, which has a simple ferritin core located on the tail, has a circle size of 8.9 kb and could have arisen only if replication had started at the right end. Some molecules, such as the one with a circle size of 35.5 kb shown in C, could have started replicating at the left or the right end, and their origin of replication could not be identified. Thus, replication can begin at either the left or the right end of Mu. Restriction enzyme analysis of these structures was also carried out, which again showed that replication could begin at either one of the ends of Mu. These results rule out schemes that propose diffusible copies or single-stranded intermediates in transposition and lend support to those in which double-stranded ends of transposable elements invade nonhomologous DNA sites to insert copies of themselves.

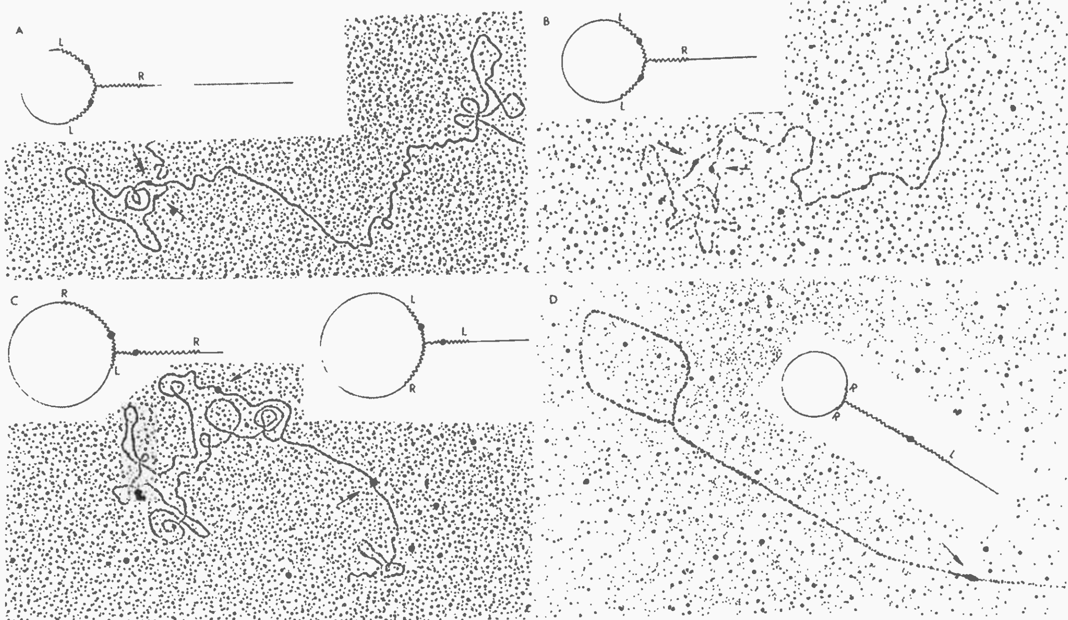

Figure 1
Analysis of key structures showing *lac* operator sequences. The ferritin cores (ferritin-conjugated anti-antibody-*lac* repressor antibody-*lac* repressor complexes) are indicated by arrows. The drawings show schematically the disposition of the ferritin cores. The locations of the *lac* operator sequences (contained in Mu) correspond to the predictions of Harshey and Bukhari (1981).

Packaging of Host Sequences Attached to the Ends of Mu DNA

M. George

A characteristic feature of Mu DNA is the presence of host DNA at its ends; the host DNA is heterogeneous in length and in sequences. These sequences result from packaging of Mu DNA, linked to different host sequences during the Mu growth cycle. We previously showed that the left-end host sequences vary from 56 bp to 150 bp and fall into discrete blocks of 6 bp, with a 5-bp spacing. Thus, cutting of DNA during packaging occurs with a spacing of 5 bp between each block.

We have now found that the cutting of DNA during packaging is staggered. The experiment was done as follows: (1) The 3′ end of the host DNA was labeled with a single [α-^{32}P]triphosphate and Klenow polymerase. (2) The 5′ end of the host DNA was labeled with [γ-^{32}P]ATP and polynucleotide kinase. (3) We used all four [α-^{32}P]triphosphates, and Klenow polymerase, to fill in the ends. We then isolated the left end of the *Hin*dIII fragment, which is 1000 bp cut it with *Hin*f, which makes a cut 7 bp away from the left end of Mu, and ran the digest on an 8% sequencing gel to size the fragments from the three reactions.

The results of the experiment indicated that the 5′ end of host DNA at the left of Mu is 2 bp longer than its complementary strand.

Transmid: A Vector for Shotgunning Bacterial Chromosomes without Restriction Enzymes

Bacteriophage Mu has many uses in molecular genetics because of its remarkable genetic properties. When Mu transposition functions are fully active, it can fuse two DNA molecules or it can dissociate a DNA molecule into smaller molecules at a high frequency. If a circular DNA molecule undergoes dissociation, it results in the formation of two smaller circles, each containing Mu DNA. These circles normally do not have a replication origin (other than Mu DNA, which replicates by a special mechanism). Thus, the heterogeneous circles formed after Mu induction in bacterial cells are lost and cannot be rescued. We have now cloned a ColE1 replication origin within Mu, which allows the circles to replicate as plasmids. Since the circles contain random host sequences, isolation of the circles amounts to cloning of host-DNA segments into a plasmid. These "plasmids" can be isolated by merely inducing Mu in the presence of a "transmid," a mini-Mu sequence containing a replication origin. A Mu transmid can replicate by transposition and can also be packaged in Mu phage particles. Various aspects of this system are currently being analyzed.

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YEAST AND PLANT GENETICS

J. Hicks, A.J.S. Klar, J.N. Strathern, *J. Abraham, P. Creatura, S. Dellaporta, L. Haas, J. Ivy, G. LoFranco, C. McGill, J. McIndoo, R. Malmberg, K. Nasmyth, D. Prudente, S. Weisbrod, J. Wood*

The past year has been marked by two important developments in the growth of what has been known as the "Davenport Yeast Group." First, we are no longer residents of Davenport Laboratory. With the completion of our year-round laboratory and office addition in the summer of 1981, the entire complex was renamed in honor of the late Professor Max Delbrück. Second, our yeast genetics research has been expanded to include new research on the genetics and molecular biology of higher plants. This progress has been made possible both by the increased space available in Delbrück Laboratory and the addition to our group of plant biologists Russell Malmberg and Steven Dellaporta. We have also enjoyed the presence of Visiting Scientists Jeremy Thorner from the University of California at Berkeley and George Sorger from McMaster University in Hamilton, Ontario. Dr. Thorner continued his work on the regulation of genes in the mating pathways of yeast, and Dr. Sorger has pursued a search for DNA clones of the nitrate-inducible genes of *Neurospora crassa*.

As in the past most of our work this year has centered on the structure of the mating-type genes in the yeast *Saccharomyces cerevisiae* and the mechanism by which mating types are alternated in dividing yeast cells. The general layout of the mating-type genes has been described in previous reports and is diagrammed in Figure 1. The important features of this scheme can be summarized as follows. Mating behavior is determined by the *MAT***a** and *MATα* cassettes that alternatively occupy the *MAT* locus on chromosome III. These alleles are identical in DNA sequence with the exception of an internal segment of 642 bp in *MAT***a** and 747 bp in *MATα* (see Fig. 1). Mating-type switching involves the replacement of the *MAT***a** sequence with the *MATα* sequence (or vice versa) and is the result of transposition of information to *MAT* from either of two additional cassette sites, *HML* and *HMR*, on the left and right ends of the same chromosome. The transposition event is catalyzed by the product(s) of the HO (homothallism) gene located on chromosome I. The *HMLα* and *HMR***a** storage cassettes are not normally expressed but are under the negative transcriptional control of four genes known as *MAR1, SIR1, SIR3,* and *SIR4*. In strains defective in any one of these *MAR/SIR*

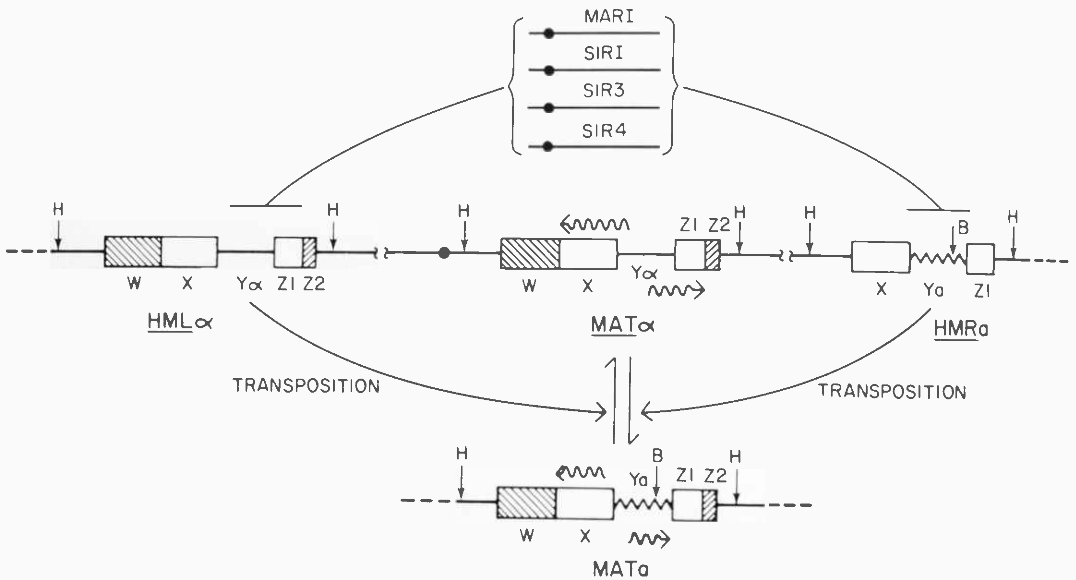

Figure 1
Diagram of the mating-type cassettes on chromosome III of Saccharomyces cerevisiae. See text for genetic description of *MAT, HML,* and *HMR*. W, X, Y, Z1, and Z2 represent regions defined by homology between *MAT* and the *HML* or *HMR* locus: The W region is 723 bases in length and is found at *MAT* and *HML;* the X region is 704 bases in length and is found at *MAT, HML,* and *HMR; MAT, HML,* and *HMR* can have either the **a**-specific sequence Y**a** (642 bases) or the α-specific sequence Yα (747 bases); Z1 is 239 bases in length and is found at *MAT, HML,* and *HMR;* finally , Z2 is 88 bases in length and is found only at *MAT* and *HML*. The *HML* and *HMR* copies of the mating-type genes are normally kept silent by the *MAR* and *SIR* genes (see text). Interconversion of *MAT***a** and *MATα* involves a unidirectional transposition-substitution event from *HML* or *HMR* to *MAT* and is regulated and/or catalyzed by the *HO-gene product.*

genes, all three cassette-containing loci are fully expressed. Our work this year has centered on the mechanism of mating-type transposition and the regulaton of the silent cassettes.

Switching Intermediates

Last year we demonstrated that DNA from a population of cells that is switching efficiently contains a significant portion that is cut in the *MAT* DNA. The cut is at the boundary of the Y and Z regions (see Fig. 1). The YZ boundaries at *HML* and *HMR* are not cut, although the DNA sequences at these boundaries are identical to that found at *MAT*. These results suggest questions about the role of this cut in the homothallic switching process and about the mechanism that defines *MAT* as the recipient and *HML* or *HMR* as the cassette donor.

We also demonstrated that switching could occur at *MAT* on a replicating plasmid. For example, *mata*$^-$ on the 2μ based plasmid YEp13 is stable in heterothallic cells but is efficientially switched to *MATα* or *MAT***a** in homothallic strains. The double-strand cut at the YZ boundary on the plasmid is also readily observed in homothallic cells.

These results suggested that in vitro mutagenesis of the *MAT* DNA could be used to identify sequences essential for switching. A set of deletions was made by *Bal*31 digestion from the *Bgl*II site in Y**a** followed by religation in the presence of *Xho* linkers. YEp13:*mat***a** plasmids with these deletions were tested for the ability to undergo homothallic switching by transformation into *HO HMLα MATα HMRα* cells. Switching was indicated by the production of YEp13:*MATα*. Deletions entirely contained within Y**a** were efficiently switched. In contrast, the deletions that extended into Z were stable as *mat***a**$^-$. For example, deletion a125, which leaves 30 bases of Y**a** to the left of Z, can be converted to *MATα*, whereas deletion a147, which deletes 9 bases into Z, is not switched at all. The site defined by these experiments is diagrammed in Figure 2.

The coincidence of the site of the double-strand cut with the site at the YZ boundary defined by the deletion analysis as essential for switching suggests that the double-strand cut is an intermediate in the switching process.

We performed similar deletion analyses of the W and X regions of *MAT* (both from the left of *MAT* and from a site within Yα) and found no single site required for homothallic switching. As long as the YZ boundary was intact and there was homology between the plasmid and sequences to the left of Yα, the deletions bearing plasmids could switch efficiently.

In a related set of experiments we tested the effect of the double-strand cut in strains unable to complete the switching process because the storage loci had been deleted. If the double-strand cut were the initiating event in switching, then cells lacking a donor locus might be unable to repair the break and would therefore die. Spores of genotype *hml*Δ *MATα* (or *MAT***a**) *hmr*Δ *HO* were observed microscopically to produce such dead cells with a frequency and pattern predicted by the established rules of mating-type switching (Fig. 3). Thus, in these strains, the *MAT* locus is cut in vivo, but in the absence of the donor loci, the resulting broken chromosome

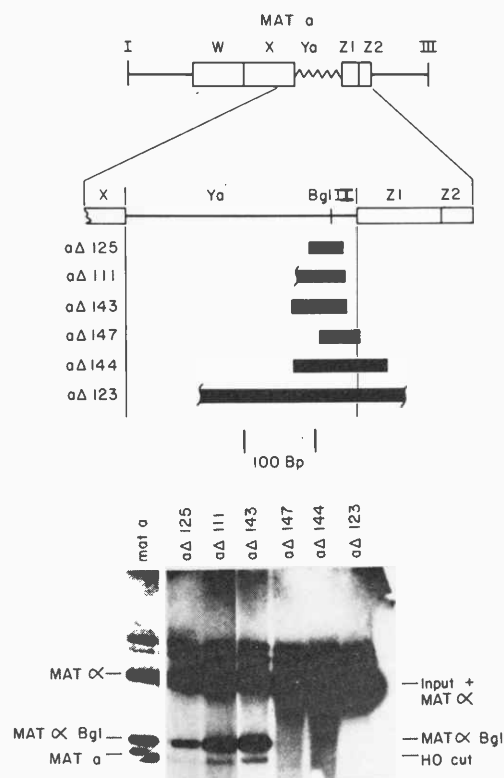

Figure 2
Deletion analysis of a cloned *MAT* cassette showing that the region of the double-strand cut is required for switching onto the plasmid from the chromosomal *HML* or *HMR* locus. The Southern blot lanes at the bottom of the figure show the assay for switching. The apparance of the "*MATα Bgl*" band indicates that the plasmid *MAT* sequence has been replaced by the *MATα Bgl* cassette containing an extra *Bgl*II restriction site.

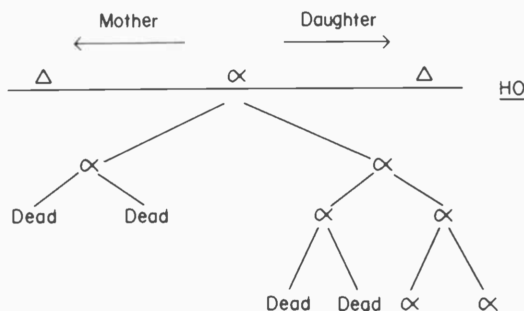

Figure 3
Appearance of dead cells in the pedigree of a homothallic strain deleted for the *HML* and *HMR* cassettes.

leads to lethality. On the basis of this result we suggest that the double-strand cut is not only an intermediate but is in fact the initiating event in transposition.

The deletion strains that yield dead cells essentially grow in a linear rather than logarhythmic fashion. This slow growth pattern provides a novel genetic screening scheme for selecting mutations in the switching pathway. Any mutant that blocks the initiation of switching would grow logarithmically and therefore could be easily spotted on solid media. Thus far we have obtained two classes of "fast growers." One class results from mutations mapping at *MAT* (substrate mutations) and the other class results from mutations at *HO*. Whether the *HO* gene itself codes for the cutting activity is now being tested.

Control of the Silent Cassettes

One of the most novel aspects of the mating-type regulatory scheme is the mechanism by which the normally silent storage cassettes at the *HML* and *HMR* loci are controlled (see Fig. 1). Each silent cassette contains fully functional mating-type genes that are kept under negative transcriptional control through the action of the *MAR*- and *SIR*-gene products. This implies that there are negative control sites at *HML* and *HMR* that are not present at the expressed *MAT* locus. All mating-type cassettes are transcribed divergently from a region of DNA that is identical in sequence whether it is located at *MAT, HML,* or *HMR*. From this result it was inferred that the required control sites lie outside the cassettes themselves, in sequence unique to *HML* and *HMR*.

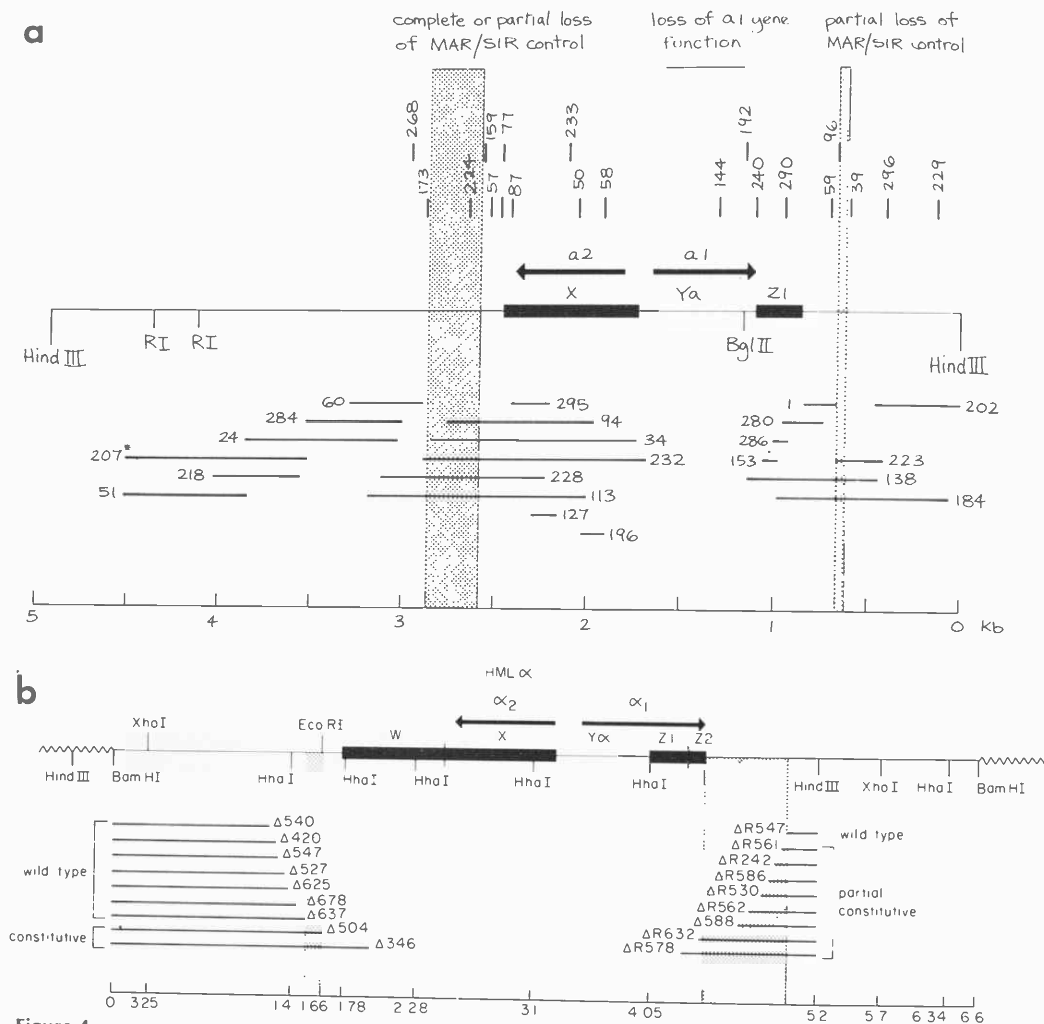

Figure 4
Deletion and insertion mutagenesis of *HMR***a** (*a*) and *HML*α (*b*) cassettes. The stippled areas indicate the regions defined as necessary for the action of the *MAR/SIR* genes. The E site is on the left in both cases, and the I site on the right.

To define the location and function of the control sites, we performed in vitro mutagenesis on cloned plasmids containing the *HML* and *HMR* cassettes. The most detailed studies were performed by Judy Abraham on the *HMR***a** locus. Random deletions followed by insertion of synthetic DNA sequences containing *Xho*I restriction endonuclease cleavage sites were introduced into the region surrounding the *HMR***a** gene, thus disrupting the normal coding and control sequences. The effects of these mutations were assayed by reintroduction of the altered plasmids into suitable yeast strains. The results of this work are summarized in Figure 4a. Two sites, one on each side of the cassette homology, were found to be required for normal *MAR/SIR* control. The two sites are not equivalent. Deletion of the E (*E*ssential) site leads to full expression of the adjacent cassette, whereas deletion of the I (*I*mportant) site yields only a slight increase in expression. Deletions bringing E or I closer to the promoter region in *HMR* do not disrupt regulation, indicating that the control mechanism does not depend on the exact distance between E, I, and the transcription start site. The E and I sequences thus define a domain of negative transcriptional control at *HMR* that extends a distance of 2000 bp.

Sites analogous to E and I were also located near the *HMLα* cassette by John Feldman, in collaboration with Dr. James Broach (State University of New York, Stony Brook). The series of deletions generated by these experiments are shown in Figure 4b. At *HML*, the domain of negative control between the E and I sequences extends roughly 3300 bp.

The role of chromatin structure in the regulation of the *HML* and *HMR* cassettes was explored by Kim Nasmyth using nuclease digestion of chromatin in intact nuclei. It is well known that chromatin structure can cause certain sites in and around genes to become "hypersensitive" to nuclease digestion. When assayed by gel electrophoresis and Southern blotting, these sites show up as dark bands, which are not present when the experiment is performed on purified DNA. The appearance of such hypersensitive sites can often be correlated with gene expression and provides a probe for changes in chromatin structure that affect regulation. Under normal *MAR/SIR* regulation, the silent cassettes yield nuclease digestion patterns distinct from those found at these loci when they are transcribed in Mar^- strains. The digestion patterns of the silent loci under *MAR/SIR* regulation also differ from those found at the *MAT* locus, indicating that the action of the *MAR/SIR* products on the silent cassettes causes a specific alteration in chromatin structure. An example of these results is shown in Figure 5.

In related experiments, Kim also compared the topology of plasmids carrying regulated (*HML* and *HMR*) and expressed (*MAT*) cassettes replicating in Mar^+ and Mar^- yeast strains. This was accomplished by separating the various forms of the plasmid population on the basis of DNA supercoil density by gel electrophoresis in the presence of an intercalating dye. These experiments showed that plasmids carrying either *HML* or *HMR* are more negatively supercoiled when isolated from Mar^+ cells than when isolated from Mar^- cells. At *HMR* the sequences required for generation of the supercoil difference are the same as those required for transcriptional regulation: The supercoil difference is completely lost if the E site is deleted and is almost completely lost if the I site is removed. The supercoil difference can be observed in plasmids lacking the promoter region and is thus independent of transcription.

MAR and *SIR* Genes

The results mentioned above clearly affirm the

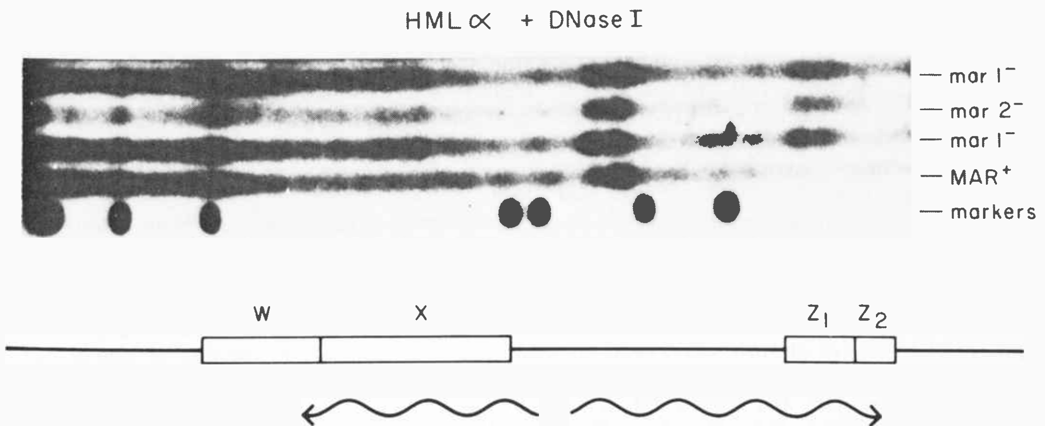

Figure 5
Partial DNase-I digestion of chromatin in and around the *HML* cassette showing two sites near the 5′ ends of the transcripts that do not respond to *MAR/SIR* control and a pair of sites at the Y-Z boundary that only appear when the cassette is in the expressed condition, either at *MAT* or at *HML* in a Mar^- background.

necessity for characterizing the products of the *MAR* and *SIR* genes and their interactions with one another before we can understand how they mediate control at *HML* and *HMR*. This work is following several different paths. Cloned molecules capable of complementing defects in the *MAR* and *SIR* genes have been isolated and, through the work of Lisa Haas and John Ivy, have been shown to correspond to the appropriate genetic loci. Preliminary results indicate that the actions of certain loci are dependent on the others. For example, raising the copy number of the *SIR3* gene on a plasmid can compensate for complete lack of activity of the *SIR4* gene, possibly indicating that expression of *SIR3* may normally be dependent on *SIR4*. Furthermore, a subfragment of the *SIR4* clone lacking *SIR4* activity can exercise a dominant effect on the *MAR/SIR* regulatory system in wild-type cells, possibly by interfering with formation of the normal regulatory complex. Complete construction of the *MAR/SIR* regulatory mechanism will, of course, depend on isolation of proteins encoded by these genes. To that end, Stu Weisbrod has identified a cell line carrying a highly amplified version of the *HML* cassette in its normal regulated form carried on a plasmid. Partial purification of this "minichromosome" may yield amplified amounts of the presumed control protein complex bound to the E and I control sites.

Directionality of Switching

Homothallic yeast strains carrying storage cassettes in the "normal" arrangement (*HML*α *HMR***a**) switch the cassette at *MAT* to the opposite allele in 90% of the cell divisions where switching can occur. Strains in which the storage cassettes are reversed (*HML***a** *HMR*α), however, switch at a greatly reduced frequency ($<6\%$). In last year's report we proposed that a control mechanism exists to direct the switching process and that the storage cassette chosen depends on the mating type of the cell. That is, cells expressing *MAT*α preferentially transpose information from *HMR* to *MAT*, and cells expressing *MAT***a** preferentially transpose information from *HML*, irrespective of the genetic information carried at the silent locus. In the normal cassette arrangement this preference leads to efficient switching. However, in cells with reversed cassettes this directional bias leads to "futile" switching events in which the *MAT*α allele, for example, is replaced by an identical α cassette from *HMR*α.

This hypothesis was confirmed through observation of switching behavior in strains carrying genetically marked alleles at *HML* and HMR. Furthermore, in strains where the preferred donor locus has been deleted, the inefficient donor becomes capable of donating efficiently. Thus, the directional control mechanism apparently involves a competition between the two storage loci for pairing with the *MAT* locus and donating genetic information. The exact nature of this control is still under study.

Plant Genetics

The overall direction of our newly initiated research in plant systems is to use the unique ability of some plants to regenerate from cell culture to study gene expression during development. In cell culture plants behave essentially as microorganisms and can be manipulated genetically in the same ways as yeast or bacteria. The cells retain the capacity for development, however, and can be stimulated to regenerate into whole plants in the laboratory. This feature allows exploitation of genetic techniques at the cell culture level on petri plates, followed by analysis of the results in the developing organism. We are therefore attempting to identify developmental mutants and to achieve genetic transformation of plant cells for the purpose of modifying and manipulating the genes of interest.

An immediate goal of Russell Malmberg's research is the selection and characterization of cell culture mutants. Since moving to Cold Spring Harbor Laboratory in May, he has made progress in two areas: (1) analyzing a temperature-sensitive mutant of tobacco and (2) improving the ability of peas to regenerate whole plants from cell culture. Previously, he had isolated the cell line *ts4* and had shown that it is defective in polyamine synthesis, although probably not in any of the structural genes of the enzymatic pathway. Gel electrophoresis studies showed that a 25,000-kD polypeptide was being overproduced in the mutant cell line. Recently, he discovered that growing wild-type cells on the polyamine synthesis inhibitor methylglyoxal Bis(guanylhydrazone) (MGBG, a spermidine analog) induces a similar polypeptide. In collaboration with the Protein Chemistry Section here, the polypeptides were shown to have identical tryptic digestion patterns. Thus, this tobacco cell line exhibits a polypeptide for which there exists a constitutive mutation (*ts4*) and a gratuitous inducer. In vitro translation studies have shown that the message for this polypeptide is present in both induced and uninduced cells, indicating that control of its expression is at the posttranscriptional level. cDNA cloning of this gene is now under way to further study its regulation.

In contrast to tobacco, peas (*Pisum sativum*) are an important food crop and have excellent whole-plant genetics but they are very difficult to work with in cell culture. Before coming to Cold Spring Harbor, Russell had shown that one particular primitive pea variety is capable of regeneration from cell culture, whereas standard peas are not. By Mendelian crosses he has now shown that the ability to regenerate is a recessive character with a fairly simple inheritance pattern, possibly coded by a single gene. The implication of this work is that it may be possible to cross the regeneration

trait into cultivated peas of agronomic and genetic interest. He is currently trying to map the genetic factors responsible and to determine the number of genetic loci.

Vectors for the genetic transformation of plant cells with cloned DNA are being constructed by Steve Dellaporta. This field has been limited by the lack of selectable markers in plant cells. In his previous work Steve had selected tobacco cells capable of growing without plant hormones after introduction of the tumor-inducing plasmid Ti from *Agrobacterium tumefaciens*. Refinements of this selection are being carried out with cloned portions of the Ti plasmid in the hope of finding an easily selectable DNA sequence, the presence of which will identify cells that have received new genes. Steve is also constructing hybrid vectors that will employ drug resistance genes isolated from bacteria as selectable markers in the hope that these genes will cause plant cells to become resistant to the same drugs and thus allow the identification of transformants. Additional components of these vectors, such as origins of DNA replication and promoters of gene transcription, are being isolated from the tobacco genome by assaying their functions in yeast, where transformation is easy and efficient. We believe that by combining yeast genetics and plant genetics, progress toward our goal of understanding the genetic control of cell differentiation will be made easier and much more interesting.

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MOLECULAR APPROACHES TO MICROBIAL PATHOGENICITY AND VIRULENCE

M. So, *C.H. Lee, T. Mlawer, K. Messina, T.F. Meyer*

The emphasis of this group is the study of the genetic and molecular basis of microbial pathogenesis. The approach is to understand the basic mechanisms underlying the acquisition of pathogenicity and virulence by the microorganism with the hope that what is learned will lead to a solution for curing the disease in question.

There are two projects within the lab at the moment. One involves enterotoxigenic *E. coli,* which produces diarrhea in man and in several agriculturally important animals. The *E. coli* must produce colonization factors that enable them to attach to and establish a niche within the host and toxins that elicit the clinical symptoms of diarrhea. We are examining the genes encoding the heat-stable toxins produced by these enterotoxigenic *E. coli* as well as the toxins themselves. In this vein, we have shown that the genes coding for one of the heat-stable toxins, STI, lies within a transposon flanked by inverted repeats of *IS1*. We have been able to get some important information regarding the toxin from the sequence of the gene and are in the process of establishing a system for studying the mode of action of the toxin itself. Colonization is an important facet of the disease process, and we are currently studying the genetics of the colonization factors specific for human intestines, CFAI and CFAII, in collaboration with D.G. Evans (University of Texas, Houston). These projects are being carried out by Chao-Hung Lee.

The second project in the laboratory also involves colonization factors. One of the major virulence factors in *Neisseria gonorrhoeae* is the pili. We are examining the genetic regulation of expression of the pilus antigen as well as the basis for its antigenic variability. We have recently shown that expression of the antigen involves chromosomal rearrangement.

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MECHANISM OF TRANSPOSITION

F. Heffron, *R. Kostriken, M. Wong, C. Moomaw*

Genetic rearrangements take place in many organisms and have important biological consequences, for example, in the expression of a diverse number of immunologlobin genes, in genetic rearrangements in corn thought to be linked with their development, and in bacteria and lower eucaryotes genetic rearrangements are used as parts of pathogenetic mechanisms. The focus of this group is the study of the bacterial transposable element Tn*3*. Procaryotic transposable elements appear to transpose by two different mechanisms. IS sequences and composite transposons such as Tn*5*, Tn*9*, and Tn*10* all appear to transpose by a mechanism that yields one of two possible end products—either a cointegrate is generated or a precise transposition of the transposable element occurs—whereas Tn*3*, and others of its class, appears to transpose by a mechanism in which cointegrates are obligate intermediates. Most of our current research involves testing the hypothesis that cointegrates are intermediates in Tn*3* transposition and determining the precise molecular mechanism for its transposition. In the last year we have shown that Tn*3* encodes a site-specific recombination system and that mutations in that system "invariably" transpose to form cointegrates (Kostriken et al., *Proc. Natl. Acad. Sci. 78:* 4041 [1981]). We have shown that during transposition, first, a cointegrate is formed in a reaction requiring the transposase and both ends of Tn*3*, and second, the cointegrate is resolved by the site-specific recombination system it encodes. The reason the word "invariably" is in quotation marks is that no careful search has ever been made to determine whether precise transposition can take place with a Tn*3* derivative containing a defective site-specific recombination system.

As one test of the mechanism of transposition, we have constructed a plasmid that allows us to assay end-deletion frequency and inversion frequency from Tn*3* and to determine the frequency of transposition in the same experiment. The plasmid contains the galactokinase gene, a gene for alkaline phosphatase, a temperature-sensitive origin of replication, as well as complete or mutant copies of Tn*3*. Using this plasmid we have determined that the frequency of precise transposition in a *tnpR*-minus Tn*3* derivative is much lower than cointegration and may be zero. End-deletion frequency is low in this plasmid, lower than the frequency of transposition but equal to the frequency of inversion formation. Neither end deletions nor inversions require the product of the site-specific recombination system (M. Wong et al., in prep.).

As another test of the mechanism of transposition we have contructed a heteroduplex transposon containing different restriction sites on the two strands. This hybrid transposon has allowed us to test the strandedness of transposition. We first constructed two Tn*3* derivatives that contain different restriction sites but differ by only a few base pairs. A heteroduplex of the two was constructed on bacteriophage λ. We can infect a cell and force the hybrid transposon to transpose onto a plasmid in the recipient cell without λ replication. The products we observe are all cointegrates (which was expected since we have not supplied *tnpR*) in which one copy of the transposon contains restriction sites exclusively from one strand of the heteroduplex and the other copy contains restriction sites exclusively from the other strand (R. Kostriken et al., in prep.).

The second general area of work is focused on the biochemistry of mating-type interconversion. In collaboration with the Yeast and Plant Genetics Section, we have established a system to study mating-type interconversion in vitro. Mating-type interconversion is thought to be initiated by a double-strand break made by the product of the *HO* gene at the *MAT* locus. In our in vitro system we have identified *HO*-dependent cutting within the mating-type locus. A number of mutant plasmids (constructed by Jeff Strathern, Yeast and Plant Genetics Section) containing genetic lesions near the *HO* cut site have been tested in vitro. Those plasmids that can undergo switching in vivo are cut in vitro and those that do not work in the cell also do not work in vitro. We have also observed that linear DNA is a substrate and that normally silent copies are cut in this in vitro system, although we are investigating what factors might keep these copies silent (R. Kostriken and F. Heffron, in prep.).

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Vertebrate Gene Structure and Expression

The development of recombinant DNA techniques over the past few years has allowed molecular biologists to investigate a considerable number of eucaryotic gene systems that were previously unapproachable. Problems relating to the control of gene expression in both normal and transformed cells can now be studied by recombinant DNA methods. In response to the rapid progress in this area, a new research division whose aim was to study several different gene systems in higher eucaryotes was formed at Cold Spring Harbor in 1980.

The study of the structure and expression of vertebrate genes has continued to expand in 1981. A Program Project Grant from the National Cancer Institute was funded starting April 1, 1981. This program comprises four distinct Sections: Mammalian Cell Genetics, Hormonal Control of Gene Expression, Genes for Major Structural Proteins, and Structure and Expression of Polypeptide Hormone Genes. These four Sections are headed by Michael Wigler, David Kurtz, Stephen Hughes, and John Fiddes, respectively.

In addition, two other Sections with related interests are within the Vertebrate Gene Structure and Expression Division. These are the Molecular Genetics Section headed by David Zipser and the Molecular Genetics of the Mouse Section headed by Lee Silver. To further the strength and unity that the program envisions, regular meetings are held at which members of the Division present and discuss their work and ideas.

MAMMALIAN CELL GENETICS

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

The DNA-mediated gene-transfer method is playing a critical role in our understanding of the genetics of animal cells, and nowhere is this more evident than in the study of cancer. This year marks the first cloning of "tumor" genes from human cells. The cloning and characterization of these genes has become a major focus of our lab. Projects on the inheritance and function of DNA methylation and the characterization of the cellular thymidine kinase (*tk*) gene continue from previous years.

Screening Human Tumor Cell Lines for Transforming Genes

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

Work from two laboratories (R. Weinberg's at MIT and G. Cooper's at Sidney Farber Cancer Institute) has demonstrated the growth transformation of normal-growth-controlled mouse cells (NIH 3T3) with DNA from certain chemically transformed rodent cell lines (Shih et al., *Proc. Natl. Acad. Sci. 76:* 5714 [1979]) lines (Shih et al., *Nature 290:* 261 [1981]; Krontiris and Cooper, *Proc. Natl. Acad. Sci. 78:* 1181 [1981]). These experiments suggested the existence of dominant-acting transforming genes of cellular origin. Such genes are of obvious interest. Since, as our success with the cloning of the chicken *tk* gene demonstrated, any gene can be cloned given a means to detect its transfer, we set about to clone transforming genes of human origin.

As a first step, we screened various human tumor cell lines for the presence of dominant-acting transforming genes by using the NIH 3T3 DNA-transfer assay system. Twenty-one human cell lines provided by Jorgen Fogh (Sloan-Kettering Institute) were screened, and five were found positive: one bladder carcinoma cell line, T24; two lung carcinoma cell lines, SK-LU-1 and Calu-1; one colon carcinoma cell line, SK-CO-1; and one neuroblastoma cell line, SK-N-SH. The transforming gene or genes contained in these cells were partially characterized by the technique of "Alu blotting" (Shih et al., *Nature 290:* 261 [1981]; Perucho et al., *Cell 27:* 467 [1981]). This technique exploits the ubiquitous presence of the repeated sequence within the human genome known as the Alu family (Jelinek et al., *Proc. Natl. Acad. Sci. 77:* 1398 [1980]). The presence of these human sequences within a transformed mouse cell are readily detected by blot hybridization. The pattern one sees in transformants is characteristic for a given gene. Using this method, as well as characterization by restriction endonuclease sensitivity, we established that three distinct transforming genes were present in our five cell lines: one unique to T24, one unique to SK-N-SH, and one common to the colon and two lung carcinomas (Perucho et al., *Cell 27:* 467 [1981]). After analyzing samples exchanged with R. Weinberg's laboratory, another human colon carcinoma cell line was found also to contain this last gene. Finding a human transforming gene common to several independently derived human tumor cell lines may indicate the existence of common or overlapping pathways for carcinogenesis in humans.

We have also screened DNAs extracted from surgical specimens of human tumors and from human tumors grown in nude mice. No DNAs from human tumors have been found positive. However, DNA from one human lung tumor, maintained in nude mice, efficiently transformed NIH 3T3 cells. These cells contained the gene common to the lung and colon carcinoma cell lines. We are continuing our screening of DNAs from human tumors both to study the distribution and frequency of occurrence of the recently identified genes and to identify new sources of novel genes.

Characterization of the Transforming Gene from T24 Bladder Carcinoma Cells

M. Goldfarb, K. Shimizu, M. Wigler

Screening studies indicate that T24 cells contain a dominant-acting transforming gene. In previous years we developed a method for isolating genes from animal cells by utilizing an essentially genetic approach ("plasmid rescue") and in this way cloned the chicken *tk* gene. An improved technique ("suppressor rescue") recently developed in our laboratory also utilizes genetic selection in *E. coli*, based on the ability of a cloned tRNA suppressor gene to complement amber mutant bacteriophage. This method has been successfully applied to the isolation of the transforming gene from T24 bladder carcinoma cells (Goldfarb et al., *Nature* [In press]). A DNA sequence of less than 5.0 kb was obtained and found to be active in transforming NIH 3T3 cells. Hybridization studies indicate that these sequences are found in all NIH 3T3 cells transformed with T24 DNA and in all normal and tumor human tissue tested. No sequences were found in NIH 3T3 cells or in NIH 3T3 cells transformed with DNA from neuroblastoma or colon and lung carcinoma cells. The locus that encodes the transforming gene of T24 is highly polymorphic in the human population with respect to restriction fragment length polymorphisms. Our best work to date indicates that this gene is not grossly rearranged in T24 cells. The T24 gene encodes an mRNA of 1100 bp that has

been found expressed in every tumor cell line examined to date, but not in human placenta or in human fibroblasts cultured in vitro.

Isolating the Transforming Genes from Lung and Colon Carcinoma Cells

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

We have adopted a different strategy to clone the common transforming genes of lung and colon carcinoma cells. From the number and sizes of gene-associated restriction fragments hybridizing with human repetitive DNA, we have estimated this gene to be in excess of 30 kb. Cloning it by purely genetic means would therefore be very difficult. We have used an alternative method based on screening genomic libraries constructed in phage λ from mouse cells transformed by these human genes and using human repetitive DNA as our hybridization probe. We obtained one phage with a 12-kb human DNA insert. This insert contained sequences that are present in all NIH 3T3 cells transformed with the common lung/colon gene but not in NIH 3T3 cells transformed with other human genes. Using the "left most" and "right most" parts of the insert, we are attempting to clone additional parts of the gene by "chromosome walking." We have not yet detected a transcript of this gene in transformed mouse cells.

Characterization of the Cellular *tk* Gene

J. Kwoh, K. Shimizu, M. Goldfarb, M. Wigler

Efforts to understand the control of the cellular *tk* gene are continuing. Jesse Kwoh has now precisely mapped the more than 80 linker insertion mutants of the cloned chicken *tk* gene and correlated these with their transformation efficiencies. Results of combined physical/genetic mapping and nuclease-S1 heteroduplex mapping suggest that the 3′ noncoding region is encompassed in an 800-base unspliced region of transcript and that the 5′ coding region consists of at least three exons (the largest no greater than 400 bases) totaling about 1000 bases. John Lewis (Molecular Genetics Section) has shown that expression of *tk* is cell-cycle-regulated in mouse cells transformed with the 3.0-kb clone of chicken *tk*, pchtk-5. This suggests that (1) the control sequences for cell-cycle regulation of *tk* expression are contained on that 3.0-kb fragment and (2) that the regulatory mechanisms have been conserved from chicken to mouse. In related work, Kenji Shimizu is isolating the human *tk* gene and John Lewis is isolating the Chinese hamster *tk* gene. Both are utilizing the technique of suppressor rescue. These genes will be useful in identifying and studying the regulatory control sequences. The isolation of the human *tk* gene will further enable us to study mutational events in the various disorders involving DNA repair that afflict humans.

The Inheritance and Function of DNA Methylation in Mammalian Cells

D. Levy, M. Wigler

In previous years we had begun work on the study of DNA methylation in mammalian cells. These studies involved methylating cloned genes in vitro with *Hpa*II methylase, a bacterial enzyme that modifies DNA at a subset of sites (CCGG) that are normally found methylated in the genomic DNA of vertebrates. We demonstrated two things: first, that methylated genes do not transform as well as the unmethylated controls; and second, that DNA methylation patterns are heritable. The first point confirmed the findings of others that methylation was roughly associated with gene activity, and the second point suggested, as had been hypothesized, that methylation might play a role in somatic inheritance. Current work has centered about these two points. First, we have established that the reduced transformation efficiency of methylated *tk* genes is likely to be the result of an effect on the expression of those genes, since methylation does not affect the efficiency of their transfer. Second, we have established that methylation of the coding regions of the herpes simplex virus (HSV) *tk* gene is sufficient to inhibit transformation, and methylation of the 5′ untranscribed region is without effect. Third, treatment with azacytidine, a cytidine analog that inhibits DNA methylation, can induce reactivation of unexpressed methylated *tk* genes. Fourth, the inheritance of methylation can be very faithful, approaching greater than 99% per cell generation in rat fibroblast cells. Our previous estimate was 95% per generation on the basis of studies in mouse L cells.

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HORMONAL CONTROL OF GENE EXPRESSION

D.T. Kurtz, *D. Bishop, M.M. Manos, L. McCullough, C. Nicodemus*

For the last several years our laboratory has been investigating the regulation of the synthesis of a male rat liver protein called α_{2u}-globulin. This protein is synthesized in the liver of adult male rats under the control of sex steroids, insulin, thyroid hormone, and glucocorticoids.

α_{2u}-Globulin Gene Structure

We have found that α_{2u}-globulin is encoded by a multigene family comprising 18–20 genes per haploid complement. Twelve different genes have thus far been isolated from a library of rat genomic DNA cloned in Charon 4A. All the genes are ~3.5 kb in length and contain six introns. Three of the twelve genes have been found to be variants (possibly pseudogenes). From their 5′ ends through their last introns, these variants are quite similar to the "normal" genes, but from that point on the 3′ sequences in these variants are completely different from those in the normal genes and different from each other. This region corresponds to the 3′ untranslated region of α_{2u}-globulin mRNA. Specific fragments from these variant 3′ ends were cloned and used as probes versus mRNA from several rat tissues. No hybridization was detected, indicating that within these limits of detection, these genes are not transcribed in vivo. Electron microscopy studies of heteroduplexes formed between one of the variant genes and a normal gene showed that in addition to the lack of homology at the 3′ end, there is a small (~100) deletion in this variant, which has been mapped to the protein-coding region, between the fifth and sixth introns. Finally, all three variant genes contain several *Msp*I sites (CCGG), whereas the normal genes contain one, or at most two, *Msp*I site in an 8-kb region surrounding the genes.

α_{2u}-Globulin Synthesis in the Salivary Gland

A cloned α_{2u}-globulin cDNA was used to screen several rat tissues for the presence of α_{2u}-globulin mRNA. It was found that in addition to the liver, α_{2u}-globulin mRNA is found in the salivary glands of both male and female rats at a level of ~100 copies/cell. This is 1/200 the level found in the liver. However, the synthesis of α_{2u}-globulin in salivary gland was found to be constitutive and not under hormonal modulation. Thyroid hormones, androgen, glucocorticoids, or estrogens had no effect on the α_{2u}-globulin mRNA level in the salivary gland.

Hormonal Modulation of α_{2u}-Globulin Gene Expression

The approach we have used to investigate the hormonal control of α_{2u}-globulin gene expression is a simple one: If the information necessary for hormonal response is encoded in the DNA sequences in or immediately around the α_{2u}-globulin gene, then when an isolated gene is put into a cell with the appropriate hormone receptor, it should be able to respond to the hormone. We have found that this indeed is the case: Purified α_{2u}-globulin genes introduced into mouse L cells, rat R2 cells, or rat XC cells respond to glucocorticoids, resulting in the induction of cytoplasmic α_{2u}-globulin mRNA. The extent of induction varies from clone to clone but it is generally eight- to tenfold. Using cloned α_{2u}-gene fragments containing progressively smaller amounts of flanking DNA sequence, we have found that a cloned gene fragment containing 275 bases of 5′ flanking sequence and ~400 bases of 3′ flanking DNA still responds to glucocorticoids, although the fold induction is somewhat less.

The different cloned α_{2u}-globulin genes were tested for hormone inducibility following transfer into L cells. All of the genes that possess the "normal" 3′ end were found to be inducible. The variant genes mentioned above, however, showed a markedly different behavior: When introduced into L cells, all of these variant genes produce mRNA constitutively at a relatively high level. Hormone administration has no effect on the level of message. The constitutive level of α_{2u} mRNA produced by these variants (200–300 copies per cell) corresponds to the fully induced level found for the hormonally inducible genes. Hybrid genes containing either the 5′ or 3′ half of an inducible gene linked to the opposite half of a constitutive gene have been constructed and will be used to investigate this phenomenon.

Cloned fragments from the 5′ end of an inducible α_{2u}-globulin gene were ligated to the structural gene for HSV thymidine kinase. These hybrids were used to transfect L*tk*$^-$ cells to a TK$^+$ phenotype. It was found the thymidine kinase enzymic activity in L cells containing this construction was induced two- to fivefold by glucocorticoids. Thymidine kinase activity in L cells containing an authentic HSV *tk* gene is unaffected by glucocorticoids, and in fact usually decreases. The induction of these hybrid constructs that contain only the 5′ promoter region of the α_{2u}-globulin gene is consistently less than that seen when the entire α_{2u}-gene is used. This could indicate that DNA sequences that respond to the hormone are found throughout the gene or that the induction

of α_{2u}-globulin by glucocorticoids is due both to an induction of transcription and a concomitant stabilization of α_{2u} mRNA in the cytoplasm. (Specific stabilization of mRNA by steroid hormones has been found in several systems.) Constructs that contain only the 5′ promoter region from the α_{2u}-globulin gene may be able to respond to glucocorticoids at the transcriptional level, but the HSV *tk* message produced would not be expected to be stabilized by glucocorticoids. To determine whether there is a component of mRNA stabilization in the induction of α_{2u}-globulin, we have constructed hybrids in which various portions of the α_{2u} structural gene have been cloned into the 3′ untranslated region of the HSV *tk* gene. Such constructs should result in an artificially elongated *tk* mRNA that contains α_{2u} information at the 3′ end. These clones have been transfected into L cells and the effect of glucocorticoids on the level of the hybrid mRNA is being investigated.

Modulation of α_{2u}-Globulin Expression by Other Hormones

The studies outlined above have been restricted to glucocorticoid hormones because the recipient cells used in these studies do not possess receptors for sex steroids or thyroid hormone. The induction of α_{2u} by thyroid hormone in vivo and the repression of α_{2u} by estrogens in vivo are much greater than the in vivo effects of glucocorticoid hormones. Several cell lines have been described that contain receptors for thyroid hormone (C3H 10T 1/2 cells) or estrogen (MCF-7 cells). A dominant-acting vector for eucaryotic gene transfer has been constructed by Doug Hanahan (Tumor Virus Section). It consists of the procaryotic neomycin resistance gene from Tn5 linked to the SV40 early promoter. If appropriate splice and poly(A)-addition sites are added, this vector seems to be highly efficient in transforming several different tissue culture lines to resistance to the neomycin analog G418. This vector will make possible studies on the molecular mechanisms of action of sex steroids and thyroid hormone and may allow us to begin to study the genetics of the hormone-response pathway.

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GENES FOR THE MAJOR STRUCTURAL PROTEINS: ORGANIZATION AND CONTROL OF EXPRESSION

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Nondefective Avian Retroviral Vectors

Retroviruses, alone among the viruses of higher eucaryotes, act as vectors in nature. The highly oncogenic retroviruses all contain genes captured from host-cell chromosomes. The high level of oncogenicity of these viruses is a direct result of the expression of the captured cellular gene. In addition to their natural qualifications as vectors, retroviruses have a number of other features that enhance their usefulness for eucaryotic gene manipulation and expression. Retroviruses are nonlytic, and when not carrying an oncogene, they have little or no effect on the growth properties or morphology of cells in vitro. There are even a few strains of retroviruses available that replicate in animals but do not cause any disease. Retroviruses associate stably with the host cells; a copy of the viral genome is inserted into host DNA as a normal part of the virus life cycle. The efficiency of infection is high: In vitro it is possible to infect essentially every cell on a dish. The infection is self-limiting, however, and the number of proviruses inserted into the host genome is usually less than four. Replication-competent viruses containing only viral genes are usually about 7.5 kb in length, making manipulations with restriction enzymes manageable. Additional genetic information can be accommodated; the parental virus, Rous sarcoma virus (RSV) is 9.5 kb in length. Of all the known retroviruses that carry oncogenes of cellular origin, only RSV has retained a full complement of viral genes. In all other cases the acquisition of the cellular gene is accompanied by the loss of part or all of one or more viral genes. Finally, the unique alternation between RNA and DNA forms as the virus replicates poses the question of how retroviruses regulate RNA splicing so that genes containing essential genetic information are not lost. The question of how newly introduced genes containing introns are handled is especially relevant, since in all of the cases studied so far, the viral copy of the acquired proto-oncogene is without intervening sequences, despite the fact that in most cases the cellular antecedent of the gene has intervening sequences.

For these reasons we have initiated a project to use retroviruses as vectors. RSV was chosen as the prototype because it should be possible to replace the oncogene (*src*) with other genes and still maintain the full complement of viral genes. Since *src* is expressed, precise replacement of *src* should lead to expression of the inserted gene. The advantages of a helper-independent vector are substantial: Most important is that there need be no selection for genes inserted into the vector. Since all the viruses present in the infection are the same, the problems caused by recombination with or overgrowth by the helper are eliminated. The vector is viable whether or not DNA is inserted in place of the *src* gene, which permits direct assessment of a vector construction without the complication of an insert.

Replication-competent *src* deletion mutants occur naturally at relatively high frequency. The first retrovirus vectors we constructed resembled these natural *src* deletion mutants; cloned viral DNA was manipulated in vitro to remove most or all of *src*. To allow the easy insertion of new genetic information in place of *src*, the endpoints of the in vitro deletions were joined with a linker containing a site for the restriction endonuclease *Cla*I, which does not normally cleave RSV DNA. The choice of *Cla*I has the additional advantage that DNA cleaved by *Taq*I, which has a 4-base recognition site, may be ligated directly to DNA cleaved by *Cla*I without further manipulations.

In the wild-type SR-A strain of RSV, from which the vectors were derived, *src* is flanked by direct repeats approximately 120 bp long. The majority of the natural deletion events that remove *src* occur within these 120-bp repeats (J. Sorge, unpublished), although it is not clear whether the deletion events are strictly homologous. The first generation of vectors retain most or all of both repeats (see Fig.1), and in all cases deletion events similar to those that remove *src* from RSV were observed. The percentage of deleted virus present at any time after the initial infection is a product of the propensity for deletion of the starting virus and the growth advantage, if any, of the deleted form. There seems to be a general tendency for infections initiated with viruses containing large inserts to accumulate a higher percentage of deletions. One apparent exception to this rule was a construction in which a small insert was introduced together with a small piece of pBR322 (325 bases of pBR322 were present). This construction deleted much more rapidly than did a very similar construction that lacked the pBR322 sequences (see Fig. 1). Nuclease-S1 mapping demonstrated an apparent deletion endpoint within the pBR322 sequences.

In some sense the selective advantage of the smaller viruses may reflect the fact that the life cycle of these viruses is more rapid. Reverse transcriptase takes several hours to copy viral RNA, and the difference in the amount of time it takes to copy the larger and smaller genomes may make the replication cycle of a deleted virus faster. There may, however, be more to the system than this: We have not been able to grow any virus containing a genome larger than about 10 kb; larger constructions all failed to show any

growth (Fig. 1), suggesting that there may be some absolute size constraint.

We wished to eliminate or vastly reduce the tendency for the virus to delete inserted genetic information at high frequency. The propensity for deletion obviously compromises the general usefulness of the vector, since sequences for which no genetic selection is available can be rapidly

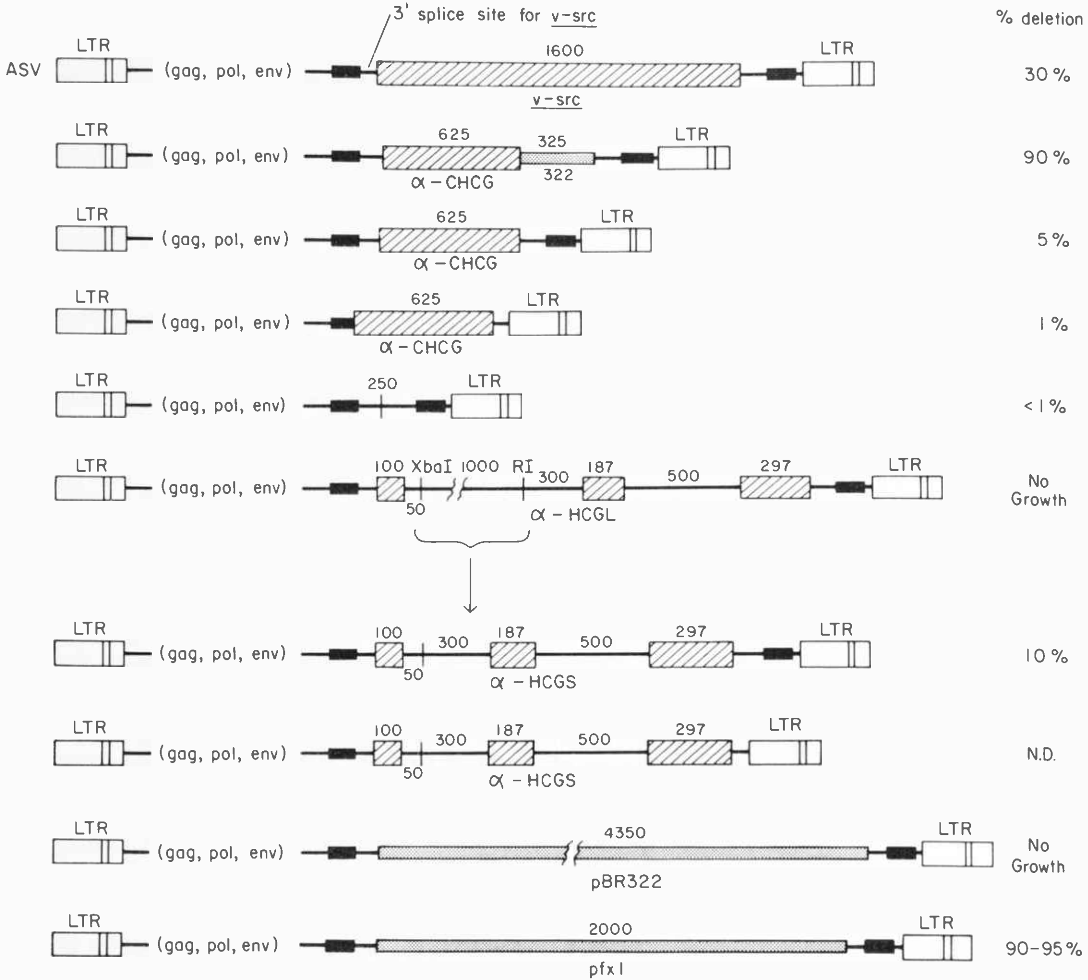

Figure 1
A comparison of some of the retroviral vectors derived from RSV. At the top of the figure is a schematic drawing of the parental virus (labeled ASV). For convenience, only the *src* region and the LTRs are drawn to scale; the *gag, pol,* and *env* genes are vastly compressed. In the SR-A strain of RSV, the *src* gene (labeled *v-src* in the drawing) is flanked by direct repeats. These are shown in the drawing as filled black boxes. The size of the *src* gene, approximately 1600 bases, is given above the *v-src* region. After passage of a pure stock of RSV, and several of the recombinant viruses as well, the region between the direct repeats is lost. The approximate percentage of deletions is given at the right. This is not an absolute value, since the proportion of deleted viruses increases with time, but the measurements were made at approximately the same time (about 4 weeks) after the initiation of infection in each case.

Immediately below the parental virus are drawings of three recombinants, each of which contains a complete cDNA copy of the α subunit of human chorionic gonadatropin (αCHCG). The uppermost of these contains, in addition, 325 bases of sequence from the bacterial vector pBR322, the lowest of the three has had removed essentially all of the right-hand copy of the direct repeat that originally flanked *src*.

The fifth drawing from the top shows one of the viral vectors without an insert; the vertical line represents the *ClaI* used to introduce DNA into the vector.

Below the drawing of the vector are three constructions that contain the complete genomic copy of the α subunit of the human chorionic gonadotropin gene. This form of the gene contains two intervening sequences; the exons are shown as cross-hatched boxes and the introns by a single dark line. The construction depicted in the uppermost of the three drawings contains a complete copy of genomic α-HCG; the lower two have had a portion of the first exon removed.

The two drawings at the bottom are retroviral vectors with plasmid inserts, either pBR 322 or the small plasmid pfx-1. We have called constructions of this sort RSVPs.

lost during virus passage. Assuming that the propensity for deletion depends on the presence of both direct repeats that originally flanked *src*, the solution was to eliminate one or both repeats. Since the splice acceptor site used in making the *src* message (see Fig.1), which should be useful for expression of inserted genes, lies between the upstream repeat and the beginning of *src*, we chose to eliminate the downstream direct repeat. A series of clones were prepared by *Bal*31 digestion and ligation to *Cla*I linkers. This created *Cla*I sites at varying distances from the U_3 region of the LTR. These fragments were combined with various cloned viral DNAs with *Cla* sites upstream to *src* to yield viral DNAs with deletions ending −10, −30, −45, etc. bases from the LTR. The −10 derivatives were not viable, but all derivatives with 30 or more bases upstream of the LTR were viable. The derivatives with almost all of the downstream direct repeat removed show a greatly reduced deletion frequency (see Fig. 1).

The behavior of inserts containing intervening sequences was tested. The initial experiments were done with derivatives that contained genomic clones of human chorionic gonadotropin (HCG), which has two intervening sequences. The original construction was about 11 kb in length and did not grow (see Fig. 1), presumably because it was too large. Removing 1000 bp from one of the intervening sequences in vitro permitted viral replication. The experiments were done in two sets of viral vectors, those that contained both repeats intact and those lacking almost all of the downstream direct repeat. After the virus had reached a high titer, virion RNA and whole-cell RNA samples were analyzed by nuclease-S1 digestion, and the genomic form of viral DNA was analyzed by the transfer procedure of Southern.

In two of the experiments, the virion RNA was a mixture of forms that did and did not retain HCG intervening sequences. Loss of the HCG intervening sequences from the provirus was documented by the transfer procedure of Southern. In the third experiment the virion RNA contained only the fully spliced form of the HCG insert. The elimination of the intervening sequences is precise, as judged by nuclease-S1 analysis. The ratio of spliced to unspliced HCG inserts in virion RNA is apparently affected by the amount of viral DNA used in the transfection. Following a high-efficiency transformation, the vast majority of the genomes that were present a few weeks after transfection contained completely spliced HCG inserts. Following a low-efficiency transfection the percentage of viral genomes containing spliced HCG inserts is nearer 50%.

It is clear that splicing is not the inevitable fate of retroviral RNA. There are three reasons for this statement: Wild-type genomic retroviral RNA is itself unspliced; there are unspliced HCG inserts present in several of the infections with the vectors; and finally, the construction with the full HCG genomic insert (exceeding the apparent size limitation) does not grow following a low-efficiency transfection.

Taken together, these data suggest separate pathways for the maturation of virion RNA and viral message. Virion RNA is apparently protected from the normal splicing machinery, presumably by a viral gene product. Such a mechanism would function as feedback loop that would regulate the ratio of spliced to unspliced RNA if the viral gene product were derived from spliced RNA. Spliced RNA is not absolutely excluded from virions; some viral message is packaged in the course of a normal infection. If the mispackaging of message is the major pathway whereby introns are removed from the genomic HCG inserts, we can account both for the fact that intervening sequences can be lost from the HCG during the retrovirus life cycle and for the fact that not all the viruses lose the intervening sequences even after several rounds of viral replication. These observations have considerable significance for the acquisition of oncogenes by retroviruses. A retrovirus with a precise cDNA copy of a gene can potentially derive from a DNA fusion event linking retroviral genes to a cellular gene containing intervening sequences. The only question that remains is how well this event works with genes that contain large intervening sequences.

We plan to test the effects of high-efficiency transformation on the growth of constructions with the full HCG genomic inserts, which apparently are too large to replicate unless some of the insert is removed either by deletion or by splicing.

Retrovirus Recombinants Capable of Replication in *E. coli*

It is now clear that retroviral genomes can act as insertional mutagens in certain specific circumstances and can in either activate or inactivate particular genes. It is also clear that retroviral DNA can insert at a great many sites in the host genome. It is not yet known whether retroviral DNAs insert essentially at random in the host genome. This is of considerable interest for two reasons: First, specificity of integration might play a role in defining differences in oncogenic potential among related viruses, some of which can activate oncogenes by integrating nearby. Second, retroviruses may be useful as general insertional mutagens for both the identification and the isolation of selectable markers. The general utility of such a proposal depends crucially on whether or not there is significant restriction on the number of sites in the host genome that a retroviral genome can enter.

For these reasons, using the RSV-derived vectors described in the previous section, we have constructed recombinants (which we call RSVPs) that have the *src* region of RSV replaced by a small (2 kb) plasmid that carries the *E. coli* origin region and the tetracycline resistance gene (*tet*)

from pBR322. Since the ultimate goal is to mutagenize the host chromosome and then to rescue retroviral genomes responsible for the mutagenic events, it is valuable, if not essential, that the vector be helper-independent. This construction grows on *E. coli* since there is an *E. coli* origin of replication and a selectable marker in the small plasmid, and it also grows in chicken cells since it contains a full complement of uninterrupted viral genes. The original RSVP construction retained the direct repeats that flank *src* in RSV and, as a consequence, deleted the plasmid sequence at high frequency during passage in chicken cells. Using a high-efficiency *E. coli* transformation protocol developed by D. Hanahan (Tumor Virus Section), it was possible to rescue onto *E. coli* RSVP DNA directly from DNA isolated from RSVP-infected chicken cells, despite the fact that 90% of the RSVP viruses present in the chicken cells lacked the plasmid insert. Based on the RSV vectors described above we are now constructing a second generation of RSVPs that have the downstream copy of the direct repeat removed (see Fig. 1). Since these have a vastly reduced deletion frequency with other inserts, we hope to use them to create RSVPs that delete their plasmids only rarely during passage on chicken cells.

Structure of the Endogenous Virus RAV-0 and the Construction of Defined Recombinants between Avian Leukosis Viruses

The avian leukosis viruses (ALVs) can cause leukosis by activating the endogenous oncogene *c-myc*. Insertion of an ALV provirus near *c-myc* can cause greatly increased levels of *c-myc* transcription and lead to cellular transformation. The endogenous avian retrovirus RAV-0, which is closely related to the ALVs, does not cause leukosis in chickens. The most obvious physiological difference between the ALVs and RAV-0 is their host range, which is determined by the *env* gene. However, in vivo ALV/RAV-0 recombinants that have acquired RAV-0 host range are still oncogenic in birds, which argues strongly that the crucial difference(s) in oncogenic potential between RAV-0 and the ALVs does not lie in *env*. A physical comparison of the RAV-0 and ALV genomes by T1 oligonucleotide fingerprinting and hybridization suggests that the most significant differences in sequence between RAV-0 and the ALVs lie in a region corresponding to the 3′ end of viral RNA. To examine this more precisely, we have cloned a replication-competent DNA copy of RAV-0 and sequenced the LTR and adjacent regions. The most striking differences between the RAV-0 and ALV sequences in the segment examined lie in the U_3 portion of the LTR. Could these differences account for the differences in oncogenicity?

There are two obvious (as well as several less likely) explanations for the lack of RAV-0 oncogenicity. Either the RAV-0 proviruses cannot integrate near the cellular oncogene *c-myc*, or if they do integrate near *c-myc*, they cannot activate *c-myc*. If there are preferred integration sites or regions in the host genome for the insertion of retroviral DNA (which is not known), models can be generated in which the LTR sequences are important determiners of this specificity. Alternatively, RAV-0 may integrate next to *c-myc* and fail to activate the endogenous oncogene. If this is the correct explanation, the crucial difference between RAV-0 and the ALVs might still reside in the LTR and, more specificially, in the U_3 region.

As a final test for the crucial region defining the differences in oncogenic potential between the ALVs and RAV-0, we are constructing in vitro defined recombinants between an oncogenic ALV parent, specifically the RSV-derived vectors described above, and RAV-0. Because it is likely that the crucial difference will reside in the LTR, the first recombinants we have constructed have all the genes, *gag, pol,* and *env*, from the ALV parent and the LTR region from RAV-0. Dr. Lyman Crittenden (USDA Poultry Laboratory) has agreed to test the oncogenicity of these viruses in birds. If, as we predict, such constructions are not oncogenic in vivo, they would serve as the prototype for an in vivo vector.

Since these recombinants have not been tested in chickens as yet, our current conclusions must rest on the sequence data alone. The sequence of the RAV-0 LTR is very similar to that of the replication-defective endogenous virus EV-1. Like the EV-1 LTR, the RAV-0 LTR is smaller (278 bp instead of 330 bp) than the LTRs of the oncogenic members of the ASV/ALV group.

The U_5 and R segments are quite similar; the majority of the changes lie in the U_3 segment. Although there is still obvious homology between the U_3 regions of RAV-0 and the oncogenic ALV/ASV viruses, there are a series of small segments present in the oncogenic viruses that are absent in RAV-0. It is possible that these missing segments could account for the differences in oncogenic potential of RAV-0 and the ALVs. The RAV-0 sequence outside the LTR region has also been compared with the available ASV/ALV sequences (Czernilofsky et al 1980; D. Schwartz, pers. commun.). The sequences between the LTR and *gag* are very similar in the Pr-C strain of RSV and RAV-0, and the region of *env* for which we have sequence is similar in the SR-A and Pr-C strains of ASV and RAV-0. The most obvious difference is at the extreme end of the *env* gene, at the C terminus of gp37. The nucleic acid sequences are obviously related, but the predicted amino acid sequence of the RAV-0 gp37 is significantly longer than the sequence of the gp37 of Pr-C, and very similar to the sequence of the gp37 of SR-A. (We have made significant corrections in the sequence of SR-A near the carboxy terminus of gp37. [J. Sorg and S. Hughes, unpubl.].)The major differences in this segment of gp37 suggest that this region probably does not play a major role in the function of the *env* protein.

The acquisition of *src* is of considerable interest. Sarcoma viruses presumably are derived from ALVs; however, *src* is also lost from RSVs at high frquency, creating ALVs. When dealing with an ALV, therefore, it is very difficult to know whether or not the ALV previously contained *src*. To understand the original acquisition of *src*, it is essential to examine a viral genome that never contained *src*. The most obvious candidate is RAV-0, which is closely related to the other ALV/ASV viruses, but almost certainly never contained *src*.

The segment of RAV-0 that lies between the end of the *env* gene and U_3 is approximately 190 bases in length. Essentially this entire segment is present between *env* and *src* in the SR-A strain of RSV. Most of this segment is also present between *env* and *src* in Pr-C; however, in Pr-C there is an apparent deletion of 40 bases in the region adjacent to *env*. In SR-A, but not in Pr-C, about half of this segment is also present between *src* and the LTR. This arrangement has implications for the mechanism by which *src* was acquired. It suggests that the *src* sequences were not merely inserted into the region between *env* and *src*, but that at least two viral genomes were involved. Through integration, or the actions of reverse transcriptase, or both, *src* was apparently added to the end of the viral genome, with the concomitant loss of the LTR. To complete the virus, a second copy of the sequence found between *env* and the LTR of RAV-0 was also acquired, together with the LTR; almost certainly a second viral genome was the donor of this segment.

Isolation and Identification of Genes Encoding Major Skeletal Proteins

We are working on protocols for rapidly identifying individual recombinant clones. Our initial strategy was to identify, from a complete genomic library, clones that encoded abundant messages. Such clones were defined as those that reacted strongly with ^{32}P-labeled cDNA prepared from poly(A)$^+$ RNA. Individual clones were identified by hybridization selection of mRNA followed by translation. The translation products were analyzed on one- and two-dimensional polyacrylamide gels. These methods allowed us to identify clones containing genes from the actin, tubulin, and 100-Å-filament families. Although we have been successful in identifying these highly ex-

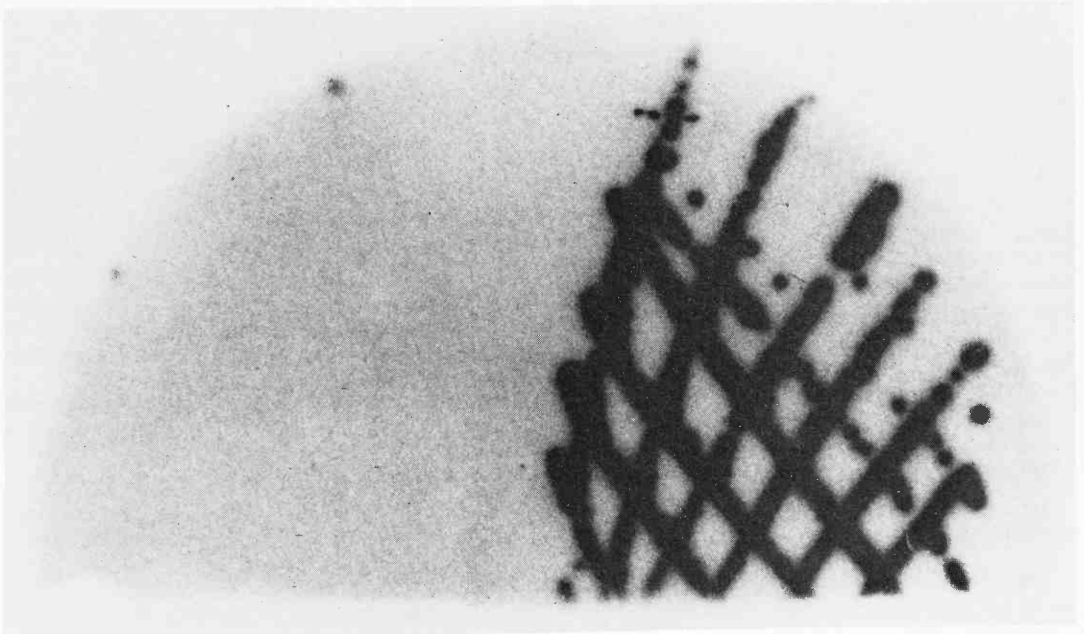

Figure 2
Immunological identification of bacterial clones producing flu virus HA antigen. Two halves of a bacterial plate were streaked with bacteria. Those containing a plasmid that expresses flu virus HA antigen were on the right; those containing a related plasmid that did not contain flu virus HA antigen were on the left. A portion of the colonies was transferred to an inert support, lysed, and cut in half, and one half of the filter was exposed first to a rabbit antibody specific for flu virus HA antigen and then to a ^{125}I-labeled goat anti-rabbit antibody. Excess antibody was washed off, and the filter was exposed to X-ray film. An autoradiogram of the film is shown in the figure. The pattern of streaks and spots on the right side of the figure corresponds precisely to the pattern of growth of the colonies producing flu virus HA antigen. A similar pattern of colonies not producing flu virus HA antigen was present on the left side of the figure; these are not detected in the assay.

pressed genes, the method is difficult and we have had little success in identifying clones containing genes expressed at lower levels. We are now trying a new strategy for identification: cloning cDNAs into plasmid expression vectors and screening the individual colonies with labeled antibodies. Several groups have published protocols of this sort, although only one of these has been used to identify an unknown clone; the others have been used only in reconstruction experiments with defined cDNAs. The available protocols are relatively laborious when compared with the nucleic acid hybridization protocols normally used to isolate clones.

We are now testing a much simpler protocol, based on the "western" protein transfer to nitrocellulose, which is straightforward and, at least in reconstruction experiments, quite sensitive. In our preliminary experiments, bacterial clones (kindly provided by M.-J. Gething, Imperial Cancer Research Fund) producing about 3000 molecules of flu virus HA antigen per cell were readily detected, and colonies not producing HA that were present on the same filter were not detected (Fig. 2). We are not constructing expression cDNA libraries and will initially screen in parallel clones for which both antibody and nucleic acid probes are available. If a reasonable percentage of the clones detected by the nucleic acid probes can be detected with the antibody probes, a purely antigenic screening will be initiated.

It is to be expected that only one cDNA clone in six has both the appropriate orientation and frame to express the same protein sequence that the parental message expresses. However, John Fiddes (Human Polypeptide Hormone Genes Section) has developed a protocol for inserting, with linkers, cDNAs in defined orientations, and with the high-efficiency transformation protocols of Doug Hanahan (Tumor Virus Section), it is straightforward to manufacture an expression library large enough (about 10^5 members) to screen for clones of even relatively rare messages. Defined antibodies are available at Cold Spring Harbor for approximately twenty of the cytoskeletal proteins. Our initial efforts will be attempts to obtain vinculin clones.

Analysis of the Chick β-Actin Gene

There is only one β-actin gene in the chicken genome. We have obtained two independent recombinant λ phage, each of which contains a complete copy of this gene. The message is about 2000 bases in length, of which about 1100 bases are required to encode the β-actin protein. There is a long 3′ untranslated region, about 600–700 bases in length; the 5′ untranslated region is much shorter, about 120–130 bases in length.

The gene itself contains four intervening sequences, all of which lie in the coding segment. All four are relatively small, the one nearest the site of initiation of transcription is about 300 bp in length. The other three are, in order, about 550 bp, 200 bp, and 350 bp in length. Most of the gene has now been sequenced. There is homology, as expected, to the yeast actin gene in the coding regions both in the predicted amino acid sequence and in the nucleic acid sequence. The amino acid sequence is much better preserved; there has been considerable genetic drift at positions in the gene that are unselected (usually third base positions). Interestingly, there apparently has not been conservation of the position of intervening sequences, suggesting that such sequences have been inserted into (and possibly lost from) this gene during the course of evolution.

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STRUCTURE AND EXPRESSION OF HUMAN POLYPEPTIDE HORMONE GENES

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

We have been interested in the structure and expression of two separate groups of mammalian polypeptide hormones, the pituitary and placental glycoprotein hormones, and the hypothalamic releasing hormones. Although structurally distinct, these two groups have a functional relationship in that the hypothalamic releasing factors are required for stimulating the synthesis and release into the bloodstream of the pituitary hormones.

The Glycoprotein Hormones

There are three pituitary glycoprotein hormones, luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), and one placental glycoprotein hormone, chorionic gonadotropin (CG). LH and FSH function synergistically in both the male and the female to promote spermatogenesis or ovulation by stimulating the synthesis of steroids by the testes or ovaries. Following conception, the placenta makes CG, which functions in a very similar way to LH in that it stimulates the corpus luteum of the ovary to produce the steroids essential for the maintenance of pregnancy. Functionally, therefore, the three hormones, LH, FSH, and CG, are called the gonadotropins. The fourth member of the group, TSH, although structurally related to the gonadotropins, is functionally distinct in that it causes the thyroid gland to make thyroxine and tri-iodothyronine.

All four glycoprotein hormones are dimeric and consist of dissimilar α and β subunits that are held together noncovalently. The α-subunit amino acid sequence appears to be common to all four hormones, whereas the β subunits are unique and confer biological specificity on the hormone. The four β subunits all show sequence homology ranging from about 80% between LH and CG to from 30% to 40% between all other pairs of β subunits. It is therefore expected that the β subunits will be encoded by a multigene family of sequences that presumably had a common evolutionary ancestor.

Previously we had isolated and characterized full-length cDNA clones for both the α and β subunits of CG by reverse transcribing human placental RNA. The cloned α-subunit gene was then used as a hybridization probe to detect chromosomal clone containing the region coding for the α subunit. Analysis of this clone, and comparison of it to the hybridizing fragments observed in total human DNA, strongly indicates that there is a single α-subunit gene that must be transcribed for all four hormones.

Recently we have been analyzing genomic clones isolated by hybridization to the βCG cDNA and have found that the situation is much more complex than with the single α gene. The β subunit of CG appears to be encoded by a family of at least seven separate genes. A total of five of these have been isolated on chromosomal clones, and the presence of the other two is indicated by Southern blotting of total human DNA.

The five genes isolated so far all appear to have the same overall structure, with a small intron close to the start of the signal peptide (between amino acids -15 and -16) and another small intron between amino acids 41 and 42 of the mature protein. Several regions of slight sequence divergence exist between the genes. For example, one gene has a single nucleotide difference in the coding region so that the proline codon for amino acid 126 is CCG and not the CCC that is present in the other genes and the initial cDNA isolate. An additional cDNA clone that has this altered codon has now been isolated, proving that at least two of the seven βCG genes are expressed. The other region of sequence difference between the genes, apart from in the intervening sequences, is in the 5′ untranslated regions. Again, distinct types of cDNA clones have been isolated, showing that several of the βCG genes are probably functional. We are presently characterizing the promoter sequences of the five genes and hope to study their expression by in vitro transcription or reintroduction of the genes into cultured mammalian cells.

All five βCG genes so far isolated are physically linked on the chromosome and span a region of about 45 kb. Southern blot analysis of human DNA implies that the two uncloned genes are also linked to this locus. Four of the five cloned genes show a most unusual structure in that they are in the form of two inverted symmetrical pairs. Transcription of each inverted pair is convergent with the two 3′ ends of the genes, being separated by about 2 kb of DNA.

Using human pituitary RNA, we are attempting to isolate cDNA clones for the β subunits of LH, FSH, and TSH to determine whether these are also encoded by multigene families and whether they are linked to the genes for the β subunit of CG.

The Hypothalamic Releasing Factors

The hypothalamus synthesizes several small peptides which include gonadotropin releasing hormone (gnRH) and thyroid-stimulating-hormone releasing hormone (TRH). These peptides pass by a direct vascular connection to the pituitary where they stimulate the release of LH and FSH (gnRH) and TSH (TRH). Although both of these peptides are produced in very minute quantities, they have been isolated and their primary structure determined. gnRH was thus shown to be a decapeptide, and TRH is a tripeptide.

We assume that these small peptides are made

in the hypothalamus as part of a larger precursor that is processed in a way similar to the proopiomelanocortin and enkephalin precursors. To understand the biosynthesis of these hormones, we are attempting to isolate cDNA clones encoding the putative precursor to one of the hypothalamic peptides, gnRH. This project is in collaboration with M. Evinger and J. Roberts of Columbia University and A. Markham of ICI, England.

Synthetic oligonucleotides, 11-16 nucleotides long, have been made to correspond to the known amino acid sequence of gnRH. Due to the degeneracy of the codons for the amino acids of gnRH, a considerable number of oligonucleotides have been synthesized. We have been using these oligonucleotides in two ways.

First, the oligonucleotides have been used to prime the synthesis of double-stranded cDNA from mRNA prepared from a tissue that should be expressing gnRH. In addition to the hypothalamus, there is evidence that gnRH is also made in the placenta, so this tissue has also been used. When this specifically primed cDNA is analyzed by gel electrophoresis, a discrete set of bands is observed. The specifically primed cDNA from human placenta has been cloned in an attempt to isolate cDNA clones corresponding to the prominent primer-dependent bands. Using this approach, a primer-extension cDNA clone has been isolated that hybridizes to an mRNA species of the same size in the placenta and the hypothalamus and that is not found in other tissues. By these criteria, this short cDNA clone is a good candidate for gnRH. We have now used the primer-extension clone to screen a complete oligo(dT)-primed cDNA library and are determining by DNA sequence analysis whether the positively hybridizing clones code for the putative gnRH precursor.

The second approach involves the construction of complete cDNA libraries by oligo(dT) priming and using either the oligonucleotides themselves or the specifically primed cDNA they generate as hybridization probes. We have been successful in creating cDNA libraries of greater than 10^5 recombinants by using a double-linked cDNA cloning technique and have screened these libraries as described above. Several positively hybridizing clones have been isolated and these are being analyzed by DNA sequencing to establish whether they encode gnRH.

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MOLECULAR GENETICS OF THE MOUSE

L.M. Silver, *G. Blose, J.S. Danska, M.I. Harper, D. Lukralle, N. Sarvetnick, J. Uman, K.R. Willison*

In our first year at Cold Spring Harbor we have set up a new program that combines classical and molecular approaches toward an understanding of the organization and expression of the mouse genome. We have an actively breeding colony of approximately 1000 mice, and this colony provides the material used in almost all of the experiments that we are conducting. A major long-term emphasis of our research program is the use of developmental mutations to understand the molecular basis of mammalian embryogenesis. In particular, we have begun a comprehensive analysis of the mouse *t* complex, which has intrigued geneticists for more than 50 years with its striking and unorthodox features.

The topics addressed in this report include: classical breeding studies of *t*-complex gene interactions; identification and analysis of *t*-complex proteins; discovery of a novel middle repetitive family of DNA sequences that has been characterized as a nomadic element within the mouse genome; efforts directed toward an analysis of the DNA sequence organization within the *t* complex; and molecular studies of another region of the mouse genome defined by the mutations *albino* and *pink-eyed dilution.*

Breeding Analysis of Developmental Mutations in the Mouse

L.M. Silver, D. Lukralle, G. Blose

The *t* complex is a large region of mouse chromosome 17 that includes the major histocompatability (H-2) complex (see Silver, *Cell 27:* 239 [1981] for a review). Variant forms of the *t* complex, know as *t* haplotypes, are found at a high frequency in wild populations. All naturally occurring *t* haplotypes exhibit a series of effects on embryonic development, sperm differentiation and maturation, and meiotic recombination. A complete *t* haplotype is operationally defined as one that suppresses recombination, in +/*t* heterozygotes, along the 12 centimorgan (cM) region of chromosome encompassing the locus *T* and the *H-2* complex (Fig. 1); all *t* haplotypes recovered from wild populations have been complete. However, partial *t* haplotypes can be obtained in the laboratory as the products of rare recombinant events in +/*t* animals (Lyon et al., *Nature 279:* 38 [1979]). All partial *t* haplotypes carry a subset of the genetic factors responsible for the various *t* haplotype effects and continue to suppress recombination only along the length of *t* chromatin derived from the parental *t* haplotype. Only a complete *t* haplotype can be transmitted at a very high ratio (greater than 95%) from +/*t* males, and it is this property that allows the propagation of the *t-H-2* complex through wild populations as an indivisible unit.

In studies done in collaboration with K. Artzt (Sloan-Kettering Institute), we demonstrated that the basis for the suppression of recombination between normal and mutant *t* forms of chromosome 17 is a lack of homology in the DNA sequence organization of the two forms of the chromosome. A basic homology in general DNA sequence does ex-

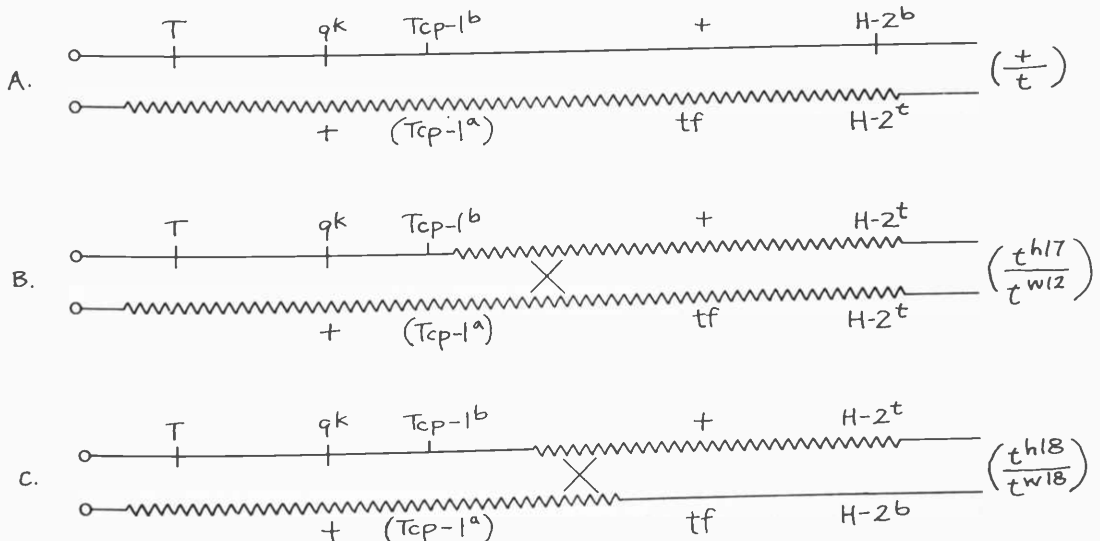

Figure 1
Genetic maps of the *t*-complex region of mouse chromosome 17. The wavy line represents *t*-haplotype chromatin/DNA. (*A*) Representative genotype of a mouse carrying a complete *t* haplotype. (*B*) Representative genotype of a mouse carrying a partial *t* haplotype opposite a complete *t* haplotype. *Recombination occurs freely only in the region of overlapping t* DNA/chromatin. (*C*) Representative genotype of a mouse carrying two different partial *t* haplotypes. Recombination occurs freely only in the isolated region of overlapping *t* DNA/chromatin.

ist among all known *t* haplotypes, so that normal recombination will occur in genotypic regions of overlapping *t* DNA (see Fig. 1).

To perform any kind of molecular analysis of the *t* complex, it is essential to breed animals that are genetically identical except for defined differences within the *t* complex region. Mouse strains that differ in an isolated region of the genome and are otherwise homogeneous are considered to be congenic. Congenic strains are produced by the repeated selection and backcrossing of a particular mutation onto a highly inbred line. We were fortunate to receive from J.L. Guenet of the Pasteur Institute a series of already congenic lines carrying different mutant *t* haplotypes (t^0, t^{w12}, t^{w2}, t^{w5}, and t^{w18}) or the unilocal *T* mutation on the highly inbred 129/Sv strain of mice. These congenic lines were carried through at least 14 successive backcross generations prior to entering our colony, where further rounds of backcrossing have been pursued. We have begun the production of ten novel congenic lines for other variant forms of the *t* complex, such as partial *t* haplotypes and deletions. In certain cases we have reached the fourth backcross generation. A continuation of this breeding program will provide a family of congenic strains to be used in all of the molecular studies of the effects of *t*-haplotype genes on development.

The studies of M. Lyon and her colleagues (Lyon et al., *Nature 279*:38 [1979]) have demonstrated the existence of three separable genetic factors that interact within a complete *t* haplotype to cause transmission-ratio distortion. We would like to understand more fully the interactions among these genes in various *cis* and *trans* combinations. Towards this goal, we are constructing a variety of genotypes for breeding analysis. Our preliminary studies indicate that only one of the three factors is active within the postmeiotic haploid genome, whereas the other two factors appear to be expressed in the diploid genome.

We are currently providing mice in a collaborative effort with G. Martin of the University of California, San Francisco, for the production of embryonic stem cell lines that are homozygous for the lethal t^{w18} haplotype. Such cell lines could provide a biochemical source for the purification of *t*-haplotype-specified proteins present only in embryonic cells. In another collaborative effort, we are providing P. Olds-Clark (Temple University) with t^{w12} males for the isolation of ejaculated sperm from the female reproductive tract. Analysis of the proportion of different sperm present from a $+/t$ heterozygous male is possible with an H-2 cDNA probe. This analysis will provide information on the physiological basis for transmission-ratio distortion.

We are maintaining congenic strains for other developmental mutations such as *Steel* and *W*, which affect hematopoesis and spermatogenesis, and *albino* and *pink eyed dilution*, which have multiple effects. Molecular studies of these mutations will be pursued in parallel with studies of the *t* complex; some of these studies are discussed below.

Identification and Characterization of *t*-Complex Proteins by 2-D Gel Electrophoresis

L. Silver, J. Uman, J. Garrels

We are using a computer-directed system of two-dimensional gel electrophoresis (see QUEST 2-D Gel Laboratory Section) for the identification and genetic mapping of protein specified by the mouse *t* complex. A comprehensive 2-D gel analysis of the *t* complex could identify proteins critical to development and differentiation, as well as provide information on the extent of polymorphism associated with naturally occurring *t* haplotypes and the relatedness of different wild *t*-haplotype groups. In our initial analysis we chose to study the testicular cell protein patterns expressed by six different lethal *t* haplotypes (five complete and one partial) that are all congenic on the 129/SvJ background. In control experiments, the 2-D gel patterns of different 129/SvJ males ranging in age from 8 weeks to 40 weeks and sacrificed on different days, were observed to be highly reproducible with no major qualitative differences. An analysis of the five complete *t* haplotypes (t^0, t^{w2}, t^{w5}, t^{w12}, and t^{w32}) allowed the identification of nine *t*-haplotype-specific proteins among the 1500 most prominent testicular cell proteins observed (see Fig. 2). Eight of these *t*-haplotype-specific proteins are expressed in an identical form by mice carrying any one of the five complete *t* haplotypes on a 129/SvJ background. All eight invariant *t*-specific proteins are also synthesized by animals carrying a complete *t* haplotype on a congenic inbred C3H background. Six of these invariant *t*-specific proteins have apparent wild-type counterparts that differ in charge but are of comparable molecular weight and intensity within the protein patterns of $+/t$ heterozygotes—all six "wild-type" proteins are missing from the 2-D patterns of compound t^0/t^{w12} animals. This last result suggests that at least six of the nine *t*-specific proteins are direct gene products of the *t* complex. The level of polymorphism associated with *t* haplotypes (9/1500 is 0.6%) is much greater than expected for simply a variant form of a region of chromosome 17 corresponding to only 1% of the total mouse genome. The accumulated data lead to the speculation that all *t* haplotypes were derived from a common ancestral pool of animals that were genetically distinct from *Mus musculus*.

We have begun the process of mapping *t*-complex-protein (*Tcp*) genes to subregions of the *t* complex by utilizing the partial *t*-haplotype strains. The preliminary results indicate that the

nine *Tcp* genes so far identified map to at least three separable regions along chromosome 17. Correlations between particular *Tcp* genes and particular factors that affect spermatogenesis and fertility have been made. Further studies along this line are being pursued.

The p63/6.9 Protein and the *Tcp-1* Gene

L. Silver

The most prominent *t*-specific protein to be expressed in the testes is called p63/6.9, and the gene that specifies this protein is called *Tcp-1*. All complete *t* haplotypes analyzed to date express an acidic p63/6.9a, whereas all wild-type mouse chromosomes 17 express a basic form of this protein called p63/6.9b. A comprehensive analysis of *Tcp-1* allelic association has been carried out with over 20 different partial *t* haplotypes and other variant forms of chromosome 17. The data indicate that the *Tcp-1*^{a} locus is separable from all of the identified *t*-haplotype factors except for one; a complete correlation has been obtained between *Tcp-1*^{a} and a proximal *t*-haplotype factor involved in effects on transmission-ratio distor-

Figure 2
2-D gel map of labeled testicular proteins from an inbred 129/Sv mouse carrying a complete *t* haplotype and wild-type alleles at *C* and *P*. Nine *t*-haplotype-specific proteins have been identified on the pattern. Other identified proteins include actin (A'), tubulin (T), and the two forms (A and B connected by arrows) of the CPR-1 protein that maps to the *albino-pink eyed dilution* region of mouse chromosome 7. The acidic side of the gel is on the left, reversed from previous work by Silver and colleagues.

tion. An initial biochemical analysis of the p63/6.9 protein has been performed. The data provide evidence that p63/6.9 is closely associated with the external surface of testicular cells but not as an integral membrane component. Chemical properties of the testicular form of this *t*-complex gene product are similar to those reported for the cell surface matrix proteins fibronectin and laminin. The possibility is suggested that primary effects of *t* haplotypes on sperm differentiation could be exerted through the extracellular matrix. p63/6.9 is also present (at a lower level) within the cytoplasm and membranes of F9 teratocarcinoma cells. It appears that the level of p63/6.9 synthesis and the exact nature of p63/6.9 intra- and/or intermolecular interactions is under tissue-specific control.

Identification of a *t*-Complex Gene Product Leads to Construction and Screening of a Testis cDNA Library

K. Willison, J. Danska, L. Silver

The p63/6.9 proteins could represent primary gene products of the *t* complex, or, alternatively, the *Tcp-1* gene could control the posttranslational modification of a precursor form of p63/6.9 that is encoded elsewhere in the genome. To determine whether p63/6.9 is a direct gene product of the *t* complex, testicular cytoplasmic RNA from mice bearing the genotypes +/+, +/*t*, and *t*/*t* was translated in a rabbit-reticulocyte-derived cell-free system, and translated products were analyzed by 2-D gel electrophoresis. The data indicate that the difference between the allelic forms of p63/6.9 is encoded within mRNA transcribed from the *t* complex. Thus, p63/6.9 is the first primary gene product identified in the *t* complex. Results obtained from both cell-free translation and pulse-chase labeling experiments indicate that the mature cell-surface associated form of p63/6.9 has not undergone detectable posttranslational modification.

Because of our interest in obtaining a molecular probe to study the organization of the *t* complex, which is thought to be rearranged relative to the wild-type form of mouse chromosome 17, we have attempted to clone the *Tcp-1* gene by exploiting its relative prominence (0.5%) among translatable testicular RNAs. A cDNA library was constructed by oligo(dT)-primed reverse transcription of testicular cell cytoplasmic poly(A)$^+$ RNA and ligation into the vector pBR322 using R_1/*Hin*dIII linkers. To identify potential *Tcp-1* clones, testicular poly(A)$^+$ RNA was fractionated by agarose gel electrophoresis, and each fraction was assayed for *Tcp-1* mRNA enrichment by cell-free translation and 2-D gel analysis of the resulting proteins. In the RNA fraction selected for screening the testis cDNA library, actin and p63/6.9 were the most prominent proteins among the cell-free translation products. We selected 30 of the most intensely hybridizing cDNA clones and analyzed them further using positive mRNA selection, cell-free translation, and SDS gel electrophoresis. None of the 30 clones selected any translatable RNA, nor did they hybridize to an actin cDNA clone (a gift from S. Hughes, Genes for the Major Structural Proteins) despite the presence of the expected number of actin clones (0.8-1%) within the complete testis library.

Therefore, we have found that within the testis, poly(A)$^+$ RNAs exist that are not translatable commensurate with their abundance. This feature of RNA populations must be considered when trying to clone minor mRNAs by probing with RNA populations characterized by their cell-free translation products. While cloning of the *Tcp-1* gene continued (see below), we became interested in characterizing the poly(A)$^+$ nontranslatable transcripts so abundant in testicular cell RNA.

A Nomadic Middle Repetitive Element in the Mouse

J. Danska, K. Willison, J. Uman, L. Silver

Much of the DNA in eucaryotes does not encode protein and is organized in moderately repetitive sequences (10-1000 copies/haploid genome) of variable length interspersed with unique sequences. The precise function of such sequences is unknown and subject to intense speculation. Repetitive DNA has been most extensively characterized in *Drosophila melanogaster,* where approximately 40 groups of moderately repetitive elements (MRE) comprise over 15% of the genome. *copia* and *412* exemplify families of MRE that reside at many chromosomal locations throughout the genome. Because different families occupy variable chromosomal positions and new arrangements have occurred within individual members of a single strain of *Drosophila,* these sequences are defined as nomadic. Another example of a nomadic MRE is the *Ty-1* family in yeast. A striking number of structural characteristics of *copia, 412* and *Ty-1* are shared by some integrated retroviruses. The MRE has persisted throughout evolution perhaps as a persistent genomic parasite or because of some conserved advantageous function or the ease with which such structures adopt new functions.

We have isolated several cDNA clones (e.g. pKWT17, see above) that recognize a moderately repetitive element in the mouse that is polymorphic among and within different inbred mouse strains. From reconstruction experiments and screening of genomic libraries (Mary Harper, see below), we estimate that between 50 and 100 copies of the element reside within the mouse genome. Restriction analysis of total genomic DNA from nine inbred lines indicates that the repeat length of greater than 16kb is highly conserved among the multiple copies of this element. Upon digestion of genomic DNA with any of the

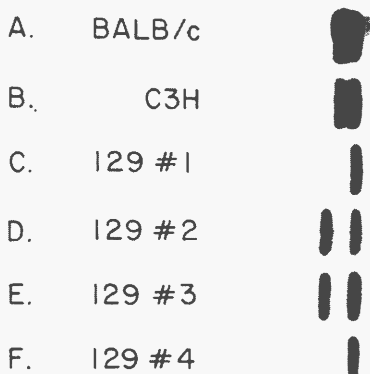

Figure 3
Comparative analysis of mouse genomic DNA with the pKWT17 probe. DNA prepared from six different mice was restricted with *Eco*RI and electrophoresed through a 0.7/ agarose gel. The 9 kb–20 kb region of the blot is shown. Polymorphism within the 129/Sv strain is clearly observed among the four 129/Sv patterns shown.

six cutter endonucleases used, the pKWT17 probe detects a simple restriction pattern of one or two major bands (see Fig. 3). In contrast, much more complex restriction patterns, containing many fragments, are obtained when the mouse genome is probed with other repeated sequences such as Alu, type-A intracisternal particle, mouse mammary tumor virus, and murine leukemia virus sequences. Therefore, we have identified a novel element with a longer repeat length than any previously described mammalian moderately repetitive sequence. Analysis of human placental DNA with pKWT17 demonstrates the existence of a homologous moderately repetitive sequence in the human genome.

While the restriction pattern produced by nine of thirteen enzymes tested is identical among inbred strains, a polymorphic segment of the element is revealed by *Eco*RI, *Pvu*II, *Bcl*I, *Bgl*II digestion. Most striking among these strain polymorphisms that are currently undergoing extensive analysis is the unstable or nomadic arrangement of this sequence within the highly inbred 129/Sv strain. Despite sharing identical 2-D gel patterns (as determined in collaboration with Jim Garrels, Quest-2D Gel Laboratory), individual members of the 129/Sv line can have one of two distinct restriction patterns with these four endonucleases. Furthermore, new restriction patterns may occur within a single generation. Thus far none of the other inbred lines tested demonstrate multiple MRE arrangements, but analyses of many more animals are necessary before a conclusion can be drawn. The mechanism of rearrangement in 129/Sv is unknown, but transposition through a circular intermediate and illegitimate recombination are under consideration. Analysis of the DNA sequence and the mapping of genomic clones currently underway are required before any of these mechanisms can be tested.

Is it of interest to know the chromosomal localization of the MRE: Is it randomly dispersed over 50 to 100 sites or clustered in only a few locations? We are currently collaborating with D. Wolgemuth (Columbia University) to determine the distribution of the MRE by using in situ hybridization of genomic clones to mouse chromosomes. By combining classical breeding analysis with molecular approaches, we will attempt to define the DNA structure, disposition within the genome, and unorthodox inheritance of this mammalian MRE.

Transcription Products of a Mouse Moderately Repetitive Element

J. Danska

The nomadic gene families *copia* and *412* were first isolated because of their homology to extremely abundant poly(A)$^+$ RNA in *Drosophila* tissue-culture lines. Several hundred slightly less abundant poly(A)$^+$ RNA species, collectively composing 60% of the total cytoplasmic poly(A)$^+$ RNA, are known to be homologous to several other such gene families. These transcripts have been characterized and are often found associated with ribosomes, but they appear to be nontranslatable in cell-free systems. The function of these copious transcripts remains undefined.

Screening of the 30 cDNA clones from which pKWT17 was selected for further study demonstrated that the RNA transcripts homologous to the MRE were more frequently represented in a fraction of the poly(A)$^+$ RNA population than were any translatable mRNAs. It was of interest to characterize the tissue and species distribution of the transcripts detected by pKWT17. Dot blot hybridizations of pKWT17 to equal amounts of testis, liver, brain, heart, skeletal muscle, and spleen poly(A)$^+$-selected mouse RNA were performed. The resulting equivalent signal intensity indicated the widespread presence of MRE homologous transcripts in the mouse. RNA from rat and guinea pig testis and liver as well as human placenta and HeLa RNA also hybridized to the probe, but yeast RNA did not. To determine the size and conservation of the transcripts, Northern blots were performed using poly(A)$^+$ and poly(A)$^-$ RNAs from the cell types described above. A primary transcript of about 1850 nucleotides was common to the poly(A)$^+$ RNA from all cell types and species tested. No signal was detectable from poly(A)$^-$ RNA under identical conditions. Additional, tissue-specific, higher-molecular-weight transcripts that bear homology to the MRE were observed in mouse myeloma and human HeLa and placenta poly(A)$^+$ RNA. We estimate that these transcripts comprise 1–2% of the total poly(A)$^+$ RNA and suspect that they are RNA polymerase II transcription products.

Berk-Sharp nuclease-S1 analysis of the transcripts using recently isolated genomic clones (see section below) is in progress to determine the size and intron-exon structure of the gene. Production of a full-length cDNA clone and subsequent sequence analysis will also contribute to a broader understanding of how these genes are controlled and expressed.

Genomic Organization of a Murine Middle Repetitive Element

M.I. Harper, L.M. Silver

We have started to characterize the MRE described above by studying mouse genomic clones derived from two sources. Total DNA from a 129/SvJ strain mouse (Fig. 3D) was digested to completion with *Eco*RI and size fractionated by sucrose gradient centrifugation. The fractions containing the 13-kb and 16-kb hybridizing bands were pooled and cloned into λ Charon 4a arms. The partial library obtained was hybridized against pKWT17, and a number of positive clones have been identified. However, even after plaque purification, the DNA from each clone contains more than one *Eco*RI band hybridizing to the cDNA clone pKWT17. This suggests that the R1 mouse inserts contain some direct repeat(s) that allow recombination during virus propagation. Nevertheless, the DNA of one such clone (λLS15) only has a small ratio of a "secondary" R1 insert and it has been serving as a useful probe because its insert size (13,000 bp) makes it more sensitive than pKWT17 (330 bp). λLS15 and the cDNA clone pKWT17 give an almost identical pattern of hybridization to R1-digested total mouse DNA (129/SvJ), except that λLS15 recognizes an additional repetitive band of 6 kb. The reason for this is being investigated.

We have also probed a Balb/c mouse genomic library (kindly provided by M. Davis, NIH) with the cDNA clone pKWT17. Approximately 1×10^6 phage were initially plated out, of which 430 proved to be positive. This gives an estimate of the copy number as 50 to 60 per haploid genome. Plaque purification and characterization of the mouse genomic inserts is underway.

Our aim is now to find clones that extend in either direction from the point of hybridization with pKWT17 in order to find the length of the MRE and the nature of the variable region(s). The ends will be identified by isolating restriction fragments from the various clones and using these fragments as probes against genomic blots until the pattern produced shows hybridization to multiple flanking sequences. The ends will then be subcloned into pBR322 and sequenced. It will be of interest to find out whether this murine MRE is flanked by direct repeats similar to those that occur with transposible elements of lower eucaryotes and procaryotes.

Cloning of DNA Encoding p63/6.9

N. Sarvetnick, G.P. Thomas

We intend to clone the DNA fragment encoding p63/6.9 that has biochemical properties of an extracellular matrix protein (see above). It is believed that these proteins play a role in cell-cell recognition during development. Genetic evidence implies that p63/6.9 might play a role specifically in sperm differentiation. We have prepared a complete cDNA library from isolated testis mRNA. Total testis RNA was passed over an oligo(dT) column to obtain a polyadenylated fraction that was used for the synthesis of cDNA. To maximize the efficiency of cloning, *Eco*RI and *Sal* linkers were sequentially ligated to each end of the cDNA molecules. The utility of this cloning technique, devised by David Kurtz and John Fiddes, is that a large cDNA library can be made and the map orientation with respect to each end of the mRNA will be known. We will screen for potential *Tcp-1* clones by making use of several previously established facts. First, p63/6.9 has an abundance that is at least 20 times higher in the testis than in the liver. Second, previous cloning experiments (described above) have produced a cDNA clone pKWT17 that represents an abundant RNA species in the same size fraction as the p63/6.9 message. Testis poly(A)$^+$ RNA will be size-fractionated on a sucrose gradient, and cell-free translation will be used to identify a fraction enriched for p63/6.9 message. A comparative screening of the library between this fraction of testis RNA and an analogous fraction of liver RNA should identify a family of clones. To maximize this differential screening, we have used liver RNA obtained from a $+/T^{hp}$ mouse. The T^{hp} form of chromosome 17 has been demonstrated both genetically and biochemically to possess a deletion that includes *Tcp-1*. pKWT17 and actin will be used as further negative screens. Potential *Tcp-1* clones can be identified by their ability to select message that can be translated into p63/6.9. Selected mRNA, obtained by hybridization to each cDNA clone, will be assayed in a cell-free translation system and the resulting proteins analyzed by SDS gel electrophoresis. Clones that select mRNA with translation products in the molecular weight range of p63/6.9 will be subjected to further analysis by 2-D gel electrophoresis. Upon identification of *Tcp-1* cDNA clones, we will be able to analyze the genomic organization of the *t* complex. We will use the cDNA clone to select genomic clones from *t*-homozygous libraries (see above) and compare their structure with corresponding wild-type genomic sequences. Subsequent analysis of *Tcp-1* gene expression during spermatogenesis and embryonic development may provide information on the mechanism by which *t* haplotypes affect these processes.

Genomic Analysis of the H-2 Complex Region Associated with Mutant *t* Haplotypes

L. Silver, S. Hughes, J. Uman

The H-2 complex is defined by a cluster of loci (within the *t* complex) that codes for cell surface antigens involved in the immune response. The most remarkable feature of the *H-2* loci is their extreme polymorphism among wild mice, with as many as 50 to 200 different alleles possible at each locus. Any one particular combination of alleles on a single chromosome defines a unique *H-2* haplotype. Surveys of large numbers of animals from around the world have demonstrated that virtually all wild mice are heterozygous at one or more *H-2* loci and that the *H-2* haplotypes carried by almost all wild (non-*t*-carrying) mice are unique. J. Klein (Max-Planck-Institut, Tubingen) and H. McDevitt (Stanford University) have discovered that in direct contrast to this situation is the restricted polymorphism in *H-2* haplotypes associated with all wild *t* haplotypes. To investigate possible relationships between the different *t*-defined *H-2* haplotypes, we have used an H-2 cDNA clone (obtained from Lee Hood, California Institute of Technology) to probe restriction digests of total genomic DNA from mice with different *t* haplotypes on the inbred 129/Sv background. Fifteen *t*-specific, *H-2*-defined *Eco*RI fragments were identified within the context of the 129/Sv background pattern. The surprising finding of this study is the identity or near-identity in *H-2*-defined restriction patterns from all complete *t* haplotypes analyzed. The available data lead to two major conclusions. First, it is clear that all *t* haplotypes were derived from a small number of closely related ancestors. Second, the H-2 complex region associated with each primordial *t* chromosome has been maintained within at least the nine different present-day *t* haplotypes analyzed so far. Hence, the *H-2* complex is an integral component of naturally-occurring *t* haplotypes.

A recent report from the laboratory of K. Artzt (Sloan-Kettering Institute) indicates that the lethal genes of several *t* haplotypes are located in the vicinity of the *H-2* complex. This suggests the possibility of using available H-2 clones as a means toward the isolation of the lethal genes. We are in the process of constructing genomic libraries of DNA from several sources including total liver DNA from a compound t^{0}/t^{w12} mouse. The libraries will be constructed by partial digestion of mouse DNA with the enzyme *Sau*3A and ligation of an appropriately large size fraction to arms of the bacteriophage vector L47.

Identification and Mapping of DNA and Protein Markers for the *albino (C)-pink eyed dilution (P)* Region of Mouse Chromosome 7

N. Sarvetnick, D. Lukralle, G. Blose, L. Silver

Albino (C) and *pink eyed dilution (P)* are linked loci on mouse chromosome 7 that were among the first used for classical studies of the mouse genome during the early part of this century. A large number of both spontaneous and induced mutations have been identified at each of these loci. Different recessive mutations have been recognized by their distinctive effects on the coat color and eye color of homozygous animals. Studies with radiation-induced deletions at each of these loci have demonstrated the existence of a family of genes within the *albino-pink eyed dilution (CP)* region that have dramatic yet specific effects on development, tissue differentiation, and fertility (see Gluecksohn-Waelsch, *Cell 16:* 225 [1979] for a complete review of the *albino* region). The nonpigmented 129/SvJ strain of mice is highly inbred, with a selection for alternate recessive alleles at the *C* locus and a recessive mutation at the *P* locus for a final genotype of $c^{ch}p/cp$.

A pigmented congenic 129 strain has been constructed at the Jackson Laboratory by repeated backcrossing of a segment of chromosome 7 from the C3H strain, which carries wild-type alleles at both the *C* and *P* loci, onto the 129/SvJ background. These congenic lines were analyzed for protein polymorphisms by 2-D gel electrophoresis. A single prominent protein difference was expressed in all tissue types studied. Further breeding analysis indicates the mapping of the gene (tentatively called *Cpr-1*) specifying this protein to the region encompassed by the *C* and *P* loci. The 129/SvJ-associated allele expresses an acidic form, and the C3H-associated allele expresses a basic form, of a 75-kD protein with an isoelectric point of 6.7 (see Fig. 2).

All mammals carry endogenous proviral sequences homologous to different retroviral genomes. In recent years, it has become clear that although the location of these proviruses within the mouse genome is highly polymorphic, they are inherited in stable Mendelian manner over scores of generations (Cohen and Varmus, *Nature 278:* 418 [1979]). In an attempt to map particular proviruses to the *CP* region, total genomic DNA was prepared from the congenic 129 lines described above and analyzed for restriction site polymorphisms with a cloned mouse mammary tumor virus (MMTV) DNA probe. The results of these experiments have allowed the identification of a specific MMTV provirus (called MMTV-V by Cohen and Varmus) that is stably integrated within the *albino-pink eyed dilution* region of the C3H genome.

To determine the relative map positions of both *MMTV-V* and *CPR-1*, the following experiment is in progress. Homozygous $c^{ch}p/c^{ch}p$ mice of the 129/SvJ strain have been mated to C3H mice which have a + +/+ + genotype in the region of interest. The F1 hybrid offspring ($c^{ch}p/+ +$) have been crossed to homozygous *cp/cp* mice of the 129/SvJ strain. By observing coat and eye colors of the offspring obtained from this second-generation cross, it is possible to identify mice carrying recombinant chromosomes obtained from the F1 parent. Nonrecombinant animals will

have a dark coat with black eyes ($++/cp$) or a light chinchilla coat with pink eyes ($c^{ch}p/cp$). Recombinant animals will have a dark coat with pink eyes ($+p/cp$) or a light chinchilla coat with black eyes ($c^{ch}+/cp$). Since *C* and *P* are separated by 14 cm we expect one out of every seven second-generation offspring to carry a recombinant chromosome. All such animals will be used for (1) protein analysis by 2-D gel electrophoresis to determine alleles at *CPR-1* and (2) DNA analysis by Southern blot hybridization with an MMTV-V probe to determine the presence or absence of the MMTV-V provirus. The results obtained from this four-point cross experiment will allow a determination of the relative map positions of four closely linked genetic loci, two of which are defined in gross morphological terms and two of which are defined in molecular terms. Further studies with radiation-induced deletions might provide tools for the molecular analysis of certain developmental genes identified within this interesting region of the mouse genome.

Isolation of a Mouse Actin Gene

M.I. Harper, K.R. Willison

The study of early mammalian development at the molecular level is only just beginning, one great handicap being the limited amount of material available. The mouse egg and preimplantation embryo are only 80–100 μm in diameter and contain approximately 25 ng of protein, 0.5 ng RNA, and from 25 pg of DNA at the one-cell stage to 500 pg at the blastocyst stage. We wish to study the expression of the early embryonic mouse genome by utilizing specific genetic probes, and it is therefore a technical requirement that we investigate genes that are transcribed at high levels during these stages. The ubiquitous structural protein actin appears an ideal candidate for such studies since it is approximately 6% of the total protein synthesized during preimplantation stages. In mammals, a family of actin genes codes for different forms of the protein in a tissue-specific manner. There are at least four α actins that are present in different muscle tissues and two cytoplasmic actins, β and γ. All three forms are synthesized in the preimplantation mouse embryo, with β actin predominating. Problems to be addressed are (1) whether all three forms are synthesized from the embryonic genome during cleavage stages or whether translation of specific forms occurs on inherited maternal message, and (2) what differentiation step first shows tissue specificity in the actin genes being transcribed by the embryo.

We have now isolated and partially characterized a genomic clone of mouse actin. The clone was first isolated from a mouse genomic λ library (kindly supplied by M. Davis, NIH) and was recognized by its hybridization to a chick full-length β-actin cDNA clone (kindly provided by D.W. Cleveland, University of California, San Francisco). Hybridization was to a 10.5-kb *Eco*RI fragment of the clone, and this fragment was subsequently subcloned into the plasmid pBR322. We are now studying two plasmid clones, pRT1 and pTR3, in which the mouse DNA is inserted in opposite orientations. A restriction map of the insert has been constructed using the chick β cDNA clone to probe Southern blots.

The pRT3 clone was used to select message from poly(A)$^+$ RNA isolated from mouse testes that was subsequently translated in vitro in the presence of [^{35}S]methionine. The translation products were separated by 2-D electrophoresis (in collaboration with Jim Garrels, Quest 2-D Gel Laboratory). Only four protein spots were visible, which correspond to the acetylated and nonacetylated forms of β and γ actin.

We now aim to find the direction of transcription of the mouse actin gene and the position of the promoter (assuming the clone does not contain a pseudogene). This is being done by (1) electron microscopic visualization of R loops between the actin clone and poly(A)$^+$ RNA and (2) comparison of hybridization to 3′-primed and randomly primed cDNAs from poly(A)$^+$ RNA. A cDNA library has previously been constructed from mouse testes poly(A)$^+$ RNA (by KRW, see above). As both β and γ actins are synthesized by testes, we plan to isolate the relevant cDNA clone for comparison with our genomic one. Using the 3′ untranslated region of the cDNA clone for RNA selection, followed by in vitro translation, we hope to identify the genomic clone pRT1/3 as either the β- or γ-actin gene. We shall then be able to probe the early embryo for specific transcripts.

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MOLECULAR GENETICS

D. Zipser, J. Lewis, L. Rodgers, L.Smith

The Bifunctional Promoter of HSV-1 Thymidine Kinase

D. Zipser, L. Rodgers, L. Smith

A large number of mutants have been generated in the 5′ flanking control region of cloned herpes virus *tk*. Both deletion mutations and restriction enzyme linker insertion mutations have been made. In addition to analyzing these physically to locate the site of mutation, we have also used DNA plasmid carrying these mutations to transform LTK$^-$ cells to a TK$^+$ phenotype. As a result of analyzing a large number of transformants, we have come to the following general conclusion: The promoter region of the herpes virus *tk* gene is bifunctional. One region of the promoter is used by animal cells to express the herpes virus *tk* gene in the absence of other herpes virus functions. The second region of the promoter is used during herpes virus infection to express herpes virus *tk* under the conditions in which cellular messages and proteins are no longer made. Expression from this part of the promoter requires herpes-coded functions. These two regions are distinct, and we have obtained mutations that expunge each of the functions without affecting the other.

We have transferred these mutations from cloned herpes viruses *tk* genes back to herpes virus to test their effect on the expression of the *tk* gene in lytic infection. There is only an initial burst of *tk* synthesis after infection with an HSV-1 with a mutation in its "herpes virus-specific" promoter region. This is expected since after shut off of cell function, this part of the promoter cannot function. There is a delayed turn on of *tk* synthesis after infection with virus with mutations in the "cell-specific" part of the promoter. This indicates that the herpes-virus-specific promoter sequences do not function until the cell functions are shut off.

Another project is an attempt to identify the herpes virus proteins that specifically interact with the *tk* promoter region. Since we are in possession of mutants that alter this region in a variety of ways, we have strong controls for the specificity of DNA interaction of either specified herpes virus proteins or cellular proteins. We plan to use the technique developed previously in this lab to identify the specific DNA interaction of the repressor protein of phage Mu.

In addition to the above projects we are also following up some minor observations obtained in the course of generating mutations in the herpes virus *tk* gene. In particular, we found that mutations in the 5′ end of the structural protein region did not completely obliterate *tk* expression. Our preliminary analysis of this finding indicates that there are two starts for herpes virus *tk* protein separated by about 140 bp. Both of these seem to make functional *tk* proteins. When a mutation disrupts the first protein start, the second one appears to suffice. To confirm this initial hypothesis, we are constructing plasmids that will express the *tk* gene from the *lac* promoter using each of the two different proposed starts. Using these plasmids in bacteria, we should be able to determine whether both starts make active enzyme.

Regulation and Cloning of Cellular Thymidine Kinase

J. Lewis

In the early 1950s autoradiographic analysis of eucaryotic cells maintained in culture revealed that cellular DNA synthesis was initiated several hours after cell division and completed several hours before the subsequent division. The recognition that events of DNA synthesis occupied only a minor fraction of the cell division time contributed to the development of the concept of a cell division cycle. The pre-and post-DNA-synthesis periods were proposed to be essential for the preparations of DNA synthesis and cell division and presumably were required for the activation and repression of gene programs essential for these dramatic cell routines. With the development of methods for the induction of synchronous cell division in in vitro eucaryotic cell cultures, analysis of protein concentrations and enzyme-specific activities were made feasible. Such studies confirmed suspiciouns that numerous gene products were active in a cyclical fashion. Not surprisingly, it was demonstrated that the specific activities of many enzymes central to the biosynthesis and metabolism of DNA nucleotide precursors and enzymes important to DNA synthesis were maximal during the S (synthetic) phase of the cell cycle.

The enzyme thymidine kinase, which contributes to the metabolism of thymidylate, shows just such an S-phase-specific activity maximum. The specific activity, readily assayed, is regulated at the nucleic acid level; the thymidine kinase enzyme is not apparently cyclically modified resulting in alterations of protein stability or function. The cyclic expression of the *tk* gene locus presents, therefore, an interesting contrast to gene regulation at other loci where expression is confined to terminally differentiated cells, for example, globins. We are interested in analyzing these controls at the nucleotide sequence level.

Using in vitro cell cultures synchronized by serum starvation and refeeding protocols, we have confirmed the cell cycle dependence of *tk* activity in TK$^+$ mouse L cells. Furthermore, we have demonstrated that *tk* specific activity is cell-

cycle-dependent as well as LTK$^-$ cells transformed to TK$^+$ by the introduction of the chicken *tk* gene cloned by Mike Wigler et al. (Mammalian Cell Genetics) and in LTK$^-$ transformed to LTK$^+$ by high-molecular-weight DNA from Chinese hamster cells. We interpret these results to suggest that genetic elements governing cell cycle expression of the *tk* gene have been introduced into the TK$^-$ cell along with the *tk* structural gene sequences. In contrast, we have observed that the *tk* specific activity in LTK$^-$ cells transformed to TK$^+$ by transfection with the *tk* gene of HSV is largely invariant throughout the cell cycle. In an attempt to establish a homologous genetic system in which a cloned mammalian *tk* gene can be clonally isolated, mutagenized in vitro with recombinant DNA techniques, and reintroduced into homologous LTK$^-$ cells for the study of cell cycle regulation, we have undertaken to isolate the Chinese hamster *tk* gene.

We have analyzed the restriction enzyme sensitivity profile of the hamster *tk* gene and identified *Bam*HI and *Bgl*II as enzymes that leave the *tk* activity of high-molecular-weight DNA intact. Enzyme-cleaved DNAs enriched for *tk* gene activity by gradient centrifugation have been ligated to cloned tRNA suppressor genes and applied as a calcium phosphate precipitate to TK$^-$ cell populations to derive TK$^+$ mouse cell clones.

DNAs from multiple secondary transformants derived from independent primary transformants have been analyzed by Southern blot hybridization for the presence of tRNA suppressor gene sequences. The presence of conserved tRNA suppressor bands common to many or all secondary 2^0 from a given primary argue for the location of tRNA suppressor gene sequences proximal to the transfected Chinese hamster *tk* gene.

Genomic libraries of DNA from six secondary transformants derived from six independent primary transformants have been prepared with λ phage 1059 carrying an amber mutation introduced by Kenji Shimizu (Mammalian Cell Genetics). These libraries have been screened for the presence of tRNA gene sequences by growth on a Sup$^-$ bacterial host. A fraction of these recombinant phage that grow on such hosts might be expected to have incorporated hamster *tk* gene sequences in the recombinant insert. The DNA of twelve recombinant phage isolates containing the tRNA suppressor gene are currently being analyzed for their ability to generate TK$^+$ cell transformants by transfection assay.

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CELL BIOLOGY

In 1981 the Cell Biology group expanded its efforts into several areas that may seem peripheral to its main topic, cytoplasmic organization. These areas include "water in the cytoplasmic matrix," "shock reaction," and "Golgi apparatus." In the detailed reports that follow, some of the reasons will be explained that suggest a possibly quite central role these areas may play in a deeper understanding of the dynamic behavior of the cytoplasm. Along the main line of our interests, there were new findings about intermediate filaments and vimentin-related proteins, as well as the raising of several monoclonal antibodies against structural proteins. Many new stimuli came through this year's Symposium, which focussed on the area of our main interests, namely "The Organization of the Cytoplasm".

We miss Keith Burridge, a friend and esteemed colleague of many years who left us in spring to join the faculty of the Department of Anatomy at the University of North Carolina at Chapel Hill. Two post-doctoral fellows, William J. Welch from the University of California at San Diego, and David Helfman from the Emory University at Atlanta, joined the group to work on Cell Biochemistry. We were glad to welcome Nancy Hogg from the ICRF Human Tumor Immunology Group, University College, London, as a guest in our group, while she continued the studies of her finding of a peculiar fiber formation around certain human monocytes. The group's photographer, Ted Lukralle, went into private business after many years of most valuable help for us. His successor is Philip Renna.

Detailed reports are given below.

CELL MOTILITY

G. Albrecht-Buehler, S. Blose, A. Bushnell, A. Calasso, N. Hogg, T. Lukralle, D. Meltzer, P. Renna, M. Schwartz, M. Szadkowski

Water Movement in the Cytoplasm: Does Blebbing Reveal the Convulsive Flow of Liquid and Solutes through the Cytoplasmic Meshwork?

G. Albrecht-Buehler

Blebs, like lamellipodia and filopodia, belong to the repertoire of surface projections that are found on the surfaces of most animal cells. They are hyaline, hemispherical extensions that expand from the cell surface within 3 to 5 sec. Their expansion stops rather abruptly at a diameter of 2–5 μm, which is followed by a phase of slow shrinking back into the cell surface (see Fig. 1, bleb 3).

The biological functions of belbs are not known, although one function may be the storage of surface materials. As to the mechanism of their formation, it has been suggested that intracellular hydrostatic pressure causes their expansion at weakened spots of the cell surface. Although the details of morphology and movement of blebs support quite strongly the suggestion that hydrostatic pressure is involved in their formation, two fundamentally different mechanisms remain to be distinguished: Either the volume of a newly forming bleb rushes in from another part of the cell (i.e., cell volume is constant during bleb expansion) or it is supplied by liquid pumped in from the outside (i.e., cell volume increases during bleb formation). If the first case is true, the blebbing all over a cell surface indicates a rapid "circulation" of liquid throughout the cytoplasm. If the second case is true, the blebbing indicates a "respiratory" action of the cell by which it rapidly takes in large volumes of liquid from the outside, and then, of course, "exhales" the liquid or else it would eventually burst. Either possibility seems important for our understanding of the transport of messages, metabolites, ions, etc. through the tightly organized cytoplasm of animal cells.

A whole cell is too large to allow detection of any volume changes concommitant with the formation of a new bleb. Therefore, we used our recently found microplasts for the experiments. Blebbing microplasts are small enough to allow detection of volume changes upon bleb expan-

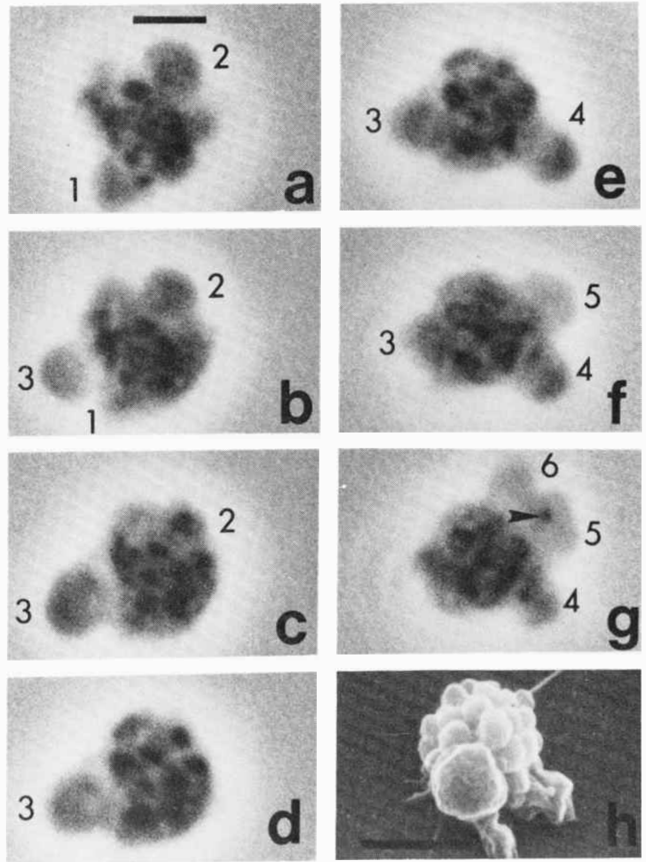


Figure 1
Blebbing microplast from a Swiss/3T3 cell. (Bars indicate 2 μm.) (*a–g*) Time sequence (interval 10/sec) showing that new blebs (e.g., 3) form at the expense of existing ones (e.g., 1 and 2). (*h*) Scanning electron micrograph of a blebbing microplast.

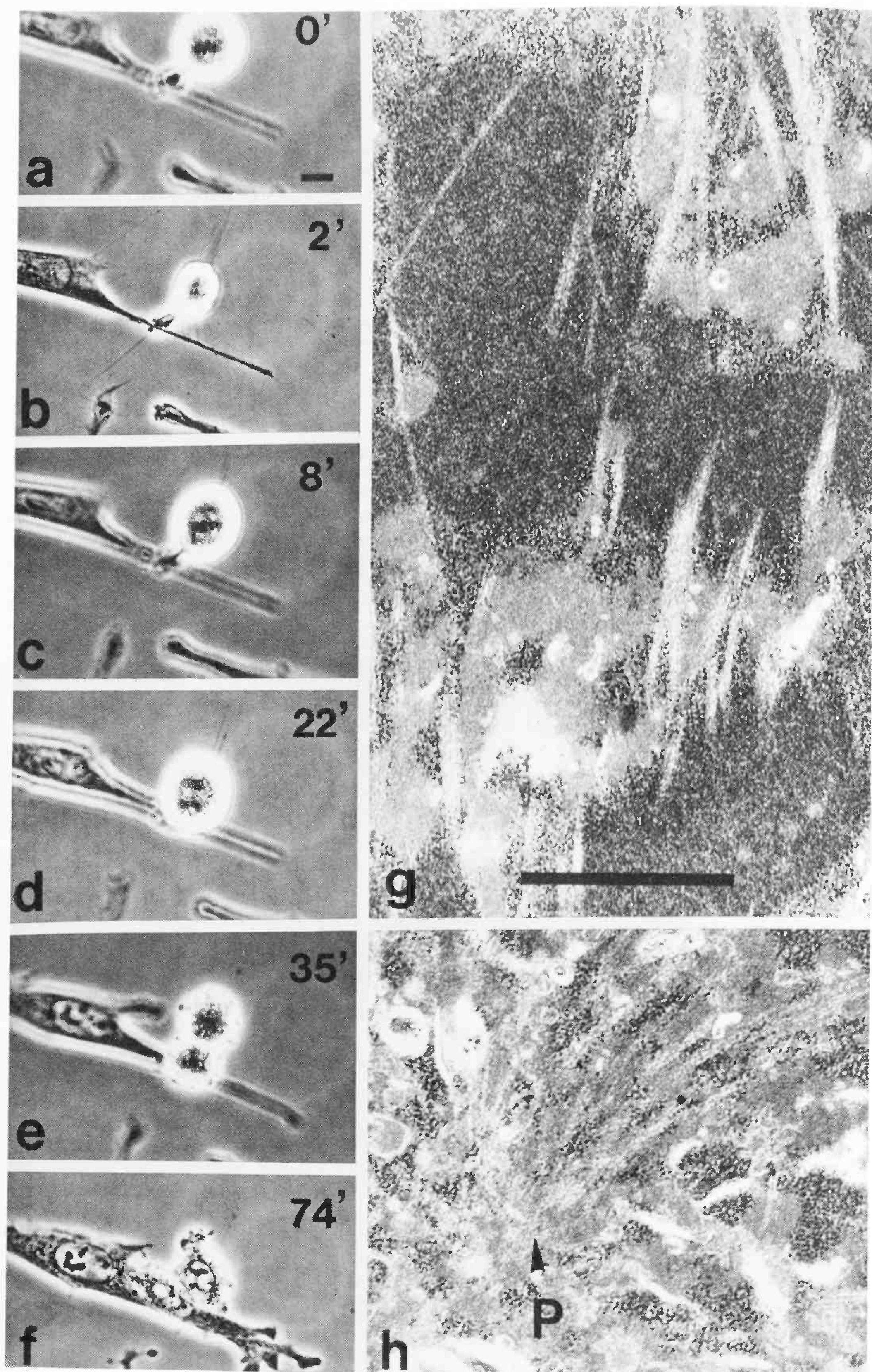


Figure 2

sion. Indeed, we found that for every newly forming bleb, the other existing blebs shrunk by the same volume (Fig. 1). Studying the rapid gyrations of particles inside blebs, we found that the blebs are filled with a liquid of a viscosity between that of water and serum.

The data suggest that the cell volume is constant during blebbing. In other words, the cytoplasm is able to push liquid through its meshwork in convulsive ways and in changing directions as blebs form on varying sites of the cell surface. It is intriguing to speculate that the well-known saltatory movement of cytoplasmic organelles in non-blebbing, flat-ended cells may be caused by the same convulsive mechanism, if it meets a higher flow resistance than rounded blebbing cells may offer.

Reversible Compression of Cytoplasm

One possible mechanism of the convulsive flow of liquid through the cytoplasm is a rapid, local compression inside the cytoplasm. Conceivably, the "free" water is pushed out of these areas and flows toward the cell surface. In this case, the cytoplasm must be able to undergo rapid and reversible local compression. It would be most interesting to study condensed cytoplasm, provided it can reversibly enter such states.

There have been earlier reports about the survival of cultured cells after the exposure to hyperosmolar media, such as media containing up to 1 M sorbitol. Sorbitol is a poorly metabolized glucose analog. Since such media can be expected to partially dehydrate, and thus condense, cytoplasm, we examined the motile behavior and ultrastructure of such treated cells.

We tested human fibroblasts, BHK cells, 3T3 cells, neuroblastoma cells, and PtK1 cells and found an astonishing reversibility of the hypertonic treatment. More than 60% of human fibroblasts survived 60 min in 2 M sorbitol. We chose a standard incubation of 3 min in medium containing 1 M sorbitol. The following observations refer to this standard incubation and to one or several of the above-mentioned cell types.

In less than 1 min the cells lost 50% to 75% of their water content. Upon return to normal medium, their ultrastructure and morphology returned to normal within 3 min. Mitoses continued normally (Fig. 2a–f). Migration patterns and growth curves also returned to normal. An intriguing aspect of irreversibility was observed when blebbing microplasts switched to ruffling. They would have continued to bleb without the prior episode of dehydration in sorbitol.

During incubation with 1 M sorbitol, the shrunken cells appear to store excess surface area in numerous, rapidly forming microvilli. The cytoplasm is also altered. Bundles of intermediate filaments and microfilaments condense into homogeneous streaks of materials that retain birefringence. In contrast, microtubules display a clearly visible exclusion zone around them (Fig. 2 g,h). Sharply outlined patches of tiny granules appear in the electron micrographs, as if ground substance had condensed into unit packages (Fig. 2 g,h).

The ultrastructural data rest exclusively on transmission electron microscopy and, therefore, must be treated with caution, until an independent method of verification is found. Taking the data at face value, however, it appears that the cytoplasm is able to condense reversibly down to one quarter of its former volume at a moments notice. In view of the various interrupted functions of the cytoplasm at the moment of partial dehydration, this seems a quite remarkable quality of the cellular control systems.

The appearance of many outlined domains in the condensed cytoplasm opens the possibility that cytoplasm is, in fact, a mosaic of interlaced domains of various shapes. This concept may allow us a new understanding of the divisibility of cytoplasm and the regeneration and replication of cytoplasm as well as of its visco-elasticity.

Human Monocytes Have a Direct Role in Coagulation

N. Hogg

Human monocytes belong to a class of cells that originate in bone marrow, circulate in blood, and then, under stimuli about which little is known, migrate into tissues where they are termed macrophages. These cells are collectively termed mononuclear phagocytes. They are best known as phagocytic or scavenger cells, arriving at sites of inflammation and tissue damage to clear the debris. They also play a crucial role early in the immune response in processing foreign material, presenting it in a suitable form to T lymphocytes. Macrophages are found in many locations in the body; the microglia of the brain, kupffer cells in the liver, and alveolar cells in the lungs are several unequivocal examples.

Figure 2
Response of mitotic BHK cells to incubation in 1 M sorbitol. (Bars indicate 10 μm.) (*A–F*) Reversibility of sorbitol treatment. The metaphase cell in *A* was incubated in 1 M sorbitol for 3 min (*B*). Note the shrinkage of the cells' outline and the disappearance of nucleoli in the phase-contrast image. After return to normal medium (*C*), the cell proceeded through mitosis normally and the visibility of nucleoli reappeared (*D–F*). (*G*) Ultrastructure of spindle microtubules and chromosomes 3 min after incubation in 1 M sorbitol. Note the while exclusion zones around the microtubules and the granular appearance of the ground substance. (*H*) One of the spindle poles (*P*). Centrioles are not visible in any of the sections, although their visibility returns immediately upon return to normal medium or extraction of the cytoplasm with Triton.

There are hints that monocytes and tissue macrophages may have other functions in the body than those mentioned. I have been studying a process in which macrophages have not been previously implicated. In an effort to define monoclonal antibodies specific for monocytes and macrophages, a monoclonal antibody, named UC45, was isolated which reacted with needlelike projections or fibers that formed on the surfaces of about 20% of monocytes from any particular individual when the cells were adherent to a glass or plastic surface (Fig. 3). In the presence of plasma, these projections formed within 15 sec and double-labeling experiments with UC45 and a rabbit antibody to human fibrinogen suggested the "needles" might be composed of fibrin. This was confirmed when the needles were isolated and biochemically analyzed. The monoclonal antibody reacted with the α chain of the three-chain fibrinogen molecule. The polymerization of fibrinogen to fibrin by monocytes was prevented by UC45, which suggests that plasma is the source of fibrinogen composing the needles and that the UC45 antigen may be crucial for polymerization.

I am now attempting to establish how monocytes can accomplish this coagulation. Studies with coagulation-factor-deficient plasmas suggest that monocytes bear a prothrombinase on their membranes. If this is so, they then have the capability of short-circuiting the usual routes of coagulation, which consist of about a dozen factors in the intrinsic pathway and about a half dozen in the extrinsic pathway. The ability of macrophages to coagulate fibrinogen suggests that they may be hitherto unrecognized participants in processes such as wound healing, they may respond to inflammation in a manner not previously appreciated, and the involvement of macrophages in the coagulation associated with metastasizing tumors would be worth investigating.

The monoclonal antibody UC45, which, as stated above, has specificity for the α chain of fibrin, a molecule of 66 kD, also reacts with the mitochondria of all cell lines tested. UC45 appears not to react with internalized α chain but with another molecule, the biochemical characteristics of which have been difficult to ascertain, as it seems to be easily degraded. A major reactive entity is 200 kD. The reaction of UC45 with the α chain of fibrinogen and the mitochondrial protein may be another example of the cross-reactivity between different proteins which is being observed with increasing frequency as monoclonal antibodies are being more widely tested.

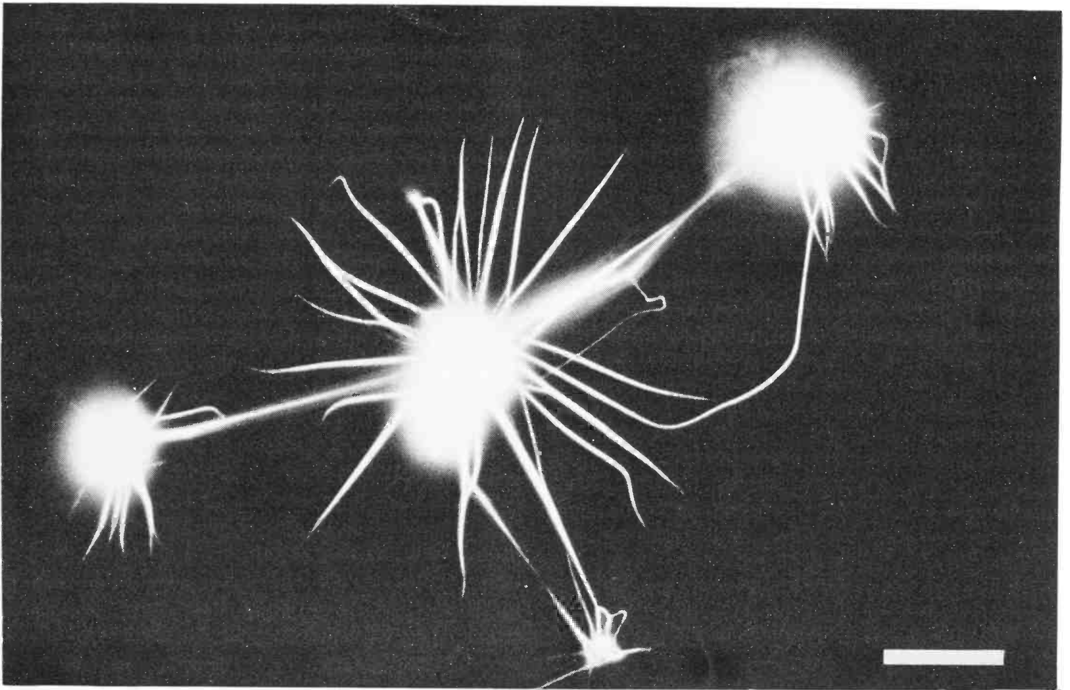


Figure 3
Fluorescent micrograph of a human monocyte bearing needles of fibrin which are labeled with the monoclonal antibody UC45. (Bar indicates 10 μm.)

Immunoelectron Microscopy of 10-nm Filaments and Microfilaments Decorated with a Monoclonal Antibody that Recognizes Both Vimentin and Tropomyosin

S. Blose, F. Matsumura, J. Lin

In recent years the molecular structure and function of the cytoskeleton have been under intensive study. Using antibodies prepared against the constituent proteins of the cytoskeleton, immunofluorescence and immune electron microscopy have revealed that many of these proteins are organized into extensive filament arrays found inside cells. The detailed structural organization of vimentin and tropomyosin, constituent proteins of the 10-nm filaments and actin-containing microfilaments, respectively, are currently under study. We have previously shown that a mouse IgM monoclonal antibody, LCK-16, can recognize a common antigenic determinant on tropomyosin and vimentin (Blose et al., *Cold Spring Harbor Symp. Quant. Biol. 46:* 455 [1982]). This antibody has been used to probe the molecular organization of these proteins in the cytoskeleton at the ultrastructural level. Gerbil fibroma cells (IMR-33) were grown in culture dishes and then extracted with 0.2% Triton-X100 in stabilization buffer (0.1 M PIPES-KOH [pH 6.9], 5 mM $MgCl_2$, 1 mM EGTA, 4% polyethelene glycol-6000, and 1.5 mM GTP) at 37°C for 3 min. This procedure leaves the cytoskeleton intact and allows the antibody access to the filaments. The LCK-16 antibody was supplied in stabilization buffer and incubated at 37°C, 100% humidity. After 45 min the antibody was washed off, and the cells were fixed in 2% glutaraldehyde and 2% tannic acid. The cells were then processed by standard methods (Blose and Chacko, *J. Cell Biol. 70:* 459 [1976]), and the thin sections were examined in a Phillips 201E electron microscope. LCK-16 decorated the 10-nm filaments in a spiral fashion (Fig. 4) as observed in thin section. The inclusion of tannic acid in the fixation procedure greatly enhanced the visualization of the antibody decoration by acting primarily as a mordant between the osmium-treated structures and the lead stain. Longitudinal profiles of the 10-nm filaments revealed the antibody to decorate with a mean interval spacing of approximately 31 nm ($n=101$, 30.9 ± 2.4 nm). The antibody-decorated filaments cut in cross-section (section thickness 60–80 nm) demonstrated triangular profiles. The triangular profiles were reinforced by three 120°-angle Markham rotations. The antibody in cross-sectional profiles added on three projections (the points of the triangle) that protruded ~12.2/nm off the 10-nm filament (diameter 9.9 ± 2.1 nm) surface. In control experiments, extracted cells were incubated with a mouse monoclonal IgM antibody to actin, JLA-20 (Lin, *Proc. Natl. Acad. Sci. 78:* 2335 [1981]). The 10-nm filaments were not decorated. Although the IgM molecule is large (~30 nm in diameter with a M of ~900,000) and could impose modest steric hindrance so that all the antigenic sites on the vimentin filaments are not bound, it is interesting that the sites that are available cause the antibody to be bound in a helical fashion with a 31-nm repeat. This repeat appears to be smaller than the 140-nm repeat of the 195K protein of neurofilaments reported by Willard and Simon (*J. Cell Biol. 89:* 198 [1980]). Small and Sobieszek (*J. Cell Sci. 23:* 243 [1977]) have shown a repeat of 31 nm of a major reflection obtained from optical diffraction patterns of negatively stained 10-nm filaments of smooth muscle. A comparison of these spacings cannot be directly made because each study employed different preparations and methods of fixation. Although the significance of the finding of these spacings has yet to be determined, it does imply some fundamental repeat in the 10-nm-filament structure. In cross-section the triangular profiles suggest that three antibody molecules bind per 31-nm period. This seems plausible because each section is ~80–100 nm thick, representing two to three period repeats, which results in the reinforcement of a triangular profile.

Based on the models of IgM binding to large antigens by Feinstein (Feinstein and Beale, in *Immunochemistry: An Advanced Textbook,* p. 263, Wiley, London [1977]; Feinstein, in *The Immune System,* p. 24, Blackwell Scientific Publications, London [1975]), it appears that LCK-16 binds to the 10-nm filament in the "table" or "staple" configuration. That is, each molecule of LCK-16 IgM has its Fab arms folded down from the central $(Fc)_5$ disc of the IgM to contact the 10-nm filament. The Fab arms then become the legs of the table, with the table's top formed by the Fc central disc. In this configuration, the monoclonal IgM is clearly visualized in the electron microscope, and because it is relatively easy to use, monoclonal IgM gives distinct advantages over primary antibody labeling or detection with a second antibody.

When the ventral microfilament bundles of the cells stained with LCK-16 were examined in thin section, the microfilaments appeared more dense than the controls, but no discernable periodicity was observed. Therefore, reconstituted thin filaments (F-actin + tropomyosin) were employed to assay at the ultrastructural level the LCK-16 binding to tropomyosin. In the negatively stained specimens, LCK-16 caused the thin filaments to laterally aggregate into large bundles as compared with the control (Fig. 5). Furthermore, LCK-16 produced a prominent periodicity of ~38 nm ($n=46$, 37.5 ± 1.18 nm) along the thin filaments. In control experiments, JLA-20 (anti-actin) decorated the thin filaments with a fuzzy coat. JLA-20 appeared to cause fragmentation of the thin filaments as compared with the control; however, JLA-20 did not induce the lateral aggregation of filaments observed with LCK-16. We have interpreted the 38-nm period to correspond to the spacing of the tropomyosin molecule on

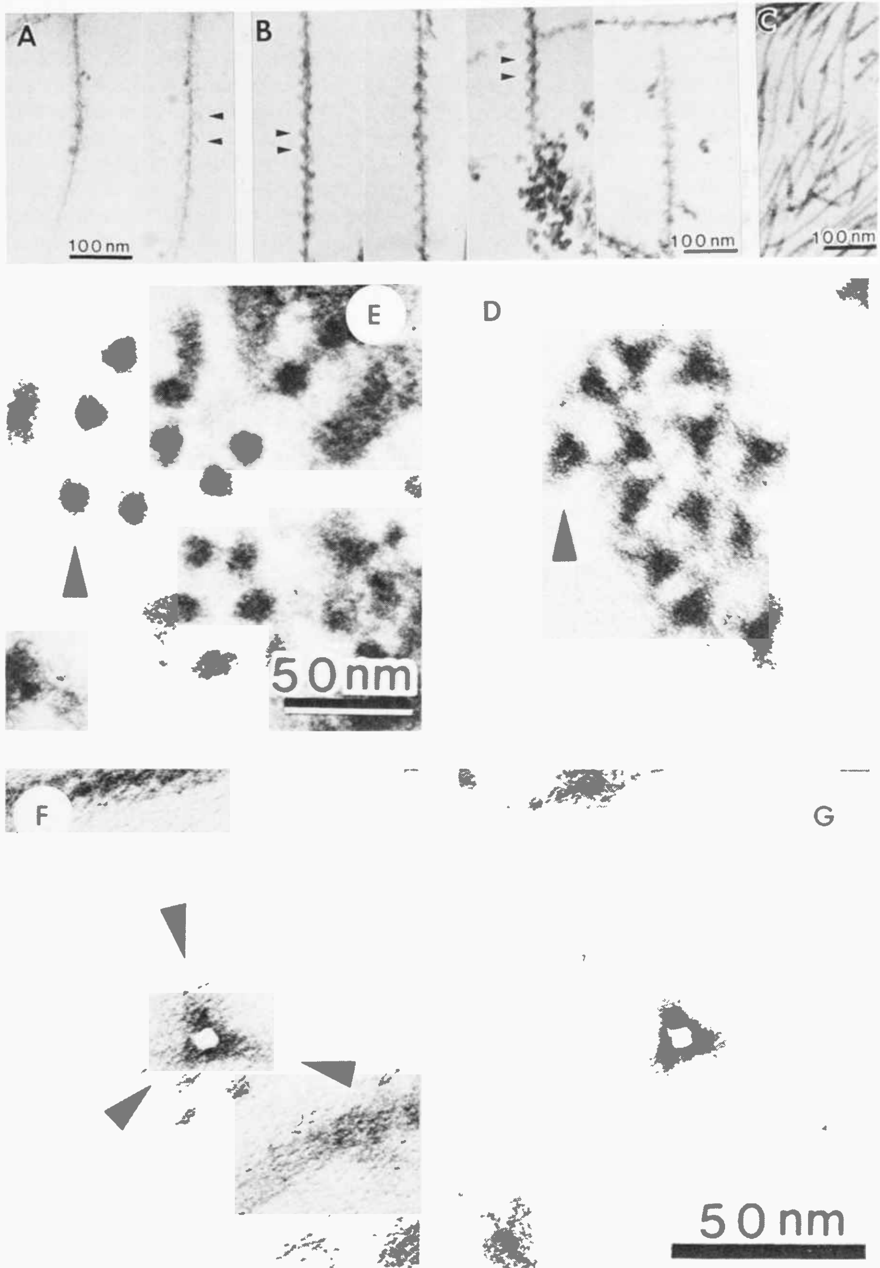


Figure 4

F-actin, on the basis of the model of Ebashi et al. (*Quart. Rev. Biophys. 2:* 351 [1969]).

Several monoclonal antibodies (Lane and Hoeffer, *Nature 288:* 167 [1980]; Dulbecco et al., *Nature 292:* 772 [1981]; Pruss et al., *Cell 27:* 419 [1981]; Klymkowsky, *Nature 291:* 249 [1981]), including LCK-16, have now been described that recognize an antigenic determinant common to different proteins. We have shown that LCK-16 recognizes a common antigenic determinant on tropomyosin and vimentin molecules. This is not surprising since recently Geisler and Weber (*Proc. Natl. Acad. Sci. 78:* 4120 [1981]) have reported a stretch of homologous sequence found in tropomyosin and desmin (a major subunit of intermediate filaments of muscle cells). The sequence they have reported is located near the C terminus (from the 220th to 228th amino acid) of the tropomyosin molecule. By observing the negative staining samples of tropomyosin paracrystals reacted with LCK-16 antibody, we have been able to identify the end of the tropomyosin molecule as the antibody-binding site (F. Matsumura and J. Lin, in prep.). Therefore, it is possible that LCK-16 antibody may recognize this homologous sequence in the tropomyosin and vimentin molecules.

By using LCK-16 antibody we have been able to demonstrate, for the first time, the organization of the tropomyosin molecule on reconstituted thin filaments. The success of these experiments at the ultrastructural level illustrates another advantage of monoclonal antibodies; that is, one molecule of monoclonal antibody recognizes only one antigenic determinant (or one molecule of the antigen).

Monoclonal Antibodies to α- and β-Tubulin

S. Blose, D. Meltzer

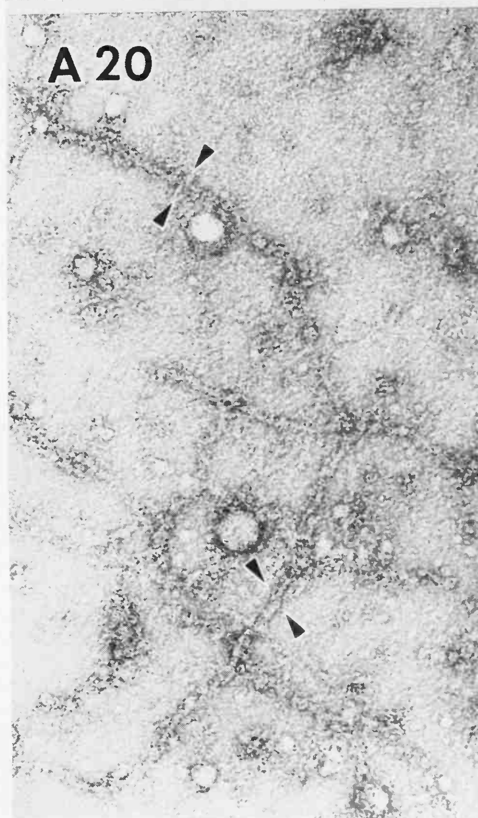
Tubulin, a ubiquitous cytoskeletal protein, is found in all eucaryotic cells. The protein is composed of two monomer subunits, α- and β-tubulin, that differ to a small degree in molecular mass, isoelectric point, and amino acid sequence. The α and β monomers associate to form α-β heterodimers that polymerize into 24-nm-diameter microtubules. During cell division the microtubules assemble into the mitotic spindle, which guides the chromosomes into daughter cells. In interphase cells, cytoplasmic microtubules radiate from the cell center to the cell's border. In interphase, microtubules influence the generation of: (1) ansiometry in cell shape, (2) cell movement, and (3) cell extensions such as cilia. Microtubules operate in the directed shuttling of intracellular organelles (i.e., mitochondria, lysosomes, and phagosomes), and in transformed cells the number and orientation of microtubules is altered (Dustin, in *Microtubules,* p. 426, Springer-Verlag, Berlin, [1978]). Other evidence suggests that microtubules associate with 10-nm filaments to anchor the cell's nucleus and organelles in the cytoplasmic matrix.

To probe the function of microtubules in cells, we have generated monoclonal antibodies against tubulin. Mice were immunized with native chick brain tubulin that had been purified by two cycles of reversible temperature-dependent assembly as described before (Blose, *Cell Motility 1:* 417 [1981]). The mice received four injections (250 μg of tubulin each time) over one month. They were then sacrificed and monoclonal antibodies were made according to the method of Kohler and Milstein (*Nature 256:* 495 [1975]), as modified by Lin et al. (in *Cell and Muscle Motility,* (ed. R.M. Dowben and J.W. Shay) Plenum Press, New York [in press]). Five hybridomas were obtained that produced monoclonal antibodies against tubulin's subunits: DM1A (IgG_1) and DM3A1 (IgG_1) were anti-α-tubulins; DM1B (IgG_1) and DM3B3 (IgG_1) were anti-β-tubulins; and DM3B2 (IgM) recognized both α- and β-tubulin. These antibodies stain the extensive cytoplasmic arrays of microtubules in interphase cells and can resolve single microtubules near the cell border (Fig. 6, A and B). Anti-tubulins stained cells entering mitosis

Figure 4
Electron micrographs of 10-nm filaments in gerbil fibroma cells decorated with mouse monoclonal antibody LCK-16. All micrographs are of thin-sectioned (60–80 nm thick) specimens. (*A*) Two examples of single 10-nm filaments decorated with LCK-16 in which tannic acid was excluded from the fixation protocol. The filaments were observed to be decorated with a very fine lace of antibody, and in some regions a helix or period could be seen (arrowheads). (*B*) Several examples of single 10-nm filaments decorated with LCK-16 and fixed with tannic acid. The antibody decorated the filaments in a periodic-helical fashion (arrowheads) that was more clearly observed by the inclusion of the tannic acid stain (cf. *A*). The antibody decorated the filaments with a period of ~31 nm. (*C*) Control: 10-nm filaments incubated with JLA-20 monoclonal antibody against actin and stained with tannic acid. As expected, the 10-nm filaments did not decorate with the actin antibody. (*D–G*) Electron micrographs of cross-sectional profiles of 10-nm filaments in gerbil fibroma cells decorated with mouse monoclonal antibody LCK-16 and stained with tannic acid. (*D*) A cluster of 10-nm filaments decorated with LCK-16 revealed that the antibody imparted a triangular profile to the individual filaments (arrowhead) when compared with the control (*E*). The antibody produced small surface projections of the "points" of the triangle that appeared to link adjacent filaments. (*E*) Control: 10-nm filaments incubated with monoclonal antibody to actin (JLA-20). No decoration was observed; instead, the filament profiles remained round and individual filaments (arrowhead) measured ~10 nm in diameter (9.9±2.1 nm). (*F*) A single 10-nm filament decorated with LCK-16 shows the triangular profile. Arrowheads indicate the points of the triangle produced by antibody decoration. Each point protrudes ~12.2 nm of the surface of the 10-nm filament. The filament in *F* was used for Markham rotation in *G*. (*G*) The triangular profile of LCK-16-decorated 10-nm filament was reinforced by three 120°-angle Markham rotations.

LCK 16

~38 nm



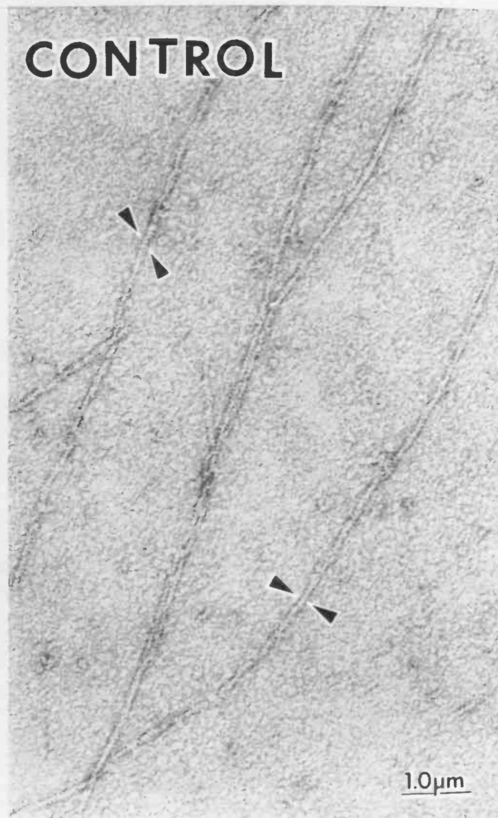


Figure 5

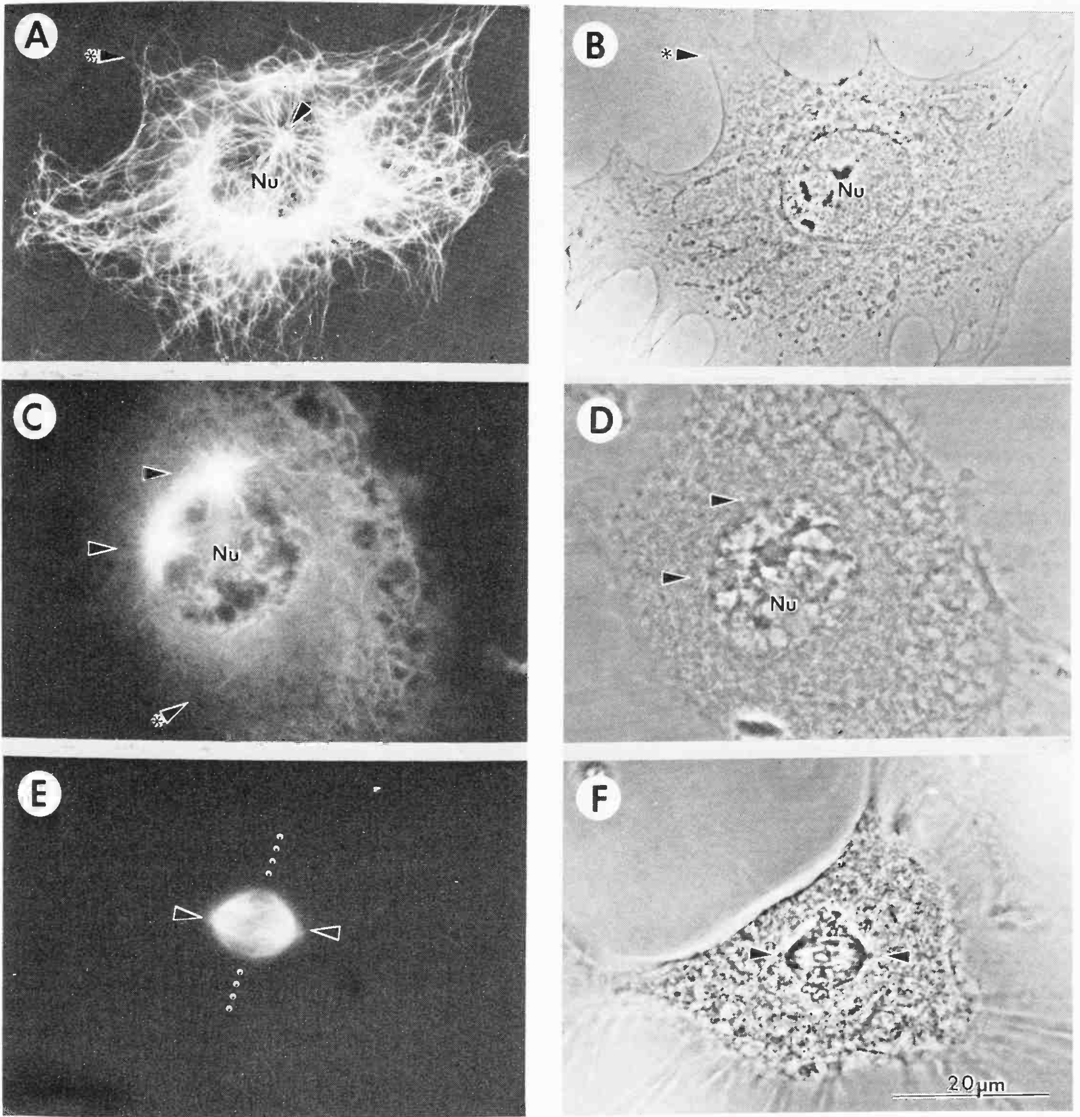


Figure 6
Fluorescence (*A*) and phase-contrast (*B*) micrographs of an interphase gerbil fibroma cell (IMR-33) that has been indirectly stained with DM1A, a monoclonal antibody to α-tubulin. Cytoplasmic microtubules radiate from the cell center located over the nucleus (Nu) (arrowhead) and extend to the cell's border (*). Fluorescence (*C*) and phase-contrast (*D*) micrographs of a gerbil fibroma cell in early prophase that has been indirectly stained with DM3A1, a monoclonal antibody to α-tubulin. The cell has begun to round up, and as compared with the cell in *A* and *B*, the ctyoplasmic microtubules have broken down. Two asters of microtubules are observed (arrowheads) radiating into the condensing chromosomes in the nucleus. Fragmented microtubules (*) are seen in the cytoplasm. Fluorescence (*E*) and phase-contrast (*F*) micrographs of a gerbil fibroma cell in metaphase stained with DM1B, a monoclonal against β-tubulin. The chromosomes have migrated to the equatorial plate (dotted line), and the microtubules have assembled into a complete mitotic spindle. Arrowheads indicate the spindle poles.

Figure 5
Electron micrographs of thin filaments incubated with monoclonal antibodies and negatively stained with 2% aqueous uranyl acetate. LCK-16 caused the thin filaments to laterally aggregate into bundles (between arrowheads). The LCK-16 antibody also decorated the thin filaments with a prominent ~38-nm periodicity. The periodicity is interpreted to correspond to the length of a tropomyosin molecule on the actin filament. When the thin filaments were decorated with JLA-20, monoclonal antibody to actin, a fuzzy coat of antibody was observed on individual filaments (between arrowheads). JLA-20 did not cause lateral aggregation of the thin filaments; it did cause some fragmentation of the filaments. In the control, the reconstituted thin filaments (tropomyosin + actin) observed between the arrowheads are long and helical. LCK-16 and JLA-20 were reacted with the same preparation of thin filaments seen in the control. All micrographs are at the same magnification.

and showed that the cytoplasmic microtubule pattern is replaced by the mitotic spindle (Fig. 6C–F). These antibodies are currently being microinjected into cells to evaluate the effect on organelle transport, cell shape, and mitosis.

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CELL BIOCHEMISTRY

K. Burridge, J. Feramisco, J. Lin, *N. Haffner, D. Helfman, F. Matsumura, S. Matsumura, B. McLaughlin, W. Welch*

During the past year we have continued to investigate the structural and the functional roles of the cytoskeletal elements in eucaryotic cell motility and these efforts have evolved into new directions concerning the cell biology of the "stress response" and the disposition of the Golgi apparatus in these cells. It is our aim to understand the relationships among the structural proteins and supramolecular systems in these cells and their functions in the motility and maintenance of the cell.

Ca^{++}-sensitive Components of the Actin Microfilament System of Nonmuscle Cells

K. Burridge, J. Feramisco, D. Helfman, W. Welch

Actin-containing microfilaments are believed to provide at least a force-generation function in many types of cell motility. Ca^{++} is thought to partake in the regulation of the force-generation system. Thus, we have been investigating the biochemistry of several of the components of the microfilaments of nonmuscle cells and their response to Ca^{++}

α-Actinin

α-Actinin was first described as a protein component of the Z line of striated muscle. It is in this region of muscle that the actin filaments terminate and presumably are cross-linked together by the action of α-actinin. In nonmuscle cells, α-actinin is localized primarily along the actin microfilament bundles in densities that appear to be related to the Z lines of striated muscle.

To compare the structural and biochemical features of the muscle and nonmuscle forms of α-actinin, we have purified these proteins from muscle and from HeLa cells using a slight modification of our original procedure developed for the purification of the muscle form (Feramisco and Burridge, *J. Biol. Chem. 225:* 1194 [1980]). Two forms of α-actinin which are structurally similar to smooth muscle α-actinin, have been purified from HeLa cells (105,000 daltons and 100,000 daltons). Peptide maps, amino acid compositions, $S_{20,w}$ values, Stokes' radii, immunological cross-reactivity, and two-dimensional gel electrophoresis have indicated that the HeLa proteins are similar but not identical to the smooth muscle protein. As reported last year, the HeLa forms of α-actinin are capable of binding to actin and causing the gelation of actin, an activity similar to that of the smooth muscle form of α-actinin. Upon analysis of the ionic conditions of the gelation activity, we have found that the activity of the nonmuscle forms of α-actinin, but not that of the smooth muscle form of α-actinin, is regulated by Ca^{++}. Whereas the smooth muscle form is equally active under all Ca^{++} concentrations tested, the nonmuscle forms are active in the absence of Ca^{++} and totally inactive in the presence of 10^{-5} M Ca^{++}

In terms of the several other Ca^{++}-sensitive actin-binding proteins isolated from nonmuscle cells—such as actinogelin (Mimura and Asano, *Nature 272:* 273 [1978]), platelet 105K protein (Rosenberg et al. *J. Biol. Chem.* [in press]), *Dictyostelium* 95K protein (Hellewell and Taylor, *J. Cell Biol. 83:* 633 [1979]), *Acanthemoeba* 85K protein (Pollard et al. *J. Cell Biol. 87:* 223a [1980]), gelsolin (Yin and Stossel, *Nature 281:* 583 [1979]), villin (Bretscher and Weber, *Cell 20:* 839 [1980]), and platelet 95K protein (Wang and Bryan, *J. Cell Biol. 87:* 224a [1980])—our results indicating the Ca^{++} sensitivity of nonmuscle α-actinin lead us to propose the following. In nonmuscle cells, there are at least two classes of actin-binding proteins—one corresponding to the nonmuscle α-actinin class and the other to the villin/gelsolin class. On the basis of their similarities in physical and functional properties, the proteins actinogelin, platelet 105K protein, *Dictyostelium* 95K protein, and *Acanthemoeba* 85K protein belong to the α-actinin class, whereas the others belong to the villin/gelsolin class.

Vinculin, Myosin Light-chain Kinase, and a Higher-molecular-weight Form of Vinculin

Other observations that seem relevant to cell motility and to the finding that nonmuscle α-actinin is inhibited by Ca^{++} in its actin-gelating activity are (1) that nonmuscle vinculin is stimulated by Ca^{++} in what appears to be its binding to the ends of actin filaments, and (2) that the myosin light-chain kinase, which is also stimulated by Ca^{++} in its activation of the contractility of actomyosin, is also a component of the actin microfilament system of nonmuscle cells. These two observations are summarized below.

First, vinculin is a component of both smooth and nonmuscle cells, and a portion of it is localized (at least in nonmuscle cells) at the ends of actin microfilaments where they terminate into the plasma membrane. In vitro analysis of vinculin has shown that it binds to the ends of actin filaments (Burridge and Feramisco, *Cold Spring Harbor Symp. Quant. Biol. 46:* 587 [1982]; Jockusch and Isenberg, *Cold Spring Harbor Symp. Quant. Biol. 46:* 613 [1982]; Lin et al., *Cold Spring Harbor Symp. Quant. Biol. 46:* 769 [1982]) and thus appears to block further elongation of the

filaments. By isolating the protein from nonmuscle HeLa cells, we have found that it, too, appears to bind to the ends of actin filaments. Unlike the activity of vinculin from smooth muscle, however, the nonmuscle protein is stimulated by Ca^{++} in this action.

Second, as we indicated in last year's report, by immunofluorescence analysis, the Ca^{++}-dependent regulatory enzyme myosin light-chain kinase was found to be localized along the actin microfilaments of nonmuscle cells (in collaboration with P. deLanerolle and R. Adelstein of NIH). This finding is supportive of the idea that this protein kinase is in part reponsible for stimulation of the actin-activated myosin ATPase of nonmuscle actomyosin and thus for the stimulation of the contractile force in these cells.

The summation of the Ca^{++}-sensitive biochemical activities of α-actinin, vinculin, and the myosin light-chain kinase and their intracellular locations lead us to several hypotheses concerning the roles of these proteins and Ca^{++} in actin-based cell motility, some of which we are testing currently. We hope that by altering the intracellular Ca^{++} concentration through microinjection, we will be able to follow alterations in the distribution and activities of these potential regulatory elements. Moreover, by utilizing affinity chromatography and chemical crosslinking experiments, we hope to identify the component(s) in the plasma membrane that is reponsible for anchoring actin filaments and vinculn to the adhesion plaques at the membrane.

As we described in last year's report, smooth muscle contains a protein with a M_r of 152kD that is structurally related to vinculin of the same tissue. Analysis of the biochemical activity of the higher-molecular-weight form of vinculin shows that it, too, is capable of inhibiting the polymerization or reannealing of actin filaments. Interestingly, this activity is stimulated by Ca^{++}, as is the nonmuscle form of vinculin. To understand the structural basis for the differences in Ca^{++}-sensitivities of the nonmuscle and muscle forms of α-actinin, vinculin, and the 152kD protein, we have initiated experiments designed to isolate the genes encoding these proteins (D. Helfman, S. Hughes, P. Thomas, J. Fiddes, J. Feramisco, in prep.; Major Structural Proteins Section). Basically, we are using two techniques: (1) protein sequencing combined with synthetic oligonucleotides and (2) cDNA expression vectors combined with a modification of Western blotting, with both of which we hope to have success.

The Stress Response

W. Welch, J. Feramisco

Eucaryotic and procaryotic cells, when confronted with environmental insults, elicit a common and seemingly highly conserved response: "the stress response." Such a response in the cell is characterized by the dramatic accumulation of a small number of polypeptides (the stress proteins) concomitant with the decreased synthesis of most other cellular polypeptides. A diverse collection of treatments, including exposure to drugs, to amino acid analogs, and growth at elevated nonphysiological temperatures (classically referred to as "heat shock"), all give rise to the stress response. We and others have described some of the mechanistic and phenomenological aspects of the stress response in a variety of eucaryotic cells. Once activated, the stress response results in the induction of a limited suite of stress proteins which eventually become the major polypeptide products of the stressed cell both in terms of their exclusive synthesis and accumulation. The increases in the amounts of these proteins in the stressed cell appear to result from the near exclusive synthesis of mRNAs coding for these proteins as well as the reduced translation of preexisting mRNAs coding for other cellular polypeptides. The mechanisms by which such transcriptional and translational control are exerted in the stressed cell are not well understood and constitute one aspect of our research into the biology of the stressed cell (see G.P. Thomas et al., Protein Synthesis Section).

The cellular response to stress appears to be well conserved in both vertebrate and invertebrate cells. The stress proteins induced in a number of different cell types by a variety of inducing agents have similar electrophoretic mobilities in one-dimensional SDS-polyacrylamide gels. In our particular system, HeLa cells grown under stress accumulate significant amounts of proteins with molecular weights of 110,000 (110K), 100,000 (100K), 90,000 (90K), 80,000 (80K), 73,000 (73K), and 72,000 (72K) (see Fig. 1, lanes E and F). Furthermore, in such stressed cells synthesis of the normal complement of other cellular proteins is dramatically reduced. All of these stress proteins appear to be present in so-called normal, unstressed cells grown in tissue culture, although at much lower concentrations (see Fig. 1, lane D). This may be indicative of normal cellular functions for these proteins or of some degree of "stress" in the tissue culture process.

Although the identity of the induced proteins in stressed cells has been well-documented, their function and location in the cell has not been established. Therefore, as a first step in the analysis of the function and location of these proteins, we have purified four of the six stress proteins, the 100K, 90K, 73K, and 72K proteins, from stressed HeLa cells (see Fig. 1). Purification of the remaining two proteins, the 110K and 80K proteins, is now in progress. Interestingly, the 73K and 72K proteins copurify throughout the purification scheme and appear related, as determined by one-dimensional peptide mapping. The 100K and 90K proteins, like the 73K and 72K proteins, copurify throughout their purification but

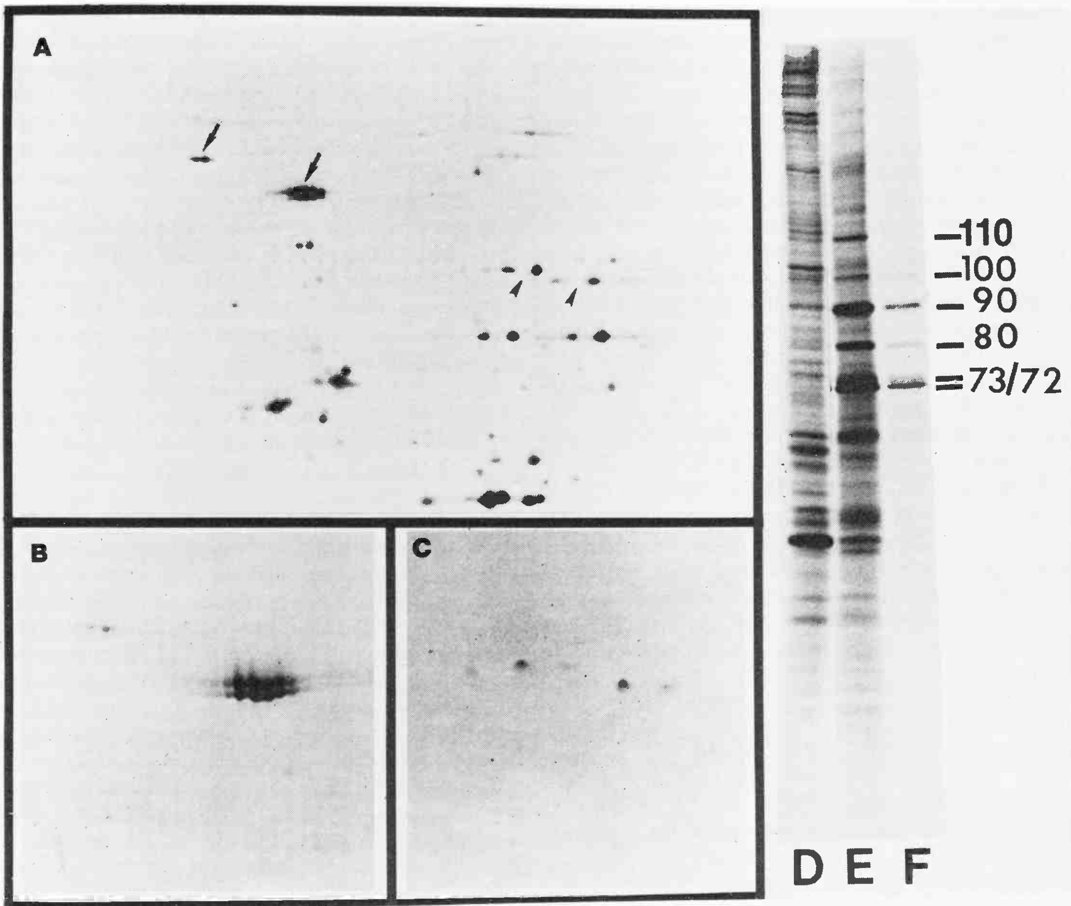


Figure 1
Two-dimensional gel analysis of purified stress proteins. The protein synthesis patterns of normal HeLa cells (lane *D*) and HeLa cells grown under stress for 16 hr (lane *E*) or for 24 hr (lane *F*) are shown. Numbers to the right indicate molecular weights of the six major stress proteins.

(*A*) A two-dimensional gel of [^{35}S]methionine-labeled polypeptides synthesized in vitro in a messenger-dependent reticulocyte lysate system supplemented with mRNA isolated from stressed HeLa cells. (*B,C*) Coomassie-blue-stained gels of our purified stress 100K and 90K proteins (*B*) and 73K and 72K proteins (*C*). Arrows in *A* indicate the 90K and 100K proteins, and arrowheads indicate the 72K and 73K proteins. Although not directly shown here, the Coomassie-blue-stained 72K/73K proteins (*B*) as well as the 90K/100K proteins (*C*) coincided exactly on the gels with their [^{35}S]methionine-labeled counterparts (*A*).

appear unrelated on the basis of one-dimensional peptide mapping. A number of physical properties, including the Stokes' radii, S_{30w} values, isoelectric points, and native molecular weights, have been determined for the purified stress proteins (W. Welch et al., in prep.). Working independently from us, Jim Lin has obtained a monolconal antibody that stains the Golgi region of the cell. Much to our surprise, the antigen recognized by this antibody appears to be the 100K stress protein. We have as yet to realize the complete significance of this location of one of the stress proteins. In addition, all of the other purified proteins have been injected into rabbits, and to date we have obtained specific antibodies to 73K and 72K proteins. We have recently begun immunizing mice with the purified stress proteins and are in the process of generating monoclonal antibodies to the stress proteins. Acquisition of the purified stress proteins as well as the appropriate antibodies should aid in the determination of the location and function(s) of the stress proteins both in normal and in stressed cells and thereby facilitate a more complete understanding of the stress response.

Monoclonal Antibodies

J. Lin, F. Matsumura, S. Matsumura

We have continued our efforts to prepare more monoclonal antibodies directed against contractile proteins of muscle cells and against structural proteins of cultured cells. Two monoclonal antibodies (JLK6 and JLK10) have been obtained and characterized as anti-vinculin antibodies. Both an-

tibodies reacted with both vinculin and vinculinlike 152K protein from chicken gizzard and with vinculin from chicken embryo fibroblasts. In fusion experiments using purified α-actinin as antigen for immunization, we have obtained three monoclonal antibodies (JLN1, JLN20, and JLN23) that gave the typical α-actinin staining pattern in cultured CEF cells. JLN1 and JLN23 antibodies appeared to be chicken-specific. On the other hand, JLN20 antibody gave the Z-line staining on myofibrils from rabbit and rat skeletal muscle. In addition, we have also obtained JLK13 antibody, which gave the typical α-actinin staining pattern, namely the periodic stress-fiber staining, and the fluorescent patches terminated at the focal points. This antibody appears to have broad species specificity. These antibodies will be used to isolate microfilaments from cultured cells.

Using skeletal muscle proteins, troponin T, and crude myosin as antigens to immunize mice, we have isolated several hybridoma clones. Among them, the clones JLT7, JLT12, and JLT36 secreted monospecific antibodies which gave I-doublet staining on myofibrils by indirect immunofluorescence microscopy. The antigen recognized by these antibodies has been identified as troponin T by using the Western blot method. The antigen cannot be detected in cultured fibroblasts. Clones FM10, FM20, and FM21 secreted antibodies that gave A-doublet staining pattern on myofibrils. According to radioimmunoassay, these antibodies are directed against C protein. The antibodies will be useful to probe the detailed organization of C protein in the myofibrils and to study myogenesis. We have also obtained two antibodies that give the doublet staining pattern at the A-I junction of myofibrils, four antibodies that give the A-doublet staining pattern, and one antibody that gives the M-line stain. The identification of the antigens recognized by these antibodies is currently in progress.

One of the advantages of the hybridoma technique is that it may generate a monospecific antibody against proteins that either exist in trace amounts or that cannot be purified to homogeneity. For example, in the fusion experiments with vinculin as antigen for immunization, we have also obtained four monoclonal antibodies (JLK3, JLK7, JLK9 and JLK16) against a high-molecular-weight (350K) protein that appears to be a trace contaminant in the vinculin fraction. The immunofluorescent micrograph showed that 350K protein is diffusely distributed throughout the whole cell. The protein appeared not to be a surface protein since iodination of intact cells did not label this protein, Affinity column will be used to purify this potein, and the biochemical properties and functions of this protein will be determined.

In addition to the above-mentioned antibodies against cytoarchitectural proteins, we have begun to raise monoclonal antibodies against the components of cytoplasmic organelles, such as Golgi apparatus, endoplasmic reticulum, plasma membrane, nucleus, and centrioles. One of the monoclonal antibodies we have prepared, JLJ5a, appears to recognize a Golgi-associated protein (see below). In addition, preliminary screening by indirect immunofluorescence shows many hybridoma clones producing antibodies that either give the nuclear staining pattern or give the endoplasmic reticulum or mitochondrial staining pattern. Characterization of these antibodies and their antigens is now in progress. Our hope is to produce specific tools capable of recognizing different organelles. These antibodies will be most useful to dissect organelle composition and to follow their fate inside cells.

Monoclonal Antibody to a Golgi-associated Heat-Shock Protein

J. Lin, F. Matsumura. S. Matsumura

Golgi proteins can theoretically be divided into three categories: (1) secretory proteins, such as serum proteins, which are transiently passed through the Golgi apparatus; (2) Golgi-specific proteins, which are unique to the Golgi apparatus and may play important roles in performing the Golgi functions such as glycosylation, sulfation, distilled fractionation, sorting, etc.; and (3) Golgi-associated proteins that are derived from or also localized in other organelles, such as NADPH-cytochrome P450 reductase, NADH-cytochrome b5 reductase. Proteins in the second and third categories are most interesting but difficult to identify and purify. However, monoclonal antibody techniques may provide an opportunity to identify Golgi-specific and Golgi-associated proteins.

We have obtained a hybridoma clone, JLJ5a, that secretes antibody directed against a 110K protein from gerbil fibroma cells, Rat-1 fibroblasts, and L6 myoblasts. This protein is mainly distributed at the perinuclear region, which we have identified as a Golgi zone by cytochemically staining for thiamine pyrophosphatase (TPP, one of the enzyme markers for Golgi apparatus). In monensin- and colcemid-treated cells, the normal Golgi stacks no longer exist and have become fragmented, as revealed by both JLJ5a antibody and TPP stainings. These results further support that the 110K protein is localized at the Golgi apparatus.

Interestingly enough, the 110K protein is not only found in the Golgi apparatus, but in the nucleus of a subpopulation of interphase cells as well. When cells were subjected to heat treatment, the number of cells with nuclear staining by JLJ5a antibody increased. It is striking that the Golgi apparatus cannot be detected by JLJ5a antibody in those cells with nuclear staining. Furthermore, in collaboration with Jim Garrels (Quest 2-D Gel Laboratory Section), we have found that 110K protein prepared from immunoprecipitation with JLJ5a antibody has electrophoretic mobility in two-dimensional gels identical to that of one of

the heat-shock proteins in L6 myoblats. Thus, 110K protein recognized by JLJ5a antibody is a Golgi-associated heat-shock protein. The accumulation of 110K protein in the nucleus in response to heat shock may suggest some important function for this Golgi-associated protein.

Microinjection of Monoclonal Antibodies into Living Cells

S. Blose, J. Lin, J. Feramisco

With some degree of understanding as to the intracellular locations and, in certain cases, the biochemical activities of several cytoskeletal proteins, we have turned our attention to the functions of these proteins in the living cell. Through the microinjection into fibroblasts of well-defined monoclonal antibodies directed against specific components of the cytoskeleton, we have been able to perturb the intracellular location of the intermediate filament system, one cytoskeletal system that has as yet unknown functions. Preliminary experiments indicate that it may be possible to alter the function of tubulin and/or the microtubules as well.

As explained in last year's report, injection of a monoclonal antibody directed against a component of the intermediate filaments caused the reorganization of these filaments into a tight cap near the nucleus. Organelle movement, cell shape and spreading, and blebbing and ruffling were seemingly unaffected in these cells lacking the normal distribution of intermediate filaments, suggesting that these filaments are not involved in these motility events.

Because of suggestions that there is a relationship between the cytoskeleton and protein synthesis (e.g., Zumbe and Trachsel, *Eur. J. Cell Biol. 22:* 376 [1980]), we have begun to examine the effects on protein synthesis of the alteration of cytoskeleton elements. To carry out this analysis, we have developed a method for confining 1-100 cells in a microwell, all of which are injected with antibodies or control solutions. These cells are then labeled for 1-5 hr with radioactive amino acids, harvested, and subjected to two-dimensional gel electrophoresis. Our first investigation was to look at the protein synthesis patterns of cells lacking the normal distribution of intermediate filaments and compare these with control cell patterns (Fig. 2). As can be seen, few detectable changes in the protein synthesis occurred even though the intermediate filament system had been drastically altered by the injected antibody.

We are carrying out similar experimental analysis of the distribution of the cytoskeletal elements, organelle movement, cell shape, and protein synthesis patterns in cells after the injection of monoclonal antibodies directed against tubulin. In addition, we are examining the effects of Ca^{++} injection on the distribution of α-actinin, vinculin, and actin to aid in the elucidation of the mechanism of the putative Ca^{++} control of function of the actin microfilament system of these cells.

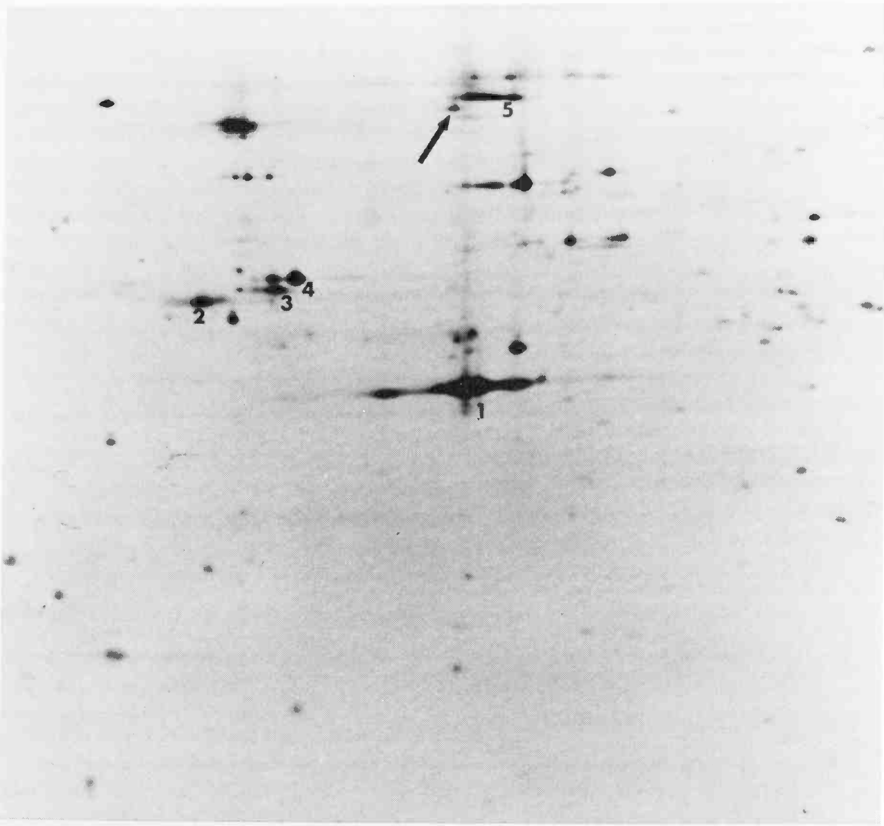
Calcium-dependent Mobility Changes of Cytoskeletal Proteins in SDS-Polyacrylamide Gels

F. Matsumura, S. Matsumura, J. Lin

It has been known that the calcium-binding protein calmodulin can be identified in the crude protein preparation by its mobility change in the SDS-polyacrylamide gels that contain additional calcium (Burgess et al., *Biochim. Biophys. Acata 623:* 257 [1980]). Since calcium appears to play a regulatory role in the interaction between cytoskeletal proteins, we have used our slab gel system (low bisacrylamide content) to test whether there is a similar calcium-dependent mobility change for the cytoskeletal proteins such as actin, myosin, tropomyosin, and troponin T, C, and I from rabbit skeletal muscle and actin, myosin, tropomyosin, α-actinin, filamin, and vinculin from chicken gizzard. The rationale was that these or other muscle-related proteins may qualify as calcium-binding proteins, suggesting that their function in vivo may be regulated by calcium concentration in cells.

In addition to troponin C (a known calcium-binding protein in skeletal muscle), we have found two proteins, gizzard α-actinin and slow component of gizzard tropomyosin, that appear to migrate faster in the Ca^{++} gel. The mobility change of these proteins in Ca^{++} gels is rather specific; in the Mg^{++}-containing gels these proteins do not show any mobility change and only a small change can be noticed in the Sr^{++}-containing gel. Ca^{++}-binding experiments show that troponin C binds 4 moles of calcium, and smooth muscle tropomyosin (dimer) binds 1 mole

Figure 2 (for figure, see p. 116)
Protein synthesis patterns in cells either containing or lacking a normal distribution of intermediate filaments. Approximately 40 cells (gerbil fibroma) were microinjected with a sufficient amount of the monoclonal antibody against a 95K protein to cause the aggregation of the intermediate filaments (see Lin and Feramisco, 1981). Ten hours postinjection the cells were labeled with [^{35}S]methionine for 2 hr, harvested, and analyzed by two-dimensional gel electrophoresis (by the Quest 2-D Gel Laboratory). The top panel shows the fluorogram of the proteins made by control cells, and the bottom panel shows the proteins made by the cells injected with the antibody. The arrow points to the antigen against which the antibody is directed. Much of the pattern is unchanged when compared with that of control cells (except perhaps for the apparent reduction in synthesis of one of the stress proteins!). (*1*) Actin isotymes; (*2,3*) tubulins; (*4*) vimentin; (*5*) α-actinin.



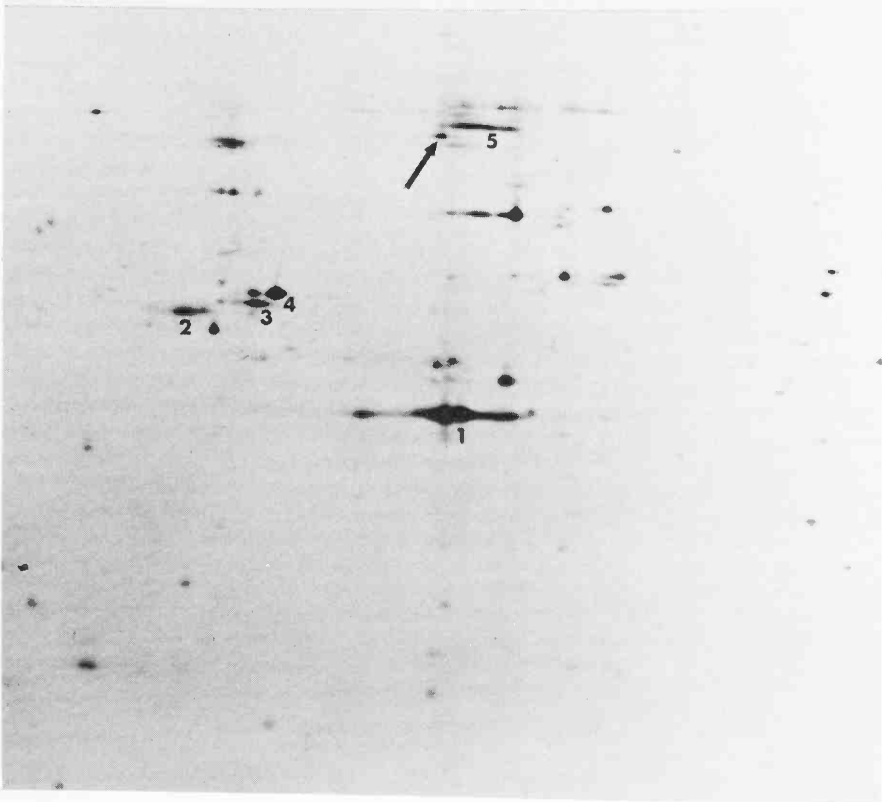


Figure 2 (See p. 115 for legend)

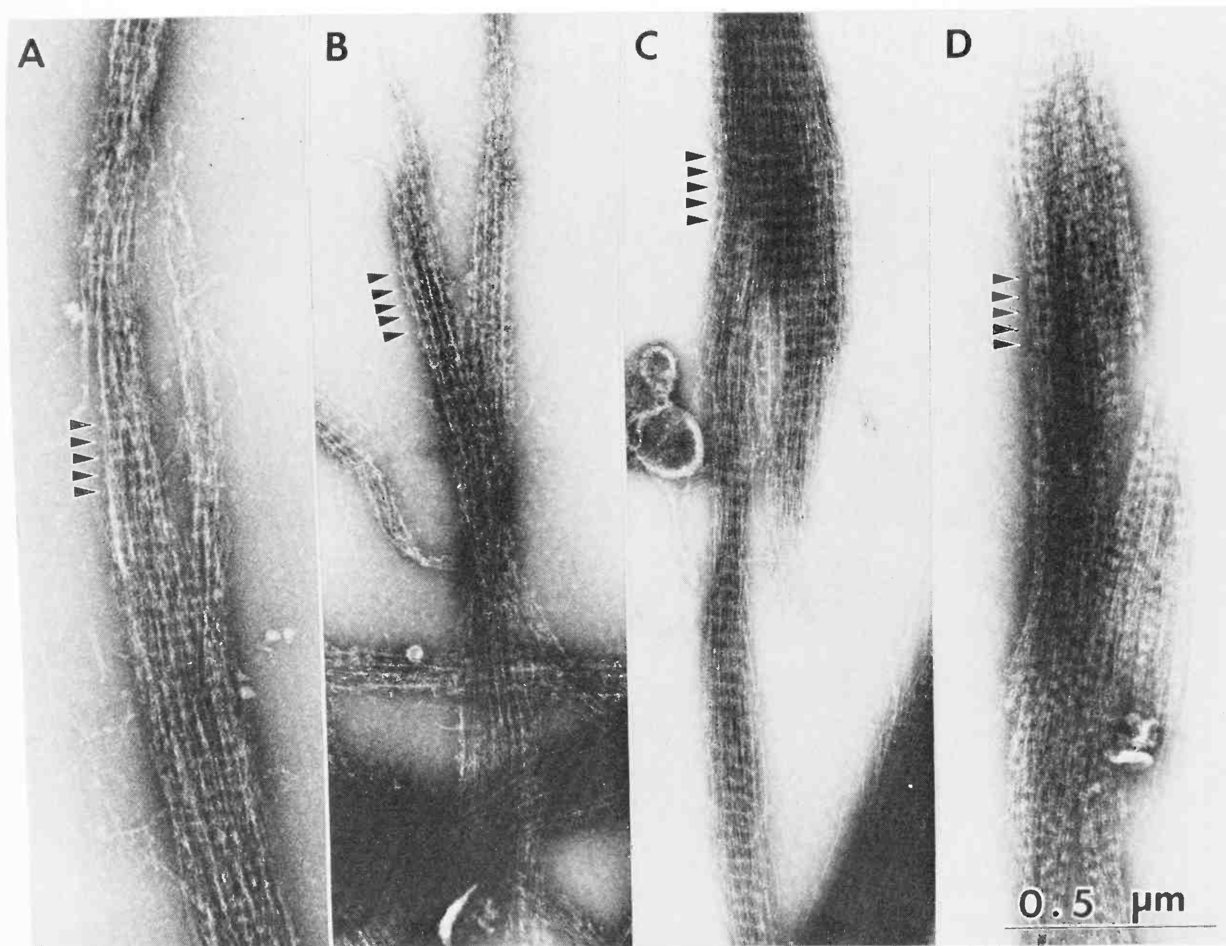


Figure 3
Ordered bundles of (*A*) thin filaments and (*B-D*) microfilaments formed by anti-tropomyosin monoclonal antibody JLF15. (*A*) Thin filament bundles isolated from smooth muscle (*Taenia coli* of guinea pig). (*B-D*) Microfilament bundles isolated from (*B*) BHK-21 fibroblasts, (*C*) L6 myoblasts, and (*D*) gerbil fibroma cells. The periodicity is indicated by arrowheads.

of calcium in the native state. Surprisingly, smooth muscle α-actinin does not bind calcium at all. Therefore, the mobility change of a protein in Ca^{++} gels can be misleading if used to identify a calcium-binding protein. Still, the observed change of mobility may indicate that such a protein may undergo a conformational change after the interaction with calcium ions. The conformational change may be important for the protein to perform its regulatory function in cells.

Use of Monoclonal Antibodies to Isolate Microfilaments from Cultured Cells

F. Matsumura, S. Matsumura, J. Lin

Microfilaments appear to play central roles in cell motility and cell-shape changes. One of our goals in studying the function of microfilaments is to determine whether the protein composition or ultrastructural organization of microfilaments changes concomitantly with changes in biological activities such as spreading, mitosis, movement, etc. For this purpose, it is quite essential to isolate microfilaments in their intact form from cultured cells. We have chosen anti-tropomyosin monoclonal antibodies (JLF15, JLH2, and LCK16) to isolate intact microfilaments for the following reasons: (1) Since tropomyosin is one of the ubiquitous components of microfilaments, it is suitable as an antigen for immunoprecipitation of microfilaments; (2) monoclonal antibodies to tropomyosin caused the aggregation of microfilaments into ordered bundles, which can be easily collected by low-speed centrifugation.

The procedure to isolate microfilaments is very simple and rapid. Briefly, monolayer cells are first extracted with Triton X-100 glycerol solution to stabilize the cytoskeleton, and the residues are homogenized in the presence of Mg-ATP. After centrifugation, the supernatant is incubated with monoclonal antibodies. Dispersed microfilaments in the supernatant are aggregated by antibodies into ordered bundles (Fig. 3), and the resultant bundles are collected by low-speed centrifugation and analyzed by electron microscopy and biochemical methods such as SDS-polyacrylamide gel analysis and actin-activated myosin ATPase activity. Such isolated microfilaments appear to be native with respect to the following criteria: (1) they preserved the typical filament morphology, namely, double helical structure and periodic arrangement of tropomyosin on actin filaments, and (2) the microfilament fraction could activate myosin ATPase activity to almost the same extent as skeletal muscle F actin does.

We applied this method to examine changes in the protein components of microfilaments from L6 cells during myogenesis. As Figure 4 shows, the composition is clearly different between these two microfilament fractions. Tropomyosin species changed from nonmuscle type to muscle type during myogenesis. In addition, troponin appeared in the microfilament fraction from myotubes. More interestingly, an as yet unidentified protein of 83K molecular weight found in the myoblast-microfilament fraction was missing from the myotube-microfilament fraction. The characterization of this new protein, including production of monoclonal antibody to this protein, is in progress.

The method assumes that Triton-glycerol extraction immediately freezes the microfilaments at the state identical to that occuring in in vivo microfilament bundles. This is still to be proven preferentially by demonstrating that the isolated bundles express pronounced differences according to the biological state of their source. Consequently, we will use the monoclonal antibodies that we have prepared (such as anti-actin, anti-filamin, anti-α-actinin, and anti-vinculin) to isolate other sets of microfilament fractions. Thus, the protein composition, and perhaps organization, along microfilaments can be analyzed in detail

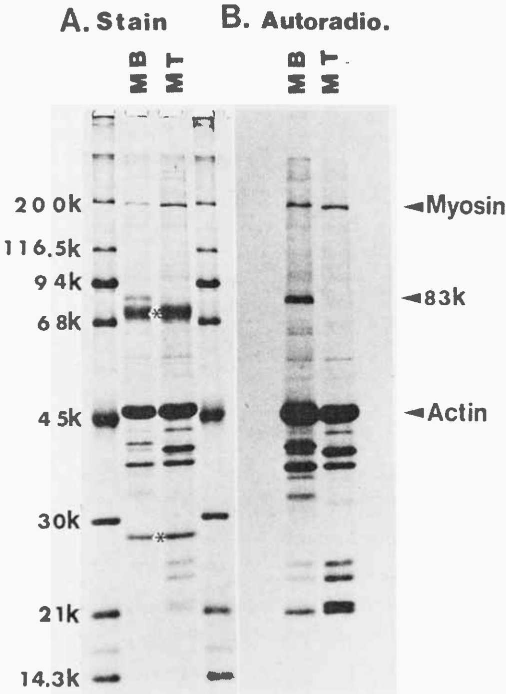


Figure 4
SDS-polyacrylamide gel analysis of microfilament fractions immunoprecipitated by monoclonal antibody JLF15 from [^{35}S]methionine-labeled L6 myoblasts (MB) and myotubes (MT). (*A*) Coomassie blue stain; (*B*) autoradiography. Asterisks indicate heavy and light chains of IgM immunoglobulin. Note that 83K protein found in the myoblast-microfilament fraction (lane MB of *B*) appears to be missing in the myotube-microfilament fraction and that tropomyosin changes qualitatively and quantitatively during L6 differentiation.

and correlated to their functions. Two examples of the usefulness of this technique are shown in the next sections.

Analysis of Microfilament Components from Cultured Normal and Virus-transformed Cells

S. Matsumura, F. Matsumura, J. Lin

Microfilament bundles in transformed cells have been reported to be in a more dispersed state and to have reduced thickness as judged by immunofluorescent microscopy. Recently, Hendricks and Weintraub (*Proc. Natl. Acad. Sci. 78:* 5633 [1981]) reported that Rous sarcoma virus (RSV)-transformed CEF contain a lesser amount of tropomyosin. In collaboration with Margaret Hightower and Bill Topp (Tumor Virus Section), we have prepared microfilaments from normal and two independent isolates of SV40-transformed rat embryo fibroblasts using our new method to isolate intact microfilaments from cultured cells by immunoprecipitation with anti-tropomyosin monoclonal antibody. Analysis by SDS gel electrophoresis (Fig. 5) showed hardly any difference between the amounts of tropomyosin in the microfilaments of transformed cells versus the normal cells. However, we could detect one protein band (37K) present only in the microfilaments of transformed cells and two protein bands (250K and 32K) significantly increased in the microfilaments of the transformed cells. The further characterization of these proteins is in progress.

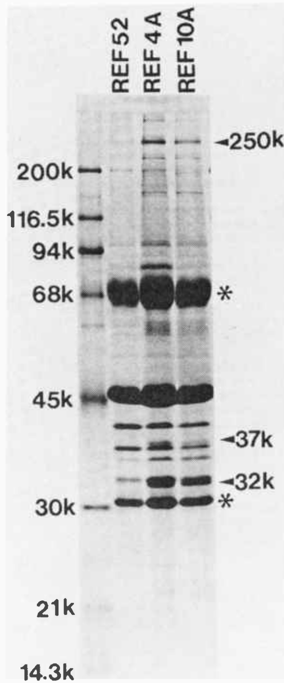


Figure 5
SDS polyacrylamide gel analysis of microfilament fractions immunoprecipitated by monoclonal antibody JLF15 from normal rat embryo fibroblasts (REF52) and its SV40-transformed lines (REF4A and REF10A). Gel was stained with Coomassie blue. Asterisks indicate the heavy and light chains of JLF15 IgM antibody.

Decoration of Microfilaments and Smooth Muscle Thin Filaments with Monoclonal Antibodies to Tropomyosin

F. Matsumura, S. Matsumura, J. Lin

Monoclonal antibodies have the advantage over conventional antibodies in that they recognize only one antigenic determinant. Therefore, one can expect to obtain higher resolution in localizing that determinant within cytoskeletal filaments by electron microscopy. We have used three different monoclonal antibodies (LCK16, JLH2, and JLF15) against tropomyosin to localize this antigen along smooth muscle thin filaments and microfilaments from nonmuscle cells. As Figure 3 shows, monoclonal antibodies caused the aggregation of smooth muscle thin filaments and microfilaments from nonmuscle cultured cells (BHK-21 fibroblasts, L6 myoblasts, gerbil fibroma cells) into ordered bundles which displayed cross-striations with a periodicity of 37 ± 1 nm and 36 ± 1 nm, respectively. In contrast, conventional rabbit antiserum to tropomyosin distorted and aggregated the thin filaments without generating cross-striations. Therefore, monoclonal antibodies to tropomyosin allow us, for the first time, to observe directly the distribution of tropomyosin molecules along thin filaments of smooth muscle cells and microfilaments of cultured cells. A similar approach using other monoclonal antibodies that we have prepared will be applied to show the ultrastructural arrangement of other cytoskeletal proteins such as α-actinin, filamin, and vinculin along microfilaments.

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QUEST 2D-GEL LABORATORY

J.I. Garrels, *F. Matsumura, S. Matsumura-Yamashiro, J. Leibold, T. Kelly, L. Cascio, S. Norris, J. Emanuele*

Our work in the past year has focused heavily on the improvement of our methods for computer analysis of two-dimensional gels and on the establishment of protein data bases for human, rat, and mouse proteins. Although our laboratory is routinely producing high-quality fluorograms to resolve thousands of proteins from cells in different states of transformation and differentiation, these studies will not be complete until we can compare the images in detail and have a database structure for storage and manipulation of the data. Here we report our recent work in these areas, as well as the status of several current projects and some new findings regarding proteins of particular interest.

Towards Computerized Protein Data Bases

Our work prior to this year had given us a set of programs for spot detection, integration, and pattern matching. It had also given us many ideas for improved speed, improved user interaction, and more convenient data file structures. These enhancements, we realized, would be necessary to achieve the throughput and ease of use required to build a large protein data base accessible to many users. Since we had already set up a successful system (the QST data management system) that allows our laboratory technicians to interact with their data (records of samples, gels, exposures, etc.) and to run programs to manage this data with no special computer knowledge, we decided that this system should be extended to control the image analysis programs and to manage the protein data files.

These goals have been realized in the development of a second-generation system of 2D-gel analysis programs. These programs now give us the speed, reliability, and ease of use that we need for routine gel analysis. The operator can begin a scan by entering data into a simple, self-explanatory form (the SCAN form) on the screen of an ADM42 terminal. The detection of spots, conversion of density to dpm, and the integration of spots is carried out by a series of programs that are run automatically by the system. Each program writes some summary information, such as number of spots found, total dpm detected, total execution time, and the input and output data file numbers, into a permanent data record that can be displayed on the SCAN form. The scanning process, which puts a smoothed image of the film onto disk, requires only about 4 minutes. The series of programs for spot detection and integration requires about 30 minutes for an image of over 1000 spots. To scan many films during the day without waiting for completion of the analysis programs, we have written a queue manager utility. The queue manager directs the flow of the programs as time allows, and the unfinished processing for the scans of the day is easily completed overnight. The image, intermediate, and spot list files are managed automatically by the system.

The spot integration programs generate a sizable data record for each spot. In this data record are stored such parameters as total density, total dpm, and Gaussian curve-fitting parameters (height, position, and half-width) and codes to indicate unusual shapes, uneven backgrounds, saturation, and degree of overlap. When integration is complete, the list is sorted, and for every spot a list of the ten nearest neighbors is compiled. Finally, a synthetic image is constructed from the spot list by computing the density at each location in the image based on the fitted Gaussian spot parameters. These postintegration programs require only a few minutes to run. At this stage, the synthetic image can be displayed on the color monitor or plotted, and the intensities of each spot can be read by the user in terms of density, dpm, or fraction of counts applied to the gel. If comparison to another image or a standard image is desired, then a matching program is run. The neighbors list, the detailed spot records, and the synthetic image provide the necessary input for the matching programs.

We have previously developed, and are just completing the enhancement of a matching program for comparison of two gel images. These images can be any two related spot patterns, but usually one is a standard pattern representing a compilation of spots from many previous gels. Our approach requires only that the two images be superimposable in local areas and that many of the spots are common to the two images. Matching is done primarily by neighbor relationships (positional information), but now consideration of spot shapes, spot intensities, saturated spots, and other flags in the data files is possible. One or more landmark spots and their matches are initially selected by the operator. Then the computer matches up to ten neighbors surrounding an initial match. One of these new matches will then be selected as the next reference match and ten neighbors around it will be matched, as so on. In the end, each spot has been matched based on its position with respect to each of its neighbors. If two or more spots are potential matches for a standard spot, the ambiguity can often be resolved by choosing the spot most similar in shape or intensity.

There are, of course, cases in which a spot on the new image could be one of two possible standard spots that run very close together. Our matching strategy allows these ambiguities to remain temporarily unresolved. Since most of our samples are applied to several gels of different

acrylamide concentrations in the second dimension, we can often use the data from a second gel to resolve the ambiguity. In a gel of different composition, the spot pattern and the neighbor relationships will be somewhat different. The ambiguities of matching found on a 10% gel in general will not occur in a 12.5% gel. Therefore, the last phase of our spot matching process is the resolution of these ambiguities by comparison of match lists from two different gels.

Our protein data-base structure has been worked out in detail, and a standardized spot map of rat proteins has been prepared. In our system, each spot in the standard map is assigned a four-digit number. As a convenience for users of the maps, we let the first digit denote position along the pH coordinate and the second digit denote position along the molecular-weight coordinate. The numbers have been assigned in proportion to the spatial distribution of the proteins, and plenty of unused numbers are available for newly discovered proteins.

In the permanent data files, space is reserved to enter the intensity of each of 10,000 spots for each sample. We presently have enough space on our disks to hold these lists for 500 samples for each data base (human, rat, mouse). Knowing only the sample number and the standard spot number, the system will be able to obtain the intensity of any spot in any sample very quickly. Since cross-referenced data to describe all experiments and samples is already in our laboratory data base, it will be easy for us to set up searches to find, for example, proteins present only in muscle cells or proteins that increase in transformed cells.

Another important aspect of the data base, in addition to the master lists of protein intensity, are records to hold textual information about each protein. These records are handled by the QST system and can be displayed on forms on the terminal screen. We have created files called HUMANSPOT, RATSPOT, and MOUSESPOT, and a data record has been allocated for each of the 10,000 potential proteins. In these records we can enter the pH and molecular-weight coordinates of each spot, its name or attributes when known, and pointers to a variety of other files to hold such information as literature references, amino acid composition, posttranslational modification data, tissue-specificity information, subcellular localization data, etc. A user can obtain the number for a spot of interest from standard maps mounted on the wall and then call up the corresponding HUMANSPOT (or RATSPOT or MOUSESPOT) record on the terminal to determine what is known about this spot. Entries are now being made for known proteins and for proteins of particular interest from our past experiments.

Although not complete, our software for 2D-gel analysis and data management is now a well-structured, modular system that uses consistent names and conventions throughout. We have begun to distribute our software package, which we call QUEST, to other scientific laboratories. The software we have distributed includes the QST record management system and the image-processing programs for scanning, calibration, spot detection, and integration. More programs will be added to the distribution tapes as they are completed.

Studies of Human Disease

A major part of our effort in the gel lab in the past year has been to prepare samples and resolve the proteins from human fibroblast lines representing normal individuals and patients with genetic disease. A total of 16 normal lines, 17 lines from patients either heterozygous or homozygous for cystic fibrosis, and 11 lines from patients with Huntington's disease have been examined. All lines were obtained from either the American Type Culture Collection or the Human Genetic Mutant Cell Repository. The Hungtington's disease study has been done with Dr. Irwin Fand from Stony Brook. At least two samples have been prepared from each line, representing high- and low-density cultures, and most lines have been examined at two or more different passage levels. Each sample has been run on gels of three different pH ranges (5-7, 6-8, and 3.5-10), and both 10% and 12.5% gels have been used in the second dimension. The images obtained from these samples are very similar, but differences can be found. Most of these differences are due to polymorphisms in the human population. A full analysis of the data from these samples is now being carried out by our new data analysis technician, Jane Emanuele.

Projects in Cell Biology

We are working closely with the other members of the Cell Biology Section to identify and characterize cytoskeletal and other abundant proteins of cultured cells. In addition, we are characterizing many of the less abundant proteins of rat cells with regard to state of phosphorylation, kinetics of synthesis and turnover, and tissue specificity.

The heat-shock proteins are a set of abundant proteins with unknown functions. We have worked in collaboration with Paul Thomas, Jim Feramisco, and Bill Welch to study these proteins in HeLa and chick cells (see Protein Synthesis Section). In addition, we have examined the heat-shock response in the rat muscle cell line L6. In many cell types the major response occurs in a 90K and a 72-74K protein. In cultures of differentiated L6 cells, however, the primary inductions are in a 100K and a 80K protein. Each of these is induced approximately 8-to 12-fold within 4 hours of a mild (42°) heat shock. Both the 100K and 80K proteins have phosphorylated components, and the phosphorylated forms are induced to the same extent as the major unphosphorylated

forms. Each of the heat-shock proteins is a major component of cultured L6 cells under normal culture conditions, and no dramatic changes of synthesis occur during muscle differentiation, although some decrease in the rate of synthesis of the 100K protein is consistently observed. The 80K heat-shock protein is apparently the same protein that was reported by Wu et al. (*J. Biol. Chem. 256:* 5309 [1981]) to be induced by calcium ionophores.

The 100K protein has been observed in other stress response systems, although seldom as the major response. We have made a fortunate observation through a separate project to identify proteins immunoprecipitated by monoclonal antibodies produced by Jim Lin. One of these monoclonal antibodies, JLJ5a, has high specificity for the 100K "heat-shock" protein. Jim Lin has already characterized this antibody and has localized its antigen by immunofluorescence (see Cell Biology Section). The localization of this protein in normal cells is coincident with the Golgi apparatus, and its localization in shocked cells is under investigation.

Protein phosphorylation has been studied recently in the L6 and Rat-1 cell lines to determine the phosphorylation patterns of particular proteins and, more importantly, to determine the overall extent of phosphorylation. We have found in cultures of L6 cells, containing both differentiated and undifferentiated cells, that about 13% of the 467 most prominent proteins contain phosphate. Our detection is based on fluorograms of gels containing ^{33}P-labeled proteins, labeled by growth of cells in phosphate-poor media containing the isotope for 24 hr. Phosphorylation could be found on some of the relatively abundant proteins, as well as on minor proteins, with no significant preference for any abundance class. There does seem to be more tissue specificity among phosphoproteins than among other proteins. About 14% of the phosphoproteins analyzed in mixed myoblast/myotube cultures are not detectable in myoblast cultures, and 41% of the same phosphoproteins are not detectable in Rat-1 fibroblasts.

These studies will be extended to many more proteins and to other cell lines. In our L6 studies, over 75% of the detected phosphoproteins did not correspond to any ^{35}S-labeled protein at the level of exposure used. This indicates that the incorporation and sensitivity for detection of ^{33}P was much better than the detection of ^{35}S-labeled proteins in this experiment and that large numbers of minor phosphoproteins remain to be characterized. Indeed, we have obtained high-sensitivity phosphoprotein gels of Rat-1 cells in which over 700 phosphoproteins can be detected. To reliably match this many minor proteins to their amino-acid-labeled counterparts is best done by a double-label experiment in which proteins labeled by each isotope are run in the same gel. Detection will be by differential decay and computer analysis.

Collaborative Experiments

Our laboratory has engaged in several major collaborations with other groups at the Cold Spring Harbor Laboratory. These experiments include studies of the stress response of HeLa and chick cells (being done with Paul Thomas, Protein Synthesis Section), identification of mouse proteins specified by mutant haplotypes of the t complex (being carried out with Lee Silver, Molecular Genetics of the Mouse Section), and studies of mouse cells transformed by DNA from human tumor lines (being carried out with Mike Wigler, Mammalian Cell Genetics Section). The progress of these studies is reported in the corresponding sections of this Annual Report.

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NEUROBIOLOGY

A new type of Neurobiology course which teaches state-of-the-art techniques that are in obvious demand was introduced last year and is proving to be very effective. The topics change every year or every second year and are a refreshing addition to our basic core program. This past summer, for example, we taught Single-channel Recording and Electrophysiology of the Mammalian Brain Slice. Likewise, our year-round program is in flux. It is slowly growing in scope and promises to become more integrated into the laboratory's scientific activity, where originally it had subserved a more peripheral function.

LEECH NEUROBIOLOGY

B. Zipser, *C. Schley*

Our laboratory uses monoclonal antibodies to probe into the molecular basis of neural recognition. We use the leech as an experimental preparation because, in principle, in this limited-size nervous system each individual neuron can be identified. Thus, our strategy is to focus on those antibodies that are specific for single kinds or small sets of identifiable neurons. Our major thrust has been and will continue to be directed at generating large numbers of specific monoclonal antibodies, since we consider it vitally important to obtain a clear understanding of the different kinds of sets of antigenically related neurons that exist in the brain. Obvious questions that we are beginning to address are, for example, how many different specific molecular markers occur in a given identified neuron? We need to know whether a neuron has just a handful of markers, such as those that exist to carry out the well-understood phenomenon of synaptic transmission and modulation, or whether there is a significantly larger pool of specific molecules that might specify synaptic connectivities. Thus, we pay attention to the recurrence of individual neurons that are part of different molecularly homologous sets of neurons. The other approach is to analyze a given set of antigenically related neurons in detail by means of electrophysiological methods. Here we search for a common functional denominator that might be a coherent function, such as, for example, reproductive behavior. Collaborative efforts are in progress with other laboratories to intensify information on such a particular antibody-labeled set of neurons by characterizing the antigens biochemically and as developmental markers. Although understanding of neural connectivities will very likely be a long-term effort, our monoclonal antibodies have turned out to be a rich resource for shedding new light on specific cell and neurobiological problems of long-standing interest in the leech.

Generation of New Monoclonal Antibodies

Our real hit among the new monoclonal antibodies that were generated last year is an antibody specific for a subset of mechanosensory neurons that respond to noxious stimulation of the skin. Our first antibody, Lan3-2, binds to full sets of nociceptive neurons, whereas our new antibody is a hyperfine immunochemical probe that can distinguish between receptive fields. Other antibodies were generated that (1) also bind to neurons in segmental ganglia innervating the animal's peripheral tissues or (2) bind only to neurons in the head brain and are likely to exercise a higher-order control over lower brain centers through widespread antigenically related processes.

Biochemical Characterization of Leech Antigens

Much of last year was spent in collaboration with Nancy Hogg in beginning the biochemical analysis of leech antigens. Nancy introduced to Cold Spring Harbor the technique of staining immunoblots from acrylamide gels through horseradish peroxidase (HRP)-conjugated second antibodies. Out of 17 antibodies tested, 7 stained bands on immunoblots with molecular weights ranging from 20,000 to 135,000. Many of these antibodies give different staining patterns depending on whether the immunblots were prepared with central nervous system tissue or with our control tissues—the gut and penis. Correlative immunocytochemistry on the gut and penis showed that in these peripheral tissues the antibodies bind to neurons and often epithelial cells. The highlight of this peripheral tissue histochemistry is the discovery of a neuron just below the epithelial gut surface that sends a thick neurite up to contact the gut lumen. This neuron, densely packed with intracellular antigen, is often surrounded by a cloud of antigenically related granular material, which may be the neuron's own neurosecretory products.

The chemistry of the antigens identified by antibodies binding to nociceptive neurons was studied in more detail. Both antibodies, Lan3-2, specific for the full set, and Laz2-369, specific for a subset, bind to fuzzy, high-molecular-weight bands. Both antibodies bind to surface antigens and Felicia Henderson and John Smart have already shown the Lan3-2 antigens to be a glycoprotein.

Using Antibodies as Developmental Markers

In collaboration with R. Stewart and E. Macagno (Columbia University), we started using antibodies as markers to study the development of central and peripheral nervous systems in the leech species *Haemopis* against which the monoclonal antibodies were generated. Since other leech species are generally used for leech embryology, Macagno and Stewart are characterizing early development of *Haemopis* embryos.

Two of our monoclonal antibodies have already provided interesting data on the development of specific nerve cells. For example, Lan3-6, the antibody that binds to about 40 neurons in the adult ganglia during early development (10–11 days), first binds to only a single pair of nerve cells (Fig. 1). At later stages, more cells begin to stain, and by day 11–12 in ganglia 3, 11 cells stain; a day later, there are already 18 stained cells. At all ages, fewer neurons stain in the more posterior ganglia, indicating an anterior-to-posterior gra-

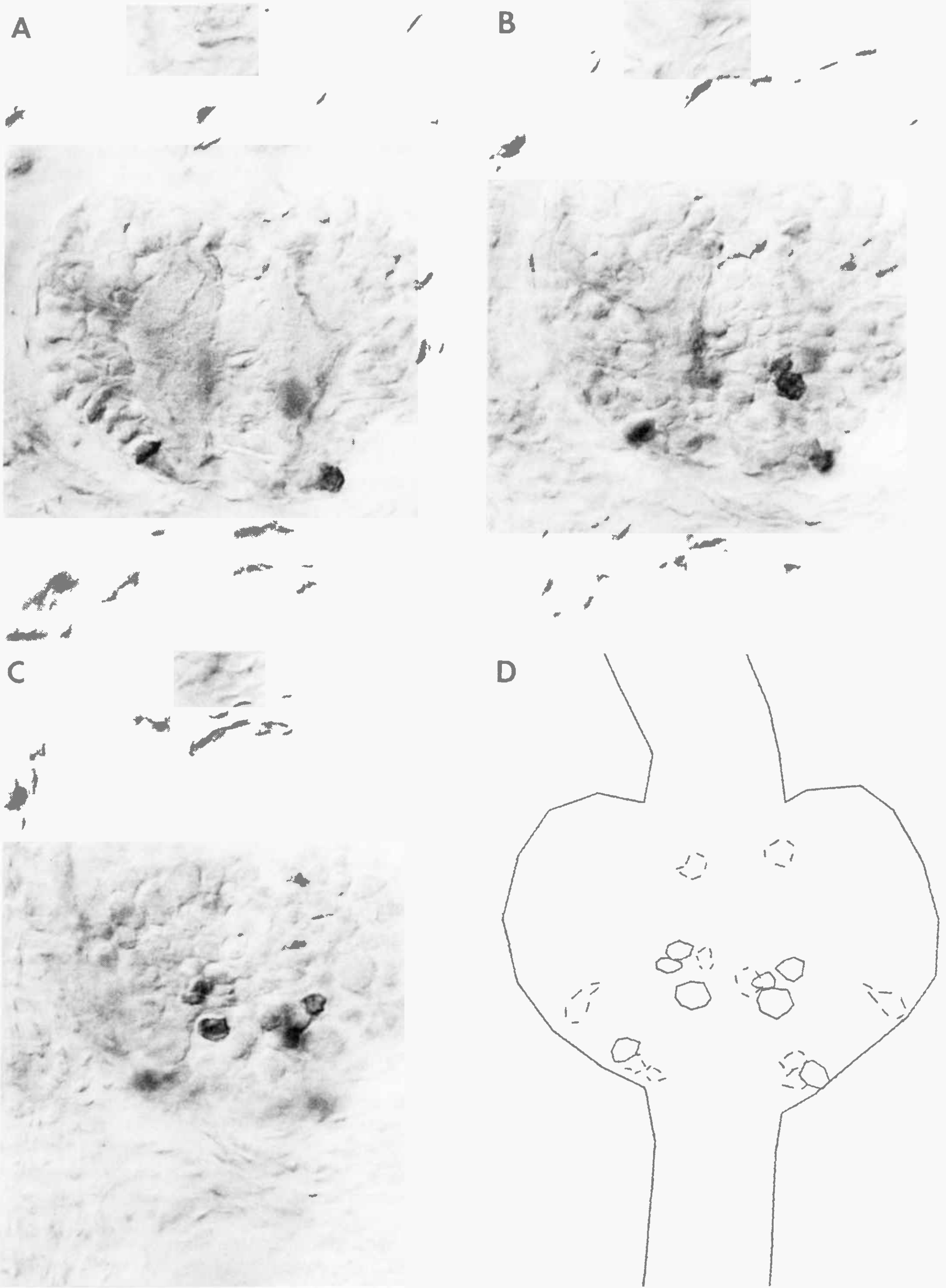

Figure 1
The monoclonal antibody Lan3-6 stains neuronal cell bodies in the 12-13-day-old embryo. (*A-C*) Three different focal planes of ganglion 3 going from the doral to the ventral surface. (*D*) Composite drawing of all labeled cell bodies, the dashed lines showing dorsal and the solid lines ventral cell bodies.

Figure 2
The staining patterns of two antibodies, Lan3-6 (*A*) and Lan3-5 (*B*) that overlap in the pressure cells (arrows). Both antibodies bind to four pressure cells in the standard 400-neuron ganglion.

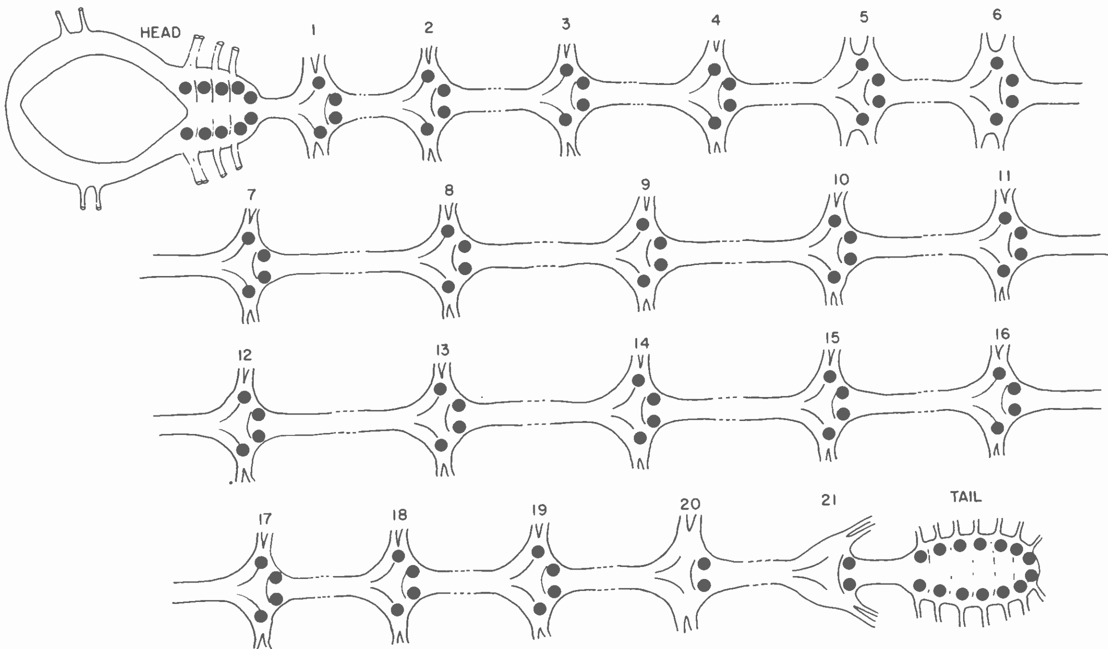

Figure 3
Whole leech nerve cord diagram with pressure cells that jointly appear in the Lan3-5 and Lan3-6 patterns.

dient of nervous system differentiation. An interesting aspect of Lan3-6 is that it stains sensory neurons in the leech central nervous system and sensory receptors on the leech skin. Stewart and Macagno have the first preliminary evidence that there might be a gradient of sensory maturation in the peripheral sensory organs as well.

Mapping Antigenically Homologous Neurons Along the Entire Extent of the Central Nervous System

Monoclonal antibodies are a new tool for studying molecular neuroanatomy. In a simple organism like the leech, monoclonal antibodies can be used to map the whole central nervous system distribution of sets of chemically homologous neurons.

One cell type that was mapped along the entire nerve cord is the primary mechanosensory cells responding to pressure applied to the skin of the leech. For this map, we used two monoclonal antibodies, Lan3-5 and Lan3-6, whose staining patterns overlap in the pressure cells (Fig. 2). Figure 3 illustrates the pressure cell distribution for the whole leech nerve cord.

Publications

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McGlade, E., W.J. Higgins, and B. Zipser. Serotonin's potentiation of acetylcholine induced contractions in the leech penile eversion muscle. (In preparation.)

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Zipser, B. and R. McKay. 1981. Monoclonal antibodies distinguish identifiable neurons in the leech. *Nature* *289:* 549.

ANATOMICAL STUDIES WITH MONOCOLONAL ANTIBODIES

S. Hockfield, *E. Waldvogel*

The large number of monoclonal antibodies that recognize different subsets of nerve cells show us that nerve cells have specific molecular markers. Now we are impelled to determine what these molecules mean to a functioning nervous system. A primary goal of the anatomical studies is to show the subcellular binding sites of antibodies. These sites within a nerve cell can then suggest possible functions of the antigen to which the antibody binds. The antibodies have also been used successfully to explore the organization of nerve cell processes. These studies have shown us the relationships among molecularly homogeneous neural elements and also suggest possible functions of neuron-specific molecules.

Electron Microscopic Localization of Monoclonal Antibodies

We have now overcome the technical obstacles to immuno-electron microscopy. We have determined the binding sites for a number of antibodies; several have been shown to bind to surface molecules and several to internal molecules.

The antibodies that bind to surface molecules are particularly exciting because many hypotheses for how the nervous system establishes and maintains specific neuronal connections postulate surface markers that are involved in cell-to-cell recognition. (Also, nerve cell membranes are specialized to conduct electrical signals and respond to neurotransmitters. Such specialized functions may be mediated by membrane molecules.) Lan3-2, an antibody that recognizes the four neurons in each standard leech ganglion that respond to noxious stimuli, recognizes a molecule found on the surface of both neuron cell bodies and axons (Fig. 1A). The axons stained by Lan3-2 form bundles (fascicles) that are delimited from neighboring axons by processes of a glial cell. In well-preserved tissue, antibody staining is more intense and consistent between axonal and glial membranes than between axonal and axonal membranes. These observations raise several intriguing questions. Is the presence of the antigen recognized by Lan3-2 a result of an axon lying within a particular fascicle, or does the presence of such an antigen direct an axon to grow into a particular fascicle? Is the nerve cell antigen recognized by the glial cell surrounding it, and therefore, does this glial cell that enwraps many different fascicles have specialized areas of its surface to recognize particular neuronal markers? Our current studies on axonal growth and regeneration will address these questions.

Internal molecules are also intriguing since they could be involved in nerve cell metabolism or in the synthesis of neurotransmitters and other neuron-specific products. Antibody Lan3-9 recognizes ten neurons at the head of the leech and nerve cell processes throughout the length of the nervous system. These neurons could be involved in modulating and/or coordinating segmental neural activity. Our studies with Lan3-9 have shown that it recognizes an internal antigen present in the cytoplasm, but not the nucleus, of cell bodies and axons. At the electron microscopic level, antibody staining is associated with vesicular profiles in axons (Fig. 1B). The staining characteristics of Lan3-9 are similar to those shown for neurotransmitters in other systems. These anatomical observations suggest physiological studies that could determine whether Lan3-9 is associated with neurotransmitter function.

Monoclonal Antibodies Demonstrate Organization of Axons in the Leech

One of the major reasons the leech has been a popular organism for neurobiological research is that its nervous system is composed of ganglia containing only 400-neuron cell bodies. The relatively small number of neurons and their consistent positions, sizes, and shapes, which can be resolved with the light microscope, allow one to study the organization of the leech nervous system. In contrast, the connective, a bundle of axons that connects each 400-neuron ganglion to its neighbor, contains more than 5000 axons whose sizes and positions can only be resolved at the electron microscopic level, making it far more difficult to study. We have now used monoclonal antibodies to explore the organization of axons in the leech. The main goals of this study were to determine whether (1) axons carry specific molecular markers, (2) axon position is as consistent as neuron cell body position, and (3) similar mechanisms encode cell body and axon positions.

The initial stages of this study showed us that each of the monoclonal antibodies has a characteristic staining pattern among axons in the connective (Fig. 2). The positions of stained axons are essentially consistent in connectives from all the segments of a single leech, from the head to the tail. Also, these positions are consistent among leeches of the same species and, in many cases, in leeches of other species. These results demonstrate that, like neuron cell body position, axonal position is highly sterotypical and must be determined in a consistent way in all animals.

Although some antibodies stain bundles of axons (Lan3-2 and Lan3-6 in Fig. 2, for example), others stain axons that run singly in the connec-

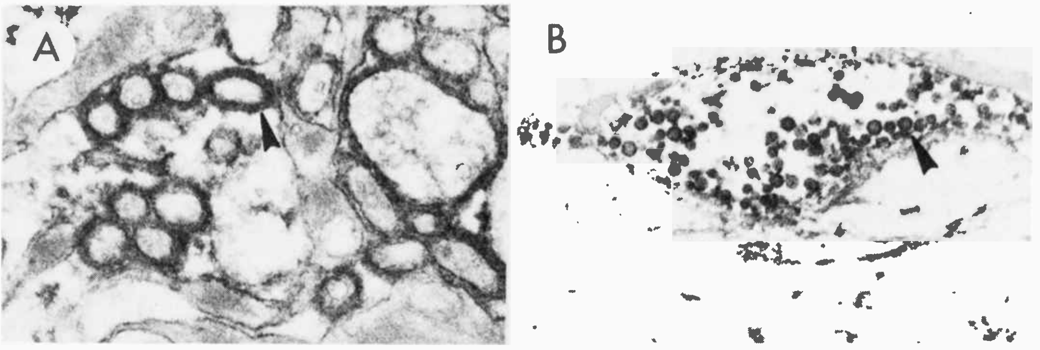

Figure 1
In electron micrographs of monoclonal antibody stained tissue one can recognize labeled axons. (*A*) Lan3-2 stains the perimeter of small-diameter axons in the connective (41,000 ×). (*B*) Lan3-9-stained axons contain HRP-darkened vesicles (10,500 ×).

Figure 2
Cross sections of the connective demonstrate staining of different groups of axons (arrows) with each of three antibodies. (*A*) Lan3-9 stains axons in the medial axon bundle of the connective and in the medial part of each lateral bundle. (*B*) Lan3-6 stains axons in the lateral part of each lateral connective (171 ×). (*C*) Lan3-2 stains fascicles of axons in the ventral part of each lateral connective.

tive (Lan3-9 in Fig. 2, for example). We therefore studied the relationship between grouping patterns of cell bodies and axons stained with a given antibody. Lan3-6 stains cell bodies that are heterogeneous in location and size but stains a distinct bundle of axons in the connective. Lan3-9 stains cell bodies that are homogeneous in location and size but stains single axons in the connective. This shows that axon grouping does not predict neuron cell body grouping. An antigen common to all axons within a group may actually be related to (by determining or as a result of) the formation of that group. In embryological studies, it has been shown that cell body position is determined before axonal outgrowth is complete. Together these studies strongly suggest that different factors govern cell body and axon positions.

Publications

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Hockfield, S. and R. McKay. 1982. Monoclonal antibodies demonstrate the organization of axons in the leech. *J. Neurosci.* (in press).

Hockfield, S., B. Zipser, and R. McKay. 1981. Light and electron microscopic localization of monoclonal antibodies to leech neural tissue. In *New approaches in developmental neurobiology,* p.25. Society for Neuroscience, Maryland.

McKay, R. and S. Hockfield. 1982. Monoclonal antibodies distinguish antigenically discrete neuronal types in the vertebrate central nervous system. (Submitted.)

Zipser, B., S. Hockfield, and R. McKay. 1981. Immunological identification of specific neurons. In *Neurobiology of the leech* (ed. K.J. Muller, J.G. Nicholls, and G.S. Stent), p.235. Cold Spring Harbor Laboratory, New York.

CHARACTERIZING LEECH NEUROANTIGENS

R. McKay, *J. Johansen, L. Kleina*

Several immediate questions were raised by our initial observation that many monoclonal antibodies could be obtained that recognize subsets of neurons in the leech nervous system. The leech nervous system is composed of a chain of ganglia containing 400 cell bodies. This structure is bilaterally symmetrical so that the basic building block can be considered to be a half ganglion of 200 cells. We found at least 30 different specific staining patterns without finding a repeat. A Poisson analysis using these numbers suggests that there is at a minimum sufficient molecular diversity in the leech ganglion for each of the 200 neurons in a half ganglion to be molecularly distinct.

In the past year we have extended our information on particularly interesting antibodies asking questions as to the molecular nature of the antigen, the distribution in the whole central nervous systems of different species of leech, and the appearance of these antigens in the development of the nervous system. In addition, we have, of course, been screening more hybridomas for interesting antibodies against the adult nervous system and, with the help of D. Weisblat (University of California, Berkeley), early embryos of the leech.

A Molecule Present in All Neuron Cell Bodies in the Central Nervous System of the Leech

In screening hybridoma cell lines for those secreting specific antibodies, we found many antibodies that bind to all neurons. One of these, Lan3-8, recognizes all neuron cell bodies in the midbody ganglia of *Hemopis marmorata*. This antibody also recognizes all cell bodies in the related species *Hirudo medicinalis* but fails to bind to neurons in the more distantly related species *Helobdella triserialis*. On Western blots the antibody recognizes a single species of molecule with a molecular mass of 62,000 in *Hemopis* and *Hirudo*. To confirm that the antigen recognized immunohistochemically is the same as that recognized biochemically, we have shown that the same 62K antigen is present in Retzius cells, nociceptive cells, and anterior pagoda cells microdissected from leech ganglia. This result is technically encouraging since it shows that our biochemical procedures are sensitive enough to identify antigens in small numbers of specific cell types, a very small mass of tissue.

Weisblat and his colleagues have shown that the majority of the central nervous system is derived from the expected ectodermal lineage. The most anterior neural structure, the supraesophageal ganglion, has a distinct developmental origin, being derived from cells that also give rise to the endoderm. We have shown both immunohistochemically and biochemically that the Lan3-8 antigen is also present on neurons in the supraesophageal ganglion. With the help of Steve Blose (Cell Biology Section), we compared the proteins in these two differently derived parts of the nervous system by two-dimensional gel electrophoresis and silver staining. We found that the majority of the proteins in these differently derived parts of the leech nervous system cannot be distinguished. However, the silver-stained 2-D gels reveal some quantitative differences between the supraesophageal and segmental ganglia consistent with observations that some of our antibodies recognize antigens present only in cell bodies in the supraesophageal ganglion whereas others recognize antigens present only in segmental ganglia. It is interesting to note here that in studies of neuronal lineages in the nematode *Caenorhabditis elegans*, neurons have been found as sisters of muscle cells in lineages that are predominantly mesodermal (J. Sulston, pers. comm.). Why some neurons are derived from lineages other than the standard ectodermal lineage is a mystery. Antibodies such as Lan 3-8 that identify all central neurons should be useful in the study of neuron differentiation and development.

An Antigen Found Only in Nociceptive Neurons

One of our antibodies, Lan3-2, recognizes the four nociceptive neuron cell bodies in the midbody ganglion of *Hemopis marmorata*. The ability of antibodies such as this to bind specifically to the cells responsive to noxious stimuli while failing to bind to other characterized cells that respond to touch and pressure has important implications. How certain can we be that the antigen recognized by this antibody is synthesized only by nociceptive neurons and not in other neurons?

Although we consistently observe intense staining of nociceptive cell bodies with this antibody, we can also sometimes see light and variable staining of other cell bodies, including P cell bodies, in *H. marmorata*. To ask whether the staining characteristics of these lightly stained cell bodies was the same as the intense staining of N cells, with Susan Hockfield we examined stained ganglia at both the light and electron microscopic levels. At the light microscopic level the N cells were intensely stained while other cells were much more lightly stained. At the electron microscopic level it became apparent that intensely stained cells had antibody bound along the external surface of the cell. In lightly stained

cells the stain was associated with cell processes that were closely opposed to a cell body that was otherwise unstained. These processes appear to derive from other cells, probably those intensely stained with this antibody. Although the cell bodies in the leech are generally free of processes, K. Muller, Carnegie Institution of Washington (pers. commun.) has recently shown by horseradish peroxidase filling that N cells do sometimes have processes that encircle other cell bodies, including P cells. These observations suggest that the light staining of other cell bodies by Lan3–2 may be due to processes of the N cell. Although these results can account for light staining of some cell types, they do not prove that the only cells to synthesize the antigen are N cells.

The antibody Lan3-2 recognizes three major bands on Western blots of proteins extracted from the nervous system of *H. marmorata.* The molecular weights of the bands are similar in the three species of leech we study. We will now be able to ask directly whether all three proteins are present in N cells since we have shown with another antibody, Lan3–8, that we can detect the antigen in small numbers of dissected cells of a particular type.

Curiously, in the other two species of leech we study, even though the molecular weights of the antigens recognized by Lan3-2 are similar to those of *H. marmorata,* no cell bodies normally bind the monoclonal antibody in immunohistochemical preparations. In all three species a subset of axons bind Lan3–2. Since an antigen recognized by Lan3–2 is only present in axons, the possibility exists that even in *H. marmorata* the fibers that bind Lan3-2 are derived from cell bodies other than N cells. We can address this question directly by double-label studies, injecting a marker into physiologically identified cell bodies and looking at cross sections of the axon for coexistence of the marker with the Lan3–2 antigen.

It is still too early to give a definitive answer to the question we posed at the beginning of this section, whether nociceptive neurons are the only neurons that synthesize the Lan3-2 antigen. However our ability to manipulate the simple nervous system of the leech allows us to expect a clear cut answer.

Physiological Studies of Nociceptive Neurons Based on Antibody Staining Patterns

An advantage in using monoclonal antibodies to examine the nervous system is that they can rapidly demonstrate the distribution of molecularly homogeneous neurons and show differences in neuron distributions between segmental ganglia. For example, Lan3-2, the antibody that stains the four nociceptive neurons in each standard ganglion binds to only two neurons in the fifth and sixth segmental ganglia. Physiological studies which Susan Hockfield and I started during the 1981 Leech neurophysiology course have shown that the two neurons recognized by Lan3-2 in these two ganglia are similar in many ways to nociceptor neurons in other ganglia.

Intracellular electrophysiological studies confirmed the immunocytochemical observation that only a single pair of N cells can be found in ganglia 5 and 6. Physiologically, these cells are similar to other N cells in action potential characteristics and in their ability to be antidromically activated by stimulating peripheral roots. Intracellular injections of tracers showed that anatomically they are also similar to other N cells in their axonal projections into the connective and roots and in the shape and distribution of axonal arbors. However, a major difference exists in the peripheral fields of innervation of these cells. Normally, each N cell innervates the skin of an entire segment. Each N cell in ganglia 5 and 6 innervates only a very small area of skin within a segment. The remaining areas are fully innervated by N cells in the next two more-rostral and more-caudal ganglia. These observations suggest that molecular characteristics of N cells recongized by antibody Lan3-2 may be independent of receptive-field characteristics but could be related to other features of N cells that distinguish them from other neurons. These features might include mechanisms by which action potential size and shape are determined (i.e., ionic channels), by which neuron cell body or axon position is determined, or other features, as yet unexplored, such as common neurotransmitter or embryological origin.

Embryology

We have used the antibody Lan3-2, to determine when in the development of the nervous system crossreacting antigens are first expressed. In contrast to other monoclonal antibodies specifically raised against leech embryos, Lan3-2 first binds to leech embryos at stage 8. This stage, which is equivalent to gastrulation, is the time at which segmental organization of the animal first becomes apparent. The antibody recognizes an antigen in the ectodermal germinal bands that will give raise to the nervous system and also in the micromere cap which is of mesodermal origin. Biochemical characterization will tell us how similar the antigens present at this early stage in development are to those found in the adult nervous system. Our preliminary observations already show that antigenically related molecules occur in cells that are mesodermally derived and not destined to form part of the nervous system.

In addition to using the antibodies generated against the adult nervous system to ask developmental questions, with the collaboration of D. Weisblat (University of California, Berkeley) we are beginning to raise monoclonal antibodies specifically against early embryonic stages of the leech. In the first series of hybridoma cell lines to be screened, most of the antibodies stained leech

embryos uniformly, but one line secreted an antibody that recognizes an antigen that is apparently restricted to ectodermal and mesodermal precursor cells. We hope to find more antibodies that identify differentiation antigens of the neural ectoderm. As with the adult nervous system, we hope that a better understanding of the cellular distribution of specific molecules will lead to an insight into the mechanisms that first differentiate neurons from other cells.

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Gooding, L.R., E. Harlow, and R. McKay. Expression of SV40 T antigen on the surface of viral transformed cells. (In preparation.)

Harshey, R.M., R. McKay, and A. Bukhari. 1982. DNA intermediates in the transposition of phage Mu. *Cell* (in press).

Hockfield, S. and R. McKay. Monoclonal antibodies demonstrate the organization of axons in the leech. *J. Neurosci.* (in press).

McKay, R. 1982. An immunoassay for sequence specific DNA-protein interactions. *Methods Enzymol.* (in press).

——. 1982. Molecular approaches to the nervous system. *Annu. Rev. Neurosci.* (in press).

McKay, R. and S. Hockfield. Monoclonal antibodies distinguish antigenically discrete neuronal types in the vertebrate CNS. (Submitted.)

Zipser, B., S. Hockfield, and R. McKay. 1981. Immunological identification of specific neurons. In *Neurobiology of the leech* (ed. K.J. Muller, J.G. Nicholls, and G.S. Stent), p. 235. Cold Spring Harbor Laboratory, New York.

Single-channel Recording Workshop, July 1-July 19

INSTRUCTORS

Patlak, Joseph, Ph.D, University of Vermont, Burlington
Horn, Richard, Ph.D., University of California, Los Angeles
Sigworth, Frederick, Ph.D., Max-Planck-Institute, Goettingen, Federal Republic of Germany
Corey, David, Ph.D., Yale University, New Haven, Connecticut
Jackson, Meyer, Ph.D., University of California, Los Angeles

The Single-channel Recording workshop gave the opportunity for 11 investigators with previous electrophysiology experience to learn the patch-clamp technique for recording the currents through single-membrane channels. Each of the five instructors set up a single-channel recording system for use during the workshop. Students ranged in experience from the graduate to the established investigator level. Lecture material presented by the instructors included extensive discussion of the techniques for obtaining high quality recordings, with special emphasis on minimizing the background noise and increasing the frequency response of the patch-clamp circuit. The 'tricks' for making electrodes, establishing gigohm seals, excising membrane, and recording from whole cells were demonstrated and practiced on the five different recording set-ups. The technique was applied successfully to a variety of preparations, including neuroblastoma, photoreceptor, smooth muscle, pituitary cells, and neurons dissociated from cortex, spinal cord, and sympathetic ganglion. Methods for the analysis of single-channel data were also discussed and demonstrated.

PARTICIPANTS

Dani, John A., Ph.D., University of Washington, Seattle
Dubinsky, Janet, B.S., University of North Carolina, Chapel Hill
French, Robert J., Ph.D., University of Maryland, Baltimore
Hahin, Richard, Ph.D., University of Iowa, Iowa City
Horn, John P., Ph.D., Harvard Medical School, Boston, Massachusetts
Levis, Richard, M.S., Rush University, Chicago, Illinois
Nowak, Linda M., Ph.D., University of Michigan, Ann Arbor
Schwartz, Eric A., M.D., University of Chicago, Illinois
Serrano, Elba E., Ph.D., Stanford Medical School, California
Singer, Joshua J., Ph.D., University of Massachusetts, Worcester
Walsh, John V., Jr., M.D., University of Massachusetts, Worcester

Electrophysiology of the Mammalian Brain Slice

August 12–August 25

INSTRUCTORS

Kelly, John S., Ph.D., St. George's Medical School, University of London, England
Andersen, Per, M.D., University of Oslo, Norway
Wilson, Wilkie A., Ph.D., Duke University, Durham, North Carolina
Alger, Bradley E., Ph.D., University of Maryland, College Park

ASSISTANTS

Crunelli, Vincenzo, Ph.D., St. George's Medical School, University of London, England
Iadarole, Michael, Ph.D., Duke University, Durham, North Carolina

In this course students were acquainted with a variety of approaches to the electrophysiological study of the in vitro mammalian brain slice, with emphasis on the hippocampal slice. Five experimental set-ups were operated. In addition to a general focus on intracellular recording techniques, special procedures represented were: (1) extracellular methods for analysis of synaptic pathways and response plasticity (Andersen); (2) analysis of drug and neurotransmitter actions using iontophoretic (Kelly and Crunelli) or constant perfusion bath application (Alger) methods; (3) single-electrode voltage-clamp recording (Wilson and Iadarola).

After an orientation period with one instructor in which pairs of students gained practice preparing and then stimulating and recording hippocampal slices, students rotated through all of the other laboratories. By the end of the course every student had successfully demonstrated an ability to prepare slices and to record intracellularly from cells in the hippocampal slice. In the evenings seminars were given by the instructors and on weekends students presented summaries of their own previous work. During the second week of the course students were encouraged to pursue individual projects with a minimum of supervision from the instructors. These projects included studies of the pharmacological effects of norepinephrine as well as extensions of the slice technique to other brain areas, including the lateral geniculate nucleus.

PARTICIPANTS

Jackson, Meyer B., Ph.D., University of California, Los Angeles
Martin, Michael R., Ph.D., National Institutes of Health, Bethesda, Maryland
Moore, Scott D., B.A., University of Virginia, Charlottesville
Neuman, Richard S., Ph.D., Memorial University, St. John's, Newfoundland, Canada
Rogawski, Michael A., Ph.D., National Institutes of Health, Bethesda, Maryland
Sanders, David J., Ph.D., University of Newcastle-upon-Tyne, England
Seifert, Wilfried, Ph.D., Max-Planck-Institute, Tubingen, Federal Republic of Germany
Smock, Timothy K., B.A., University of California, San Francisco
Vallano, Mary Lou, Ph.D., Sloan-Kettering Institute, New York, New York

SEMINARS

Andersen, P., University of Oslo. *Anatomy of the hippocampal slice.*
———. *Physiology of the hippocampal slice.*
Kelly, J.S., University of London. *Analysis of neurotransmitter actions—Classical neurotransmitters.*
———. *Analysis of neurotransmitter actions—Neuropeptides.*
Alger, B.E., University of Maryland. *Somatic and dendritic inhibition in the hippocampus.*
Wilson, W.A., Duke University. *Time-sharing single-electrode voltage clamp—Theory and practice.*

46th Cold Spring Harbor Symposium on Quantitative Biology

Organization of the Cytoplasm, May 27–June 3

Any serious scientist working with cells must of necessity view them with awe. Even the relatively simple cells of *E. coli* have the potential to make as many as 2000 different proteins and at least this number must be found at a given time in any eukaryotic cell. While we now know the main biochemical pathways through which cells utilize the energy of food (the sun) first to make cellular building blocks and later to polymerize them into macromolecules, it is highly probable that for any given cell the majority of its molecules have not yet been named, much less ascribed functional roles. Of necessity, we have largely concentrated on molecules present in relatively large numbers per cell, always attempting to understand vital cell functions (e.g., cell motility) in terms of their properties. We know, however, that even the best of our hypotheses are likely to be incomplete and that the understanding of the cell at the molecular level will have many many surprises to offer.

Until very recently the most fruitful approach for cell biologists has been to use microscopes to probe directly the morphology of cells, and with the advent of the electron microscope in the 1950s, our better cell biologists have had their field days. Happily, one after another of the objects (organelles) they have discovered (e.g., microtubules) have been dissected at the molecular level, and by now the distinction between the cell biologist and the biochemist is fading fast. The younger generation of cell biologists, in particular, know that they themselves must do much of the necessary biochemistry, with their careers in jeopardy were they to depend upon help from card-carrying biochemists. At the same time, many of our most astute biochemists know that most of their traditional goals are near complete solutions and that only by taking on the complexities of cell movement, cell differentiation, and cell division will their future lives remain exciting.

The time had thus arrived to hold our annual Symposium on cells, focusing it on "The Organization of the Cytoplasm," knowing that we had to exclude the nucleus or we would be holding another Symposium on the gene. In organizing this Symposium, we sought the advice of many experts and, in particular, we wish to thank Günter Blobel, Daniel Branton, Bill Brinkley, Werner Franke, Howard Green, Robert D. Goldman, Marc Kirschner, Keith Porter, Peter Satir, John Singer, Frank Solomon, Klaus Weber, and K. Wolfarth-Bottermann. The formal program contained 93 presentations to which the reports of several last minute presentations have been added. The total attendance was 234, a smaller number than usual, but one dictated by our desire to have virtually everyone view the meeting in person as opposed to looking at electron micrographs imperfectly through closed-circuit T.V.

The most necessary financial support that let us invite so many participants was again provided by the National Institutes of Health, the National Science Foundation, and the Department of Energy.

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

Session 1: Principles of Organization: Functional and Spatial Order in the Cytoplasm

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

E. Racker, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: A protein kinase cascade activated by transformation.

D. Branton, Dept. of Cell and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Evaluating cytoskeleton-membrane interactions.

G. Blobel, Laboratory of Cell Biology, Rockefeller University, New York, New York: Regulation of intracellular protein traffic.

F. Solomon, Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Organization of the cytoplasm for motility.

Session 2A: *Principles of Organization: Water and the Cytoplasmic Architecture*

Chairperson: R. Allen, Dartmouth College, Hanover, New Hampshire

J. S. Clegg, Laboratory for Quantitative Biology, University of Miami, Coral Gables, Florida: Interrelationships between water and cell metabolism in *Artemia* cysts. IX. Further metabolic studies and D_2O substitution.

J. W. Wojcieszyn,[1] R. A. Schlegel,[2] and K. A. Jacobson,[1] [1]Dept. of Anatomy, University of North Carolina, Chapel Hill; [2]Molecular and Cell Biology Program, Pennsylvania State University, University Park: Measurements of the diffusion of macromolecules injected into the cytoplasm of living cells.

G. Albrecht-Buehler, Cold Spring Harbor Laboratory, New York: Is blebbing of the cell surface caused by liquid streams inside cells?

K. R. Porter and K. Anderson, Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The cytoplasmic matrix of frozen-dried cells shows the same morphology as that in glutaraldehyde fixed cells.

M. Schliwa and J. van Blerkom, Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Structural organization of the cytoplasm.

Session 2B: *Elements of Organization: Microtubules I*

C. Silflow, J. Schloss, T. McKeithan, L. Keller, and J. Rosenbaum, Dept. of Biology, Yale University, New Haven, Connecticut: Expression of flagellar protein genes during flagellar regeneration in *Chlamydomonas*.

D. W. Cleveland, Dept. of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: Examination of the genes coding for α and β tubulin.

P. Wensink and L. Kalfayan, Dept. of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts: The structure and expression of the *Drosophila* α-tubulin gene family.

Session 3: *Cell Surface: Surface Organization*

Chairperson: J.-P. Revel, California Institute of Technology, Pasadena

J. P. Revel, B. N. Nicholson, S. B. Yancey, and D. J. Meyer, California Institute of Technology, Pasadena: The hepatic gap junction.

E. L. Hertzberg and N. B. Gilula, Rockefeller University, New York, New York: Studies on mammalian gap junctions.

V. Bennett, Dept. of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland: The molecular basis for membrane-cytoskeleton associations in human erythrocytes.

R. O. Hynes, A. T. Destree, and D. D. Wagner, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Relationships between microfilaments, cell-substratum adhesion, and fibronectin.

B. Geiger, Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: Substrate-attached membranes of cultured cells.

R. A. Bloodgood, Dept. of Anatomy, University of Virginia School of Medicine, Charlottesville: The flagellum as a model system for studying dynamic cell surface events.

T. Roberts[1] and S. Ward,[2] [1]Dept. of Biological Science, Florida State University, Tallahassee; [2]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Directed membrane flow on the pseudopods of *C. elegans* spermatozoa.

Session 4: *Mechanisms of Organization: Cytoplasmic and Axonal Flow*

Chairperson: N. Kamiya, National Institute for Basic Biology, Okazabi, Japan

S. Higashi-Fujime, Institute of Molecular Biology, Nagoya University, Japan: Active movement of bundles composed of muscle F-actin and myosin filaments—A simple model system for nonmuscle cell motility.

N. Kamiya, Y. Yoshimoto, and F. Matsumura, Dept. of Cell Biology, National Institute for Basic Biology, Okazaki, Japan: Contraction-relaxation cycle of *Physarum* cytoplasm—Concomitant changes in intraplasmodial ATP and Ca^{2+} concentrations.

R. D. Allen,[1] J. L. Travis,[1] J. Hayden,[1] N. S. Allen,[1] and A. C. Breuer,[2] [1]Dartmouth College, Hanover, New Hampshire; [2]Cleveland Clinic, Ohio: Cytoplasmic transport systems in eukaryotic cells.

K. E. Wohlfarth-Bottermann and I. Block, Institute of Cytology, University of Bonn, Federal Republic of Germany: The function of cytoplasmic flow in photosensory transduction and phase regulation of contractile activities in *Physarum*.

D. Taylor, J. Heiple, Y.-L. Wang, E. Luna, L. Tanasugarn, J. Brier, J. Swanson, M. Fechheimer, P. Amato, and M. Rockwell, Dept. of Cell and Developmental Biology, Harvard University, Cambridge, Massachusetts: Cellular and molecular aspects of amoeboid movement.

R. J. Lasek and S. T. Brady, Dept. of Anatomy and Neurobiology Center, Case Western Reserve, Cleveland, Ohio: The Axon—A prototype for studying expressional cytoplasm.

H. Thoenen, M. Schwab, and R. Heumann, Dept. of Neurochemistry, Max-Planck-Institute of Psychiatry, Martinsried, Federal Republic of Germany: Physiological importance and site of action of nerve growth factor (NGF) reaching the perikaryon of adrenergic neurons by retrograde axonal transport.

L. B. Chen, I. C. Summerhayes, L. V. Johnson, M. L. Walsh, S. D. Bernal, and T. J. Lampidis, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Probing mitochondria in living cells with Rhodamine 123.

Session 5: *Poster Session*

R. D. Allan,[1] J. L. Travis,[1] J. Hayden,[1] N. S. Allen,[1] and A. C. Breuer,[2] [1]Dartmouth College, Hanover, New Hampshire; [2]Cleveland Clinic, Ohio: Cytoplasmic transport systems in eukaryotic cells.

J. Avila, A. Villasante, and M. M. Valdivia, CSIC-UAM, Universidad Autónoma de Madrid, Spain: Binding of microtubule associated protein MAP_2 to chromosome components.

S.T. Brady and R.J. Lasek, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio: Axonal microtubules—A special class of brain microtubules.

D.J. Goldberg,[1] D. Harris,[2] and J.H. Schwartz,[2] [1]Dept. of Pharmocology and [2]Dept. of Physiology, Columbia University College of Physicians and Surgeons, New York, New York: Studies on the mechanism of fast axonal transport using microinjection into single giant neurons.

S.B. Horowitz, Dept. of Biology, Michigan Cancer Foundation, Detroit, Michigan: Reference phase analysis of cytoplasm.

E.-M. Mandelkow,[1] E. Mandelkow,[1] and J. Bordas,[2] [1]Max-Planck-Institute for Medical Research, Heidelberg; [2]European Molecular Biology Laboratory, Hamburg, Federal Republic of Germany: Structural transitions during microtubule assembly studied by time-resolved X-ray scattering.

F. Matsumura and J.J.-C. Lin, Cold Spring Harbor Laboratory, New York: Monoclonal antibodies to tropomyosin decorate native thin filaments with 38NM periodicity.

J.R. Morris and R.J. Lasek, Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio and Marine Biological Laboratory, Woods Hole, Massachusetts: Measurements of the concentrations of tubulin and actin as monomer and polymer in the axon.

J. Parness and S.B. Horwitz, Depts. of Molecular Pharmacology and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Taxol binds to polymerized tubulin.

D. Paulin, C. Babinet, H. Jakob, and F. Jacob, Service de Génétique cellulaire du Collége de France and de l'Institut Pasteur, Paris: Intermediate-filaments as markers of mouse early development and teratocarcinoma cell differentiation.

H. Ponstingl, M. Little, E. Krauhs, T. Kempf, W. Ade, and R. Hofer-Warbinek, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Amino acid sequence of α- andβ-tubulin from pig brain.

F.C.S. Ramaekers,[1] I. Dunia,[2] E.L. Benedetti,[2] and H. Bloemendal,[1] [1]Dept. of Biochemistry, University of Nijmegen, The Netherlands; [2]Institut de Biologie Moléculaire CNRS, University of Paris, France: Lenticular intermediate-sized filament protein, its identification, biosynthesis, and interaction with the plasma membrane.

F.C.S. Ramaekers,[1] P.H.K. Jap,[2] and G.P. Vooijs,[1] [1]Dept. of Pathology and [2]Dept. of Cytology and Histology, University of Nijmegen, The Netherlands: The use of antibodies directed against intermediate filaments in the characterization of human malignant tumors.

D.D. Sabatini, M.A. Adesnik, G. Kreibich, T. Morimoto, D. Colman, J. Sherman, and E. Sabban, Dept. of Cell Biology, New York University School of Medicine, New York: Biosynthesis of plasma membrane proteins.

J.L. Salisbury, J.S. Condeelis, N.J. Maihle, and P. Satir, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Calmodulin redistributes during capping of cell surface receptors.

I.V. Sandoval[1] and J. Vanderkerckhove,[2] [1]California Institute of Technology, Pasadena; [2]Laboratory of Histology and Genetics, Gent, Belgium: A comparative study on the capacity of MAP_2 and Tau to promote microtubule polymerization and to bind to microtubules.

E. Schulze[1] and S. Blose,[2] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Cold Spring Harbor Laboratory, New York: Maintenance of cytoplasmic continuity to small molecules between daughter cells after mitosis.

R. Singer and J. Pudney, Dept. of Anatomy, University of Massachusetts Medical School, Worcester: Morphomolecular basis of muscle differentiation.

R.W. Tucker,[1] L.-H. Liawa,[2] and M.W. Berns,[2] [1]Johns Hopkins Oncology Center, Baltimore, Maryland; [2]University of California, Irvine: DNA synthesis and centriole duplication in PTK_2 cells.

J.A. Weatherbee,[1] P. Sherline,[2] R.N. Mascardo,[2] J.G. Izant,[3] R.B. Luftig,[4] and R.R. Weihing, [1] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]University of Connecticut Health Center, Farmington; [3]University of Colorado, Boulder; [4]University of South Carolina School of Medicine, Columbia: Microtubule-associated protein of HeLa cells.

Session 6: *Elements of Organization: Intermediate Filaments I*

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

H. Green, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Differentiated structural elements in the keratinocyte.

E.H. Ball and S.J. Singer, Dept. of Biology, University of California, La Jolla: Correlations of the distributions of microtubules and intermediate filaments, and of microtubules and mitochondria, in cultured cells as determined by double immunofluorescence microscopy.

R. Goldman,[1] A. Goldman,[1] J. Jones,[1] J. Talian,[1] P. Steinert,[2] M. Whitman-Aynardi,[1] S. Yuspa,[2] and R. Zackroff,[1] [1]Dept. of Cell Biology and Anatomy, Northwestern University, Chicago, Illinois; [2]NCI, National Institutes of Health, Bethesda, Maryland: Organization and functions of intermediate filaments (IF).

H. Holtzer, G. Bennett, S. Tapscott, and J. Croop, Dept. of Anatomy, University of Pennsylvania Medical School, Philadelphia: Variations in the protein subunits of the intermediate-sized filaments associated with differentiation.

M. Shelanski, R. Liem, and J.-F. Leterrier, Dept. of Pharmacology, New York University School of Medicine, New York: Neurofilament chemistry and interactions with microtubules.

E. Lazarides, B.L. Granger, D.L. Gard, R.H. Gomer, and J. Breckler, Division of Biology, California Institute of Technology, Pasadena: Steps in the assembly of the Z-disc in muscle cells.

J.E. Célis,[1] R. Bravo,[1] J.V. Small,[2] J. Fey,[1] and P.M. Larsen,[1] [1]Dept. of Chemistry, Aarhus University, Denmark;[2] Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg: Modification of vimentin polypeptides during mitosis.

Session 7: Elements of Organization: Microfilaments I

Chairperson: K. Wohlfarth-Bottermann, University of Bonn, Federal Republic of Germany

E.A. Fyrberg,[1] B.J. Bond,[2] N.D. Hershey,[1] K.S. Mixter,[2] and N. Davidson,[1] [1]Dept. of Chemistry, [2]Division of Biology, California Institute of Technology, Pasadena: The actin genes of *Drosophila*—Protein coding regions are highly conserved but intron positions are not.

M. McKeown and R.A. Firtel, Dept. of Biology, University of California, San Diego, La Jolla: The actin multigene family of *Dictyostelium*.

S.H. Hughes, J. Sorge, J.P. Thomas, J. Feramisco, L. Chow, and J. Garrels, Cold Spring Harbor Laboratory, New York: Approaches for isolating genes encoding the cytoskeletal proteins and for reintroducing the genes into cultured cells.

T.D. Pollard, U. Aebi, J.A. Cooper, W.E. Fowler, and P. Tseng, Dept. of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland: Actin structure, polymerization, and gelation.

L. Tilney[1] and D. DeRosier,[2] [1]Dept. of Biology, University of Pennsylvania, Philadelphia; [2]Brandeis University, Rosenstiel Center, Waltham, Massachusetts: How actin filaments pack into bundles.

K. Weber and J.R. Glenney, Jr., Max-Planck-Institute for Biological Chemistry, Göttingen, Federal Republic of Germany: Calcium control of microfilament assembly and turnover.

J.A. Spudich, P.A. Simpson, J. Pardee, L. Stryer, S.S. Brown, K. Yamamoto, A. Weeds, and E.R. Kuczmarski, Dept. of Structural Biology, Fairchild Center, Stanford University, California: Control of assembly of *Dictyostelium* actin and myosin filaments.

Session 8: Cell Surface: Clathrin

Chairperson: M. Brown, University of Texas Health Science Center, Dallas

M.S. Bretscher, MRC Laboratory of Molecular Biology, Cambridge, England: Surface uptake by fibroblasts and its consequences.

M.S. Brown and J.L. Goldstein, Dept. of Molecular Genetics, University of Texas Health Science Center, Dallas: Genetic studies of the low density lipoprotein receptor.

E. Ungewickell, E.R. Unanue, and D. Branton, Dept. of Cell and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: An investigation of the structure of clathrin and its membrane interaction.

J. Wehland, M.C. Willingham, R. Dickson, and I. Pastan, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Microinjection of anti-clathrin antibodies into fibroblasts does not interfere with the receptor-mediated endocytosis of α_2-macroglobulin.

T.A. Libermann, B.A. Imhof, and W. Birchmeier, Laboratorium für Biochemie, Federal Institute of Technology (ETH), Zürich, Switzerland: Transport of newly synthesized cellular glycoproteins (e.g. fibronectin) from the endoplasmic reticulum to the golgi to the plasma membrane via coated vesicles.

Session 9: Elements of Organization: Microtubules II

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

M.W. Kirschner[1] and D.W. Cleveland,[2] [1]Dept. of Biochemistry and Biophysics, University of California Medical Center, San Francisco; [2]Dept. of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland: The regulation of tubulin monomer levels in the cell—Evidence for transcriptional control and implications for spatial organization of microtubules.

G.G. Borisy, University of Wisconsin, Madison: How microtubules lengthen, shorten, and also treadmill in the steady-state.

L. 'ilson,[1] K. W. Farrell,[1] M. A. Jordan,[1] and R. L. Margolis,[2] [1]Dept. of Biological Sciences, University . California, Santa Barbara; [2]Hutchinson Cancer Research Center, Seattle, Washington: Microtubule treadmills—Possible molecular machinery.

L. T. Haimo[1] and B. R. Telzer,[2] [1]Dept. of Biology, University of California, Riverside; [2]Dept. of Biology, Pomona College, Claremont, California: Dynein binding to spindle microtubules.

M. de Brabander, G. Geuens, R. Nydens, R. Willebrords, and J. De Mey, Laboratory of Oncology, Janssen Pharmaceutica Research Laboratories, Beerse, Belgium: Microtuble stability and assembly in living cells—The influence of metabolic inhibitors, taxol and pH.

B. R. Brinkley,[1] S. L. Brenner,[1] D. A. Pepper,[2] and R. L. Pardue,[1] [1]Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas; [2]Laboratory of Molecular Biology, University of Wisconsin, Madison: Tubulin assembly sites and the organization of microtubule arrays in mammalian cells.

M. Chalfie and J. N. Thomson, MRC Laboratory of Molecular Biology, Cambridge, England: Microtubule structure in *C. elegans* neurons.

A. S. Bajer and J. Molè-Bajer, Dept. of Biology, University of Oregon, Eugene: Microtubule (MT) arrays in enucleated cell fragments and characteristics of asters in standard amphiastral (animal) and anastral (higher plant) spindles.

Session 10: Mechanisms of Organization: Calcium-regulation and Phosphorylation of Cytoplasmic Components

Chairperson: E. Racker, Cornell University, Ithaca, New York

R. W. Wallace, E. A. Tallant, and W. Y. Cheung, Dept. of Biochemistry, St. Jude Children's Research Hospital, and University of Tennessee Center for the Health Sciences, Memphis: Calmodulin—A multifunctional modulator protein of Ca^{2+}-regulated cellular functions.

A. R. Means, J. G. Chafouleas, W. Bolton, and A. E. Boyd III, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Calmodulin may regulate the G_1/S transition during the growth cycle of mammalian cells.

A. H. Lockwood, D. Trivette, and M. Pendergast, Laboratories for Cell Biology, University of North Carolina, Chapel Hill: Association of cAMP and calcium-dependent protein kinases with the cytoskeleton—Implications for motility, transformation, and cytoplasmic organization.

R. S. Adelstein, M. D. Pato, P. de Lanerolle, J. R. Sellers, and M. A. Conti, NHLBI, National Institutes of Health, Bethesda, Maryland: Regulation of contractile proteins by reversible phosphorylation.

J. M. Scholey and J. Kendrick-Jones, MRC Laboratory of Molecular Biology, Cambridge, England: Regulation of myosin filament assembly by light chain phosphorylation.

B. Sefton,[1] T. Hunter,[1] and J. Singer,[2] [1]Tumor Virology Laboratory, Salk Institute, San Diego, California; [2]Dept. of Biology, University of California, San Diego: Cytoskeletal targets for viral transforming proteins.

L. Rohrschneider, M. Rosok, and K. Shriver, Fred Hutchinson Cancer Research Center, Seattle, Washington: On the mechanism of transformation by Rous sarcoma virus—Events within adhesion plaques.

Session 11: Elements of Organization: Intermediate Filaments II

Chairperson: M. Osborn, Max-Planck-Institute, Göttingen, Federal Republic of Germany

E. B. Lane, Imperial Cancer Research Fund, London, England: Variability in tonofilaments, with a possible marker for squamous cell transformation.

B. S. Eckert and R. A. Daley, Dept. of Anatomical Sciences, State University of New York, Buffalo: In vivo disruption of the cytokeratin cytoskeleton in cultured epithelial cells—Microinjection of antikeratin.

M. Osborn, N. Geisler, G. Shaw, and K. Weber, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Federal Republic of Germany: Expression and regulation of intermediate filaments.

W. W. Franke, E. Schmid, E.-D. Jarasch, D. L. Schiller, J. Stadler, S. Winter, R. Moll, M. Williams, R. Sommer, J. Kartenbeck, and G. Krohne, Institute of Cell and Tumor Biology, German Cancer Research Center, Heidelberg, Federal Republic of Germany: Differentiation specificity of expression of protein constituents of "insoluble" cytoskeletal elements.

P. Steinert,[1] W. Idler,[1] M. Aynardi,[2] R. Zackroff,[2] and R. Goldman[2] [1]Dermatology Branch, NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburg, Pennsylvania: Heterogeneity of intermediate filaments assembled in vitro.

G. Wiche, M. Baker, and F. Leichtfried, Institute of Biochemistry, University of Vienna, Austria: Book polypeptides are components of cultured cell cytoskeletons and interact with vimentin containing filaments in vitro.

M.S. Runge and R.C. Williams, Jr., Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Formation of a microtubule-neurofilament complex in vitro.

Session 12: *Cell Surface: Synthesis, Exocytosis, Endocytosis*

Chairperson: D. Sabatini, New York University School of Medicine, New York

S.C. Silverstein, J. Michl, J. Loike, and J. Unkeless, Laboratory of Cellular Physiology and Immunology, Rockefeller University, New York: On the mechanism of phagocytosis.

A. Novikoff and P. Novikoff, Dept. of Pathology, Albert Einstein College of Medicine, Bronx, New York: The Golgi-apparatus; Conventional wisdom.

H.F. Lodish, W. Braell, S. Fridovich, M. Porter, D. Rup, A.L. Schwartz, and A. Zilberstein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Synthesis and assembly of the VSV glycoprotein, the erythrocyte anion transport protein, and the hepatic receptor for asialoglycoproteins.

J.E. Rothman, E. Fries, and W. Dunphy, Dept. of Biochemistry, Stanford University, California: Intracellular transport of the vesicular stomatitis viral glycoprotein in cells and in cell-free systems.

D.D. Sabatini, M.A. Adesnik, G. Kreibich, T. Morimoto, D. Colman, J. Sherman, and E. Sabban, Dept. of Cell Biology, New York University School of Medicine, New York: Biosynthesis of plasma membrane proteins.

H. Pollard, C. Creutz, C. Pazoles, J. Scott, and V. Fowler, NIAMDD, National Institutes of Health, Bethesda, Maryland: Regulation of organelle movement, membrane fusion, and exocytosis in the chromaffin cell.

A. Helenius, M. Marsh, K. Matlin, H. Reggio, K. Simons, and J. White, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Are lysosomes the site of virus entry?

Session 13: *Elements of Organization: Microfilaments II*

Chairperson: K. Weber, Max-Planck-Institute, Göttingen, Federal Republic of Germany

K.E. Wohlfarth-Bottermann and W. Stockem, Institute of Cytology, University of Bonn, Federal Republic of Germany: Fine structure and organization of microfilaments in *Physarum* as revealed by chemical fixation and freeze-etching.

T. Stossel, K. Zaner, J. Hartwig, H. Yin, and F. Southwick, Dept. of Medicine, Harvard Medical School, Boston, Massachusetts: Effects of macrophage cytoplasmic proteins on actin consistency.

J. Condeelis, Albert Einstein Colllege of Medicine, Bronx, New York: Composition, structure, and function of the cortical actin lattice.

A. Asano and N. Mimura, Institute for Protein Research, Osaka University, Japan: Actinogellin, a calcium-sensitive microfilament regulatory protein.

K. Burridge and J.R. Feramisco, Cold Spring Harbor Laboratory, New York: Nonmuscle α-actinins—Calcium sensitive actin-binding proteins.

J.V. Small, Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg: Organization of actin in the leading edge of cultured cells—Influence of osmium tetroxide and dehydration on the ultra-structure of actin meshworks.

B.M. Jockusch[1] and G. Isenberg,[2] [1]European Molecular Biology Laboratory, Heidelberg; [2]Max-Planck-Institute for Psychiatry, Munich, Federal Republic of Germany: Interaction of α-actinin and vinculin with actin—Opposite effects on filament network formation.

S. Lin, D.C. Lin, M. Grumet, D.H. Cribbs, J.A. Wilkins, and W.W. Magargal, Dept. of Biophysics, Johns Hopkins University, Baltimore, Maryland: Effects of actin-containing complexes and proteins with cytochalasin-like activity on the assembly of actin filaments in vitro.

Session 14: *Cell Surface: Microvilli*

Chairperson: L. Tilney, University of Pennsylvania, Philadelphia

P.T. Matsudaira and D.R. Burgess, Dept. of Biology, Dartmouth College, Hanover, New Hampshire: The organization of the brush border cytoskeleton.

M. Mooseker, T. Keller, C. Howe, K. Wharton, and B. Grimwade, Dept. of Biology, Yale University, New Haven, Connecticut: Regulation of contractility, cytoskeletal structure, and filament assembly in the brush border.

A. Bretscher, Dept. of Cell Biology, University of Texas Health Science Center, Dallas: Purification of villin and fimbrin from brush borders and their interaction with F-actin in vitro.

D. Louvard, E. Coudrier, and H. Reggio, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Occurence in skeletal muscle of antigens related to the 110K and 140K protein of intestinal microvilli.

Session 15: *Nucleus and Cytoplasm*

Chairperson: A. Novikoff, Albert Einstein College of Medicine, Bronx, New York

L. Gerace[1] and G. Blobel,[2] [1]Dept. of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland; [2]Laboratory of Cell Biology, Rockefeller University, New York, New York: The nuclear lamina as a framework for the structural organization of the nuclear envelope.

J. E. Mullet, A. R. Grossman, and N.-H. Chua, Rockefeller University, New York, New York: Post-translational transport and assembly of chloroplast polypeptides.

G. P. Thomas, W. J. Welch, M. B. Mathews, and J. R. Feramisco, Cold Spring Harbor Laboratory, New York: Molecular and cellular effects of heat shock and related treatments of mammalian tissue culture cells.

E. Wang, D. S. Roos, and P. W. Choppin, Rockefeller University, New York, New York: Function of cytoplasmic fibers in syncytia.

S. Penman, A. Fulton, D. Capco, C. F. Tse, and M. Cervera, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The cytoskeletal and nuclear architecture—Form, functions and mode of assembly.

Summary: W. Brinkley, Baylor College of Medicine, Houston, Texas

SUMMER MEETINGS

C. elegans, May 6–May 10

Arranged by **Samuel Ward,** *Carnegie Institution of Washington,* **Robert Herman,** *University of Minnesota,* **Robert Horvitz,** *Massachusetts Institute of Technology,* **Gunther von Ehrenstein,** *Max-Planck-Institute for Experimental Medicine*

143 participants

Opening Remarks: S. Ward, Carnegie Institution of Washington, Baltimore, Maryland

Session 1: *Genes and Developmental Lineage*

Chairperson: J. Sulston, MRC Laboratory of Molecular Biology, Cambridge, England

M. Chalfie, J.E. Sulston, E. Hedgecock, J.N. Thomson, and K. Buck, MRC Laboratory of Molecular Biology, Cambridge, England: Touch-insensitive mutants.

C. Ferguson, I. Greenwald, P. Sternberg, N. Tsung, and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutations affecting vulva development in *C. elegans.*

E. Hedgecock, A. Otsuka, and C. Coulondre, MRC Laboratory of Molecular Biology, Cambridge, England: Direct isolation of cell lineage mutants.

P. Sternberg and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Postembryonic cell lineages of *Panagrellus redivivus.*

Session 2: *Muscles and Genes*

Chairperson: R.H. Waterston, Washington University, St. Louis, Missouri

J. Karn, A.R. Macleod, and S. Brenner, MRC Laboratory of Molecular Biology, Cambridge, England: Molecular studies of the *unc-54* myosin heavy-chain gene.

P. Anderson, MRC Laboratory of Molecular Biology, Cambridge, England: The physical nature of mutations affecting *unc-54.*

D.G. Moerman, T.R. Lane, and R.H. Waterston, Depts. of Genetics and of Anatomy and Neurobiology, Washington University, St. Louis, Missouri: Studies on *unc-54 I* and its suppressor *sup-3 V.*

J. Files and D. Hirsh, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: The actin genes of *C. elegans.*

L.A. Gossett,[1] L.H. Bolton,[2] H.F. Epstein,[2] and R.M. Hecht,[1] [1]Dept. of Biophysical Sciences, University of Houston Central Campus; [2]Dept. of Neurology, Baylor College of Medicine, Houston, Texas: The ontogeny of muscle proteins in the *C. elegans* embryo.

J.M. Mackenzie, D.M. Miller, L.H. Bolton, and H.F. Epstein, Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Structural studies of normal and mutant thick filaments.

D.M. Miller III, L.H. Bolton, and H.F. Epstein, Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Monoclonal antibodies as probes of muscle structure and development in *C. elegans.*

F.H. Schachat,[1] D.D. Bronson,[1] G.A. Jamieson, Jr.,[2] and T.C. Vanaman,[2] Depts. of [1]Anatomy and [2]Microbiology, Duke University Medical Center, Durham, North Carolina: Calcium regulation and phosphorylation in *C. elegans* muscle contraction.

Session 3: *The Genetics Stock Center*

Chairperson: M. M. Swanson, University of Missouri, Columbia

M. M. Swanson and D. L. Riddle, Division of Biological Sciences, University of Missouri, Columbia: *Caenorhabditis* Genetics Center.

Session 4: *Genetics*

Chairperson: J. Hodgkin, MRC Laboratory of Molecular Biology, Cambridge, England

R. Francis, D. G. Moerman, S. Plurad, and R. H. Waterston, Depts. of Genetics and of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri: Extragenic suppressors of mutations affecting muscle structure in *C. elegans.*

I. Greenwald and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetic studies of *unc-93(e1500) III.*

A. M. Rose,[1] D. L. Baillie,[2] K. A. Beckenbach,[2] and E. P. M. Candido,[1] [1]University of British Columbia, Vancouver; [2]Simon Fraser University, Burnaby, B. C., Canada: Genetic and biochemical analysis of *unc-15*—The gene coding for paramyosin in *C. elegans.*

P. S. Hartman and R. K. Herman, Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Radiation-sensitive mutants of *C. elegans.*

S. Carr M. Krause, and D. Hirsh, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Genetic mapping of DNA sequence.

D. G. Albertson and J. N. Thomson, MRC Laboratory of Molecular Biology, Cambridge, England: Kinetochore structure in *C. elegans.*

Session 5: *Embryology*

Chairperson: D. Hirsh, University of Colorado, Boulder

E. Schierenberg,[1] J. E. Sulston,[2] C. Carlson,[1] W. Sidio,[1] and G. von Ehrenstein,[1] [1]Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany; [2]MRC Laboratory of Molecular Biology, Cambridge, England: Computer-aided analysis of *C. elegans* embryogenesis.

R. Cassada, E. Isnenghi, K. Denich, K. Radnia, E. Schierenberg, K. Smith, and G. von Ehrenstein, Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Genetic dissection of embryogenesis in *C. elegans.*

K. Denich, R. Cassada, E. Isnenghi, K. Radnia, E. Schierenberg, and G. von Ehrenstein, Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Cell lineages and developmental defects of temperature-sensitive embryonic arrest mutants of *C. elegans.*

J. Priess and D. Hirsh, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Digestive tract development in *C. elegans.*

R. G. Knowlton, M. K. Edwards, M. Lauth, L. M. Donahue, P. M. Meneely, and W. B. Wood, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Gene expression in *C. elegans* embryos.

S. Strome, M. Hobbs, and W. B. Wood, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Monoclonal antibodies against tissue-specific antigens in *C. elegans*.

H. U. Certa, R. Cassada, and G. von Ehrenstein, Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Histones of *C. elegans*.

Session 6: *Poster Session*

P. F. Agris,[1] P. Schmidt,[2] J. Golden,[1] and D. L. Riddle,[1] [1]Division of Biological Sciences, University of Missouri, Columbia; [2]Dept. of Biochemistry, University of Oklahoma Health Sciences Center, Oklahoma City: In vivo phosphorous-NMR spectroscopy of *C. elegans*.

C. J. Barinaga and J. D. Willett, Dept. of Bacteriology and Biochemistry, University of Idaho, Moscow: An improved instrument for the rapid counting and simultaneous two parameter measurement of small nematodes.

M. A. Bolanowski, L. A. Jacobson, and R. L. Russell, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Water efflux from *C. elegans*.

M. Chalfie and J. N. Thomson, MRC Laboratory of Molecular Biology, Cambridge, England: Microtubule structure in *C. elegans* neurons.

V. K.-H. Chen[1] and W. F. Hieb,[2] [1]Dept. of Biophysics and [2]Division of Cell and Molecular Biology, State University of New York, Buffalo: Behavioral studies of nematodes by dynamic laser light scattering.

L. W. Cribbs, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Protease interconversion of acetylcholinesterase forms.

B. O. Davis, Jr.,[1] G. L. Anderson,[2] and D. B. Dusenbery,[2] [1]Kennesaw College, Marietta, Georgia; [2]School of Biology, Georgia Institute of Technology, Atlanta: Fluorescent pigment accumulation in aging *C. elegans*.

D. B. Dusenbery and E. A. Anderson, School of Biology, Georgia Institute of Technology, Atlanta: Cold-resistant mutants of the nematode *C. elegans*.

W. Fixsen and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A cell lineage mutation that produces multiple ectopic postdeirids and ray groups.

A. Fodor,[1] P. Deák,[1] I. Kiss,[1] and T. Timár,[2] [1]Institute of Genetics BRC, Hungarian Academy of Science, Szeged; [2]Alkaloida, Tiszavasvari, Hungary: Effects of some insect junvenile hormone and precocene analog on *C. elegans*.

P. D. Gardner, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Lectin-based purification and separation of *C. elegans* acetylcholinesterase forms.

E. Hedgecock and N. Thomson, MRC Laboratory of Molecular Biology, Cambridge, England: Neuronal guidance mutants.

D. L. Kolson, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: New alleles of *ace-1 X*.

C. Link, R. L. Russell, and L. Jacobson, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: "Age pigments" of *C. elegans*— Quantitation and physical properties.

K. R. Luehrsen, S. M. Wall, R. M. Hecht, and G. E. Fox, Dept. of Biophysical Sciences, University of Houston Central Campus, Texas: The 5S ribosomal RNA sequence of nematodes.

G. McCaffrey and T. E. Johnson, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Protein changes during lifespan—2-D gel analysis.

R. Ouazana,[1] D. Herbage,[2] and J. Brun,[1] [1]Laboratoire de Génétique Physiologique et Nématologie, C.N.R.S., Paris; [2]Laboratoire de Chimie Macromoléculaire, Université Claude Bernard, Lyon, France: Further characterization of some components of the *C. elegans* adult cuticle collagen.

R. L. Russell, O. J. Bashor, and L. Cavalier, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Structure of the pharyngeo-intestinal "valve."

S. S. Siddiqui,[1] G. von Ehrenstein,[1] E. Schierenberg,[1] P. Sams,[1] and J. G. Culotti,[2] [1]Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany; [2]Dept. of Biological Sciences, Northwestern University, Evanston, Illinois: Expression of acetylcholinesterase activity during the embryonic and postembryonic development of *C. elegans*.

Y. Tabuse[1] and J. Miwa,[1,2] [1]Dept. of Biology, Faculty of Science, Osaka City University; [2]Institute for Biomedical Research, Osaka, Japan: Mutants of *C. elegans* resistant to tumor promoters.

C. Trent, N. Tsung, and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Mutants that affect egg-laying in *C. elegans*.

B. M. Zuckerman,[1] S. P. Huang,[1] T. A. Tattar,[2] and R. A. Rohde,[2] [1]Laboratory of Experimental Biology, University of Massachusetts, East Wareham; [2]Dept. of Plant Pathology, University of Massachu-

setts, Amherst: *C. elegans*—The effects of 5-hydroxytryptophan and dopamine on behavior and development.

Session 7: Aging and the Life Cycle

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

V. Ambros and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genes that control developmental stage.

J.W. Golden and D.L. Riddle, Division of Biological Sciences, University of Missouri, Columbia: A dauerlarva pheronome.

T.P. Snutch and D.L. Baillie, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada: Inducible genes in the nematode *C. elegans*.

J. Miwa,[1,2] Y. Tabuse,[2] M. Furusawa,[2] and H. Yamasaki,[3] [1]Institute for Biomedical Research, Osaka; [2]Dept. of Biology, Faculty of Science, Osaka City University, Japan; [3]International Agency for Research on Cancer, Lyon, France: Specific effect of tumor promoters on *C. elegans*.

T.E. Johnson, C.H. Lashlee, and G. McCaffrey, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Quantitative genetics of lifespan.

M.A. Bolanowski, C. Link, R.L. Russell, and L.A. Jacobson, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Quantitative measures of aging in *C. elegans*.

Session 8: Sex Determination and Gametogenesis

Chairperson: W.B. Wood, University of Colorado, Boulder

J. Hodgkin, MRC Laboratory of Molecular Biology, Cambridge, England: A major sex-determining gene in *C. elegans*.

J.E. Kimble and J.G. White, MRC Laboratory of Molecular Biology, Cambridge, England: Control of germ cell development by distal tip cells in *C. elegans*.

L. Edgar and D. Hirsh, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Control of spermatogenesis in hermaphrodites.

M.R. Klass, Dept. of Biology, University of Houston, Texas: Regulation of the major sperm-protein gene in *C. elegans*.

S. Ward, G. Nelson, T. Roberts, and Y. Argon, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Genetic dissection of *C. elegans* spermiogenesis.

T. Roberts[1] and S. Ward,[2] [1]Dept. of Biological Science, Florida State University, Tallahassee; [2]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Directed membrane flow on the pseudopods of *C. elegans* spermatozoa.

Session 9: Poster Session

G. Anderson, School of Biology, Georgia Institute of Technology, Atlanta: Superoxide dismutase activity in dauerlarva of *C. elegans*.

T. Blumenthal, MRC Laboratory of Molecular Biology, Cambridge, England: Novel polypeptides encoded by RNA from suppressor-containing strains.

P. Deák and A. Fodor, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged: Genetics and cytology of chromosome rearrangement *SzT1* expressing dominant X-balancer and HIM phenotypes.

B. Dow and M.R. Klass, Dept. of Biology, University of Houston, Texas: Cloning of the 15K gene from *C. elegans*.

W. Fixsen and R. Horvitz, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Two loci involved in the migrations of the ventral cord precursor cells.

P. Goldstein, University of North Carolina, Charlotte: Synaptonemal complex analysis of wild-type and *him* mutants of *C. elegans*.

R.M. Hecht, S.M. Wall, and J. Jackson, Dept. of Biophysical Sciences, University of Houston Central Campus, Texas: Nuclear terminal phenotypes of the embryonic lethals of *C. elegans*.

R.K. Herman, C.K. Kari, and P.S. Hartman, Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Dominant male-throwing mutants of *C. elegans*.

E. Isnenghi, R. Cassada, K. Smith, K. Radnia, and G. von Ehrenstein, Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Maternal and other modes of expression and time of temperature sensitivity of genes affecting embryogenesis in *C. elegans*.

S. Jameel and B.A. McFadden, Dept. of Biochemistry and Institute of Biological Chemistry, Washington State University, Pullman: Decay of isocitrate lyase in *C. elegans*.

R. Jefferson,[1] P. Bazzicalupo,[2] and D. Hirsh,[1] [1]Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder; [2]Instituto Internazionale di Genetica e Biofisica, Naples, Italy: Developmental regulation of β-glucuronidase.

L. Jen-Jacobson and L. Jacobson, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Acid endoproteases of *C. elegans*.

C. Landel and D. Hirsh, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Multiple actins in the worm.

J.S. Laufer and W.B. Wood, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Behavior of cleavage-inhibited blastomeres in *C. elegans*.

R. Ouazana,[1] N. Mounier,[1] J. Brun,[1] C. Drevon,[2] and H. Yamasaki,[2] [1]Laboratoire de Génétique Physiologique et Nématologie, Université Claude Bernard Lyon-I; [2]Unit of Chemical Carcinogenesis, International Agency for Research on Cancer, Lyon, France: Effect of phorbol ester tumor promoters on *C. elegans*. Isolation and characterization of TPA-resistant mutant strains.

T.M. Rogalski,[1] D.G. Moerman,[2] and D.L. Baillie,[1] [1]Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada; [2]Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Deficiencies and lethal mutations in the *unc-22* region of linkage group IV of *C. elegans*.

R.E. Rosenbluth and D.L. Baillie, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada: Localization of *breakpoints* and demonstration of *reciprocity* for the *C. elegans* translocation, *eT1(III;V),e873*.

S.S. Siddiqui,[1] G. von Ehrenstein,[1] and J.G. Culotti,[2] [1]Dept. of Molecular Biology, Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany; [2]Dept. of Biological Sciences, Northwestern University, Evanston, Illinois: Acetylcholinesterase mosaics in the nematode *C. elegans*.

J. Sulston,[1] E. Schierenberg,[2] and G. von Ehrenstein,[2] [1]MRC Laboratory of Molecular Biology, Cambridge, England; [2]Max-Planck-Institute for Experimental Medicine, Göttingen, Federal Republic of Germany: Embryonic cell lineage.

J.R. Vanfleteren, Laboratoria voor Morfologie en Systematiek der Dieren, Rijksuniversiteit Gent, Belgium: The histones of *C. elegans*.

W. Sharrock and J. Kimble, MRC Laboratory of Molecular Biology, Cambridge, England: Adult hermaphrodite-specific proteins of *C. elegans*.

Session 10: *Neurobiology*

Chairperson: M. Chalfie, MRC Laboratory of Molecular Biology, Cambridge, England

J.G. White, E. Southgate, and J.N. Thomson, MRC Laboratory of Molecular Biology, Cambridge, England: The nervous system of *C. elegans*.

R.E. Davis and A.O.W. Stretton, Neurosciences Program and Dept. of Zoology, University of Wisconsin, Madison: Intracellular recordings from identified motorneurons in the nematode *Ascaris*.

R.L. Russell, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Computer modeling *C. elegans* movement.

J. Rand, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Choline acetyltransferase-deficient mutants of *C. elegans*.

J.G. Culotti, W.M. James, and W.L. Klein, Dept. of Biological Sciences, Northwestern University, Evanston, Illinois: Binding of muscarinic and nicotinic ligands to homogenates of wild type and cholinergic mutants of *C. elegans*.

J.A. Lewis and J.T. Fleming, Dept. of Biological Sciences, Columbia University, New York, New York: A binding assay for the levamisole receptor.

C.D. Johnson, S.M. Rotter, and A.O.W. Stretton, Dept. of Zoology, University of Wisconsin, Madison: Acetylcholine functions in *Ascaris*.

Summary: S. Ward, Carnegie Institution of Washington, Baltimore, Maryland

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Mitochondrial Genes, May 13–May 17

Arranged by **P. Slonimski,** *Centre de Génétique Moléculaire du CNRS,* **P. Borst,** *University of Amsterdam,* **G. Attardi,** *California Institute of Technology*

165 participants

Introduction: H. Roman, University of Seattle, Washington: Boris Ephrussi and the early days of cytoplasmic inheritance in yeast

Session 1: Mammalian Genes and Transcripts

Chairperson: M. Simpson, State University of New York, Stony Brook

S. Anderson, A.T. Bankier, B.G. Barrell, M.H.L. de Bruijn, E. Chen, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schereir, A.J.H. Smith, R. Staden, and I.G. Young, MRC Laboratory of Molecular Biology, Cambridge, England: Sequence and structure of mammalian mitochondrial genomes.

G. Attardi, P. Cantatore, A. Chomyn, S. Crews, R. Gelfand, C. Merkel, J. Montoya, and D. Ojala, California Institute of Technology, Pasadena: A comprehensive view of mitochondrial gene expression in human cells.

M.J. Bibb, A. Brennicke, J.N. Doda, D.P. Tapper, R.A. van Etten, M.W. Walberg, C.T. Wright, and D.A. Clayton, Dept. of Pathology, Stanford University School of Medicine, California: Sequence organization and functional properties of mouse mtDNA.

D.T. Dubin, K.D. Timko, C.C. Hsuchen, and T. Azzolina, Dept. of Microbiology, CMDNJ-Rutgers Medical School, Piscataway, New Jersey: 3′-Termini of animal cell mtRNA.

Session 2: Yeast Genes and mRNA Formation I

Chairperson: P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France

A. Tzagoloff, Dept. of Biological Sciences, Columbia University, New York, New York: Cytochrome oxidase genes in yeast.

C. Jacq, H. de la Salle, and P.P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Role of the last two introns of cytochrome *b* gene in the expression of two mosaic genes—Physical studies of mutations.

P. Pajot, M.L. Wambier-Kluppel, C. Grandchamp, O. Groudinsky, M. Labouesse, and P.P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Loss of the last two introns in the yeast cytochrome *b* gene permits the synthesis of active cytochrome *b* but prevents the formation of cytochrome oxidase.

L.A. Grivell[1,2] M. de Haan,[1,2] J.B.A. Crusius,[1] N. van Harten-Loosbroek,[1] and G.S.P. Groot,[2] [1]Section of Molecular Biology, [2]Laboratory of Biochemistry, Free University, Amsterdam, The Netherlands: Evolution and the introns in yeast mtDNA.

R. Schweyen, C. Schmelzer, H. Bruckner, B. Suchy, A. Gessner, and F. Kaudewitz, Genetisches Institut der Universität München, Munich, Federal Republic of Germany: Processing of mitochondrial transcripts in *Saccharomyces cerevisiae.*

H.R. Mahler,[1] P.S. Perlman,[2] D.K. Hanson,[1] and M.R. Lamb,[1] [1]Chemistry Dept., Indiana University, Bloomington; [2]Genetics Dept., Ohio State University, Columbus: The role of exon sequences in the processing of *cob* introns.

P.S. Perlman,[1] H.R. Mahler,[2] M.R. Lamb,[2] P.Q. Anziano,[1] and D. Hanson,[2] [1]Genetics Dept., Ohio State University, Columbus; [2]Chemistry Dept., Indiana University, Bloomington: Definition of functional domains of an intron.

Session 3: Poster Session

A.C. Arnberg,[1] J.B.A. Crusius,[2] P.H. Boer,[2] and L.A. Grivell,[2] [1]Biochemical Laboratory, State University, Groningen; [2]Section for Molecular Biology, Laboratory of Biochemistry, Amsterdam, The Netherlands: Localization of DNA sequences encoding the long 5′ leader of the mRNA for cytochrome *b* in yeast mitochondria.

G. Baldacci,[1] and G. Bernardi,[2] [1]Istituto di Fisiologica Generale, Università di Roma, Italy; [2]Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France: Transcription of the mitochondrial genome of yeast.

P. H. Boer, J. B. A. Crusius, and L. A. Grivell, Section of Molecular Biology, Laboratory of Biochemistry, Amsterdam, The Netherlands: Early events in the processing of *cob* transcripts in yeast mitochondria.

L. Bonen, Dept. of Biochemistry, Dalhousie University, Halifax, Canada: A "5S-like" sequence within the 3′ end of human mitochondrial 12S rRNA.

S. G. Bonitz and A. Tzagoloff, Dept. of Biological Sciences, Columbia University, New York, New York: The structure and nucleotide sequence of the gene coding for subunit 1 of yeast cytochrome oxidase.

H. Bruckner, A. R. Gessner, B. Sichy, and F. Kaudewitz, Genetisches Institut der Universität München, Munich, Federal Republic of Germany: Characterization of an exceptional series of mit deletions in *oxi3* and their effect on transcript splicing.

E. Ching[1] and G. Attardi,[2] [1]Dept. of Cell Biology, Rockefeller University, New York, New York; [2]Division of Biology, California Institute of Technology, Pasadena: High resolution fractionation and partial characterization of the mitochondrial translation products from HeLa cells.

M. L. Claisse, O. Groudinsky, B. Guiard, A. Spyridakis, M. Dreyfus, and P. P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: New mitochondrial high-molecular-weight translation products in *cob-box* and *oxi3* mutants.

G. Coruzzi, S. Bonitz, B. Thalenfeld, and A. Tzagoloff, Dept. of Biological Sciences, Columbia University, New York, New York: Analysis of the nucleotide sequence and transcripts in the *oxi1* region of yeast mtDNA.

M. de Zamaroczy, R. Marotta, and G. Bernardi, Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France: The primary structure of the origins of replication of the mitochondrial genome of yeast.

T. D. Fox and S. Stämpfli, Biocenter, University of Basel, Switzerland: An ochre mutation in the yeast *oxi1* gene and its supression by a second site mitochondrial mutation.

R. Gelfand[1] and G. Attardi,[2] [1]Dept. of Biology, Purdue University, West Lafayette, Indiana; [2]Division of Biology, California Institute of Technology, Pasadena: Synthesis and turnover of mtRNA in HeLa cells—The mature RNA and mRNA species are metabolically unstable.

A. Halbreich, C. Grandchamp, and M. Foucher, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: A low-molecular-weight RNA species which may arise from a trimming of the pre-mRNA of yeast mitochondrial cytochrome *b* at the 3′ end.

D. K. Hanson,[1] M. R. Lamb,[1] H. R. Mahler,[1] and P. S. Perlman,[2] [1]Chemistry Dept., Indiana University, Bloomington; [2]Genetics Dept., Ohio State University, Columbus: Homologous polypeptides accumulate in mutants of two different mitochondrial genes.

B. C. Hyman, J. H. Cramer, and R. H. Rownd, Laboratory of Molecular Biology and Dept. of Biochemistry, University of Wisconsin, Madison: Isolation of a yeast mtDNA segment conferring autonomously replicating function.

C. Julou and M. Bolotin-Fukuhara, Laboratoire de Biologie Generale, Université de Paris, Orsay, France: Mitochondrial ribosomal genes in yeast—Mutations and their suppression.

R. Marotta, R. Goursot, M. Mangin, G. Faugeron-Fonty, R. Goursot, and G. Bernardi: Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France: Suppressivity—The effect of partial or total deletions of the origin of replication of the petite genome.

J. Montoya, D. Ojala, S. Crews, R. Gelfand, and G. Attardi, California Institute of Technology, Pasadena: Mapping and structural properties of an mtDNA L-strand-coded polyadenylated RNA (7S RNA), and synthesis and processing of mitochondrial rRNA in HeLa cells.

P. Netter, C. Jacq, L. Clavilier, and P. P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Localization of mutations in the "cytochrome-*b*-homologous" intron of the oxidase gene in yeast.

G. Rödel and W. Bandlow, Genetisches Institut der Universität München, Munich, Federal Republic of Germany: A cAMP-receptor protein in yeast mitochondria.

E. Schiavon, D. Frezza, and G. Carignani, Istituto di Chimica Biologica and Centro Studio Fisiologia

Mitocondriale del CNR, Padova, Italy: Localization of mit mutations along the *oxi3* region of mtDNA in "long" strains of *Saccharomyces cerevisiae.*

B. E. Thalenfeld and A. Tzagoloff, Columbia University, New York, New York: Transcription of the *oxi2* gene of yeast mtDNA.

M. W. Walberg, J. Bird, and D. A. Clayton, Dept. of Pathology, Stanford University School of Medicine, California: Precise mapping of sequences coding for precursors of mouse mitochondrial rRNAs.

B. Weiss-Brummer, G. Rödel, and F. Kaudewitz, Genetisches Institut der Universität München, Munich, Federal Republic of Germany: Characterization of some splicing-defective mutants mapping in the intron γ/δ of the *cob* gene of *Saccharomyces cerevisiae* mtDNA.

K. Wolf,[1] B. Lang,[2] P. Anziano,[2] and P. S. Perlman,[2] [1]Genetisches Institut der Universität München, Munich, Federal Republic of Germany; [2]Genetics Dept., Ohio State University, Columbus: Structure and function of the mitochondrial genome in *Schizosaccharomyces pombe.*

Session 4: *Yeast Genes and mRNA Formation II*

Chairperson: H. Mahler, Indiana University, Bloomington

D. Levens, T. Christianson, J. Edwards, A. Lustig, B. Ticho, J. Locker, and M. Rabinowitz, Depts. of Biochemistry, Biology, Medicine, and Pathology, University of Chicago, Illinois: Transcriptional initiation of yeast mtRNA and characterization and synthesis of mtRNA polymerase.

R. A. Butow, H. P. Zassenhaus, and F. Farrelly, Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Analysis of the *var1* determinant region on yeast mtDNA.

M. E. S. Hudspeth,[1] D. S. Shumard,[1] K. R. Glaus,[2] P. S. Perlman,[2] and L. I. Grossman,[1] [1]Division of Biological Sciences, University of Michigan, Ann Arbor; [2]Dept. of Genetics, Ohio State University, Columbus: Sequence variation in the *var1* region of yeast mtDNA.

J. Lazowska, A. Gargouri, and P. P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Role of the second intron of cytochrome *b* gene—Analysis of mRNA-maturase mutations and of the 3′ region of the intron.

R. W. Davies, C. Scazzocchio, R. B. Waring, S. Lee, E. Grisi, M. M. Berks, and T. A. Brown, Dept. of Biology, University of Essex, Colchester, England: DNA sequence of mosaic genes in the mitochondrial genome of *Aspergillus nidulans.*

A. Kruszewska, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw: Nuclear and mitochondrial informational suppressors of *box3* intron mutations.

G. Dujardin, O. Groudinsky, and P. P. Slonimski, Centre de Génétique Moléculaire du CNRS, Gif-sur-Yvette, France: Further studies on phenotypic and genetic suppression of intron mutations.

A. M. Colson and C. Legrand, Laboratoire de Cytogénétique, Université de Louvain, Belgium: Properties of mitochondrial diuron-resistant mutants of *Saccharomyces cerevisiae* selected on DL-lactate.

L. A. M. Hensgens, L. A. Grivell, and G. van der Horst, Section of Molecular Biology, Laboratory of Biochemistry, Amsterdam, The Netherlands: Processing of *oxi3* RNAs is dependent on intron-encoded functions.

R. Schroeder,[1,2] M. Breitenbach,[1] and R. J. Schweyen,[2] [1]Institut für Allgemeine Biochemie und Ludwig Boltzmann Forschungsstelle für Biochemie der Universität Wien; [2]Genetisches Institut der Universität München, Munich, Federal Republic of Germany: Mitochondrial transcription and translation during yeast differentiation.

Session 5: *Replication and Ribosomal Genes in Yeast*

Chairpersons: D. Cummings, University of Colorado, Denver
C. Saccone, Istituto Chimica Biologica, Bari, Italy

G. Bernardi, G. Baldacci, G. Bernardi, G. Faugeron-Fonty, C. Gaillard, R. Goursot, R. Goursot, A. Huyard, M. Mangin, R. Marotta, and M. de Zamaroczy, Institut de Recherches en Biologie Moléculaire, Laboratoire de Génétique Moléculaire, Paris, France: The sequence organization of the mitochondrial genome of yeast.

H. Blanc[1] and B. Dujon,[2] [1]Dept. of Genetics, Stanford University School of Medicine, California; [2]The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Replicator regions of yeast mtDNA.

S. F. Cottrell, J. Blamire, and L. H. Lee, Dept. of Biology, Brooklyn College, City University of New York, New York: Evidence for the synchronous replication of mtDNA during the yeast cell cycle.

G. Faugeron-Fonty, R. Marotta, M. de Zamaroczy, and G. Bernardi, Laboratoire de Génétique

Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France: The localization and orientation of the origins of replication of the mitochondrial genome of yeast.

B. Dujon, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Polymorphic variations of the yeast mitochondrial 21S rRNA gene and their genetic implications.

H.F. Tabak, J.van der Laan, K.A. Osinga, and J.P. Schouten, Section for Medical Enzymology and Molecular Biology, University of Amsterdam, The Netherlands: The precision of RNA splicing in a yeast mitochondrial petite mutant, as assayed with a synthetic DNA oligonucleotide.

R.L. Strausberg, D.E. Lorant, and P.E. Hardin, Dept. of Biology, Southern Methodist University, Dallas, Texas: Novel mutations in the 21S rRNA region of yeast mtDNA.

C.J. Weeden, M.A. Conners, and J.A. Knight, Dept. of Biological Sciences, Mount Holyoke College, South Hadley, Massachusetts: The use of nuclear suppressors to generate unusual classes of mitochondrial chloramphenicol-resistant mutants in baker's yeast.

F. Sor and H. Fukuhara, Institut Curie, Université de Paris, Orsay, France: Nucleotide sequence of the mitochondrial 15S rRNA gene of yeast.

M. Li,[1] L. Lyon,[2] N. Martin,[2] and A. Tzagoloff,[1] [1]Dept. of Biological Sciences, Columbia University, New York, New York; [2]Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Characterization of the paromomycin resistance locus in the 15S rRNA gene of yeast mitochondria.

K.A. Osinga and H.F. Tabak, Section for Medical Enzymology and Molecular Biology, University of Amsterdam, The Netherlands: The transcription-initiation signal for the synthesis of the precursor for small rRNA in yeast mitochondria.

Session 6: *Recombination, Nucleocytoplasmic Interactions and tRNA Genes in Yeast*

Chairperson: F. Kaudewitz, Universität München, Federal Republic of Germany

A.W. Linnane, Dept. of Biochemistry, Monash University, Clayton, Victoria, Australia: Structure and expression of the *oli2* gene in yeast mtDNA.

C.W. Birky, Jr. and A.R. Acton, Dept. of Genetics, Ohio State University, Columbus: Yeast mitochondrial genes—Recombination, replication, and random changes in frequencies.

G. Michaelis,[1] G. Mannhaupt,[1] E. Pratje,[1] E. Fischer,[2] and E. Schweizer,[2] [1]Fakultät für Biologie, Universität Bielefeld; [2]Lehrstuhl für Biochemie der Universität Erlangen-Nürnberg, Federal Republic of Germany: Mitochondrial translation products of nuclear *pet* mutants in *Saccharomyces cerevisiae.*

C. Dieckmann and A. Tzagoloff, Dept. of Biological Sciences, Columbia University, New York, New York: Saturation of the nuclear genome for mutations affecting mitochondrial respiration.

L. Frontali, M. Agostinelli, G. Baldacci, C. Falcone, S. Francisci, C. Palleschi, and E. Zennaro, Istituto di Fisiologa Generale, Università di Roma, Italy: Expression of the mitochondrial genes in *Saccharomyces cerevisiae*—Influences of physiological conditions and of nuclear background.

N.C. Martin, D.L. Miller, D.R. Najarian, and K. Underbrink-Lyon, Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Yeast mitochondrial tRNAs—Genes, transcripts, and a tRNA synthesis locus.

R.P. Martin, A.-P. Sibler, R. Bordonné, and G. Dirheimer, Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Codon-reading patterns in yeast mitochondria.

A.P. Hudson, I.C. Lopez, R.A. Butow, and J.C. Mounolou, Dept. of Biochemistry, University of Texas Health Science Center, Dallas: A biochemical approach to yeast mtDNA recombination.

D.W. Deters and M. Ewing, Dept. of Microbiology, University of Texas, Austin: Mitochondrial translation products of different yeasts are distinguishable.

G.D. Clark-Walker and K.S. Sriprakash, Dept. of Genetics, Australian National University, Canberra: Mechanisms for sequence rearrangements and size diversity in mtDNAs from yeasts.

D. Wilkie and I. Bruce, Dept. of Botany and Microbiology, University College, London, England: Correlation between uptake of sugars and flocculation in yeast—Mitochondrial influence.

G. Orlowska and Z. Kotylak, Institute of Microbiology, Wroclaw, Poland: Genetic characterization of op1-type mutants.

Session 7: *Poster Session*

E. Agsteribbe, J. Samallo, and P. van den Boogaart, Laboratory of Physiological Chemistry, State University, Groningen, The Netherlands: Structural and functional analysis of *Neurospora crassa* mtDNA. II. Transcripts of the *oli* and *oxi1* region.

G. Bernardi and G. Bernardi, Laboratoire de Génétique Moléculaire, Institute de Recherche en Biologie Moléculaire, Paris, France: Intergenic and intervening sequences of the mitochondrial genome of yeast.

M. Breitenbach and R. Schroeder, Institut für Allgemeine Biochemie und Ludwig Boltzmann Forschungsstelle für Biochemie der Universität Wien, Austria: Mitochondrially inherited germination-deficient mutants of yeast.

A. Brunner, Centro de Investigaciones en Fisiologîa Celular, Universidad Nacional Autónoma de México, Villa Obregón: Recombination of mitochondrial markers during protoplast fusion in the "petite-negative" yeast *Kluyveromyces lactis.*

L. Del Giudice, Istituto Internazionale di Genetica e Biofisica, CNR, Napoli, Italy: Cloning of mtDNA from petite-negative yeast *Schizosaccharomyces pombe* in the bacterial plasmid pBR322.

S. Ferris,[1] A. Wilson,[1] and W. Brown,[2] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Division of Biological Sciences, University of Michigan, Ann Arbor: Evolutionary tree for apes and humans based on cleavage maps of mtDNA.

E. Finzi, M. Sperling, and D. S. Beattie, Dept. of Biochemistry, Mount Sinai School of Medicine, New York, New York: Isolation of cytosolic proteins which control yeast mitochondrial protein synthesis.

R. Goursot, G. Faugeron-Fonty, M. Mangin, and G. Bernardi, Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France: Suppressivity—The effect of the repeat unit length of the petite genome and of other factors.

S. L. Hajduk,[1] J. H. J. Hoeijmakers,[2] P. Borst,[2] W. B. Cosgrove,[3] and K. Vickerman,[1] [1]Dept. of Zoology, University of Glasgow, Scotland; [2]Dept. of Medical Enzymology, University of Amsterdam, The Netherlands; [3]Dept. of Zoology, University of Georgia, Athens: Kinetoplast DNA structure and mitochondrial activity in the trypanosomatid flagellate *Herpetomonas.*

J. F. Hare, R. Hodges, and G. Wilson, Dept. of Biochemistry, University of Oregon Health Sciences Center, Portland: Synthesis and assembly of the cytochrome complexes in cultured rat liver cells.

W. W. Hauswirth[1] and P. J. Laipis,[2] Depts. of [1]Immunology and Medical Microbiology; [2]J. Hillis Miller Health Center, University of Florida, Gainesville: Variational processes in bovine mtDNA.

N. Howell, Division of Radiation Therapy, Sidney Farber Cancer Institute, Boston, Massachusetts: Mitochondrial PYR-IND mutations—Cellular origin, genetics, and effects on energy metabolism.

M. E. S. Hudspeth, D. S. Shumard, and L. I. Grossman, Dept. of Cellular and Molecular Biology, University of Michigan, Ann Arbor: *Achlya* mtDNA contains an inverted repeat of rRNA genes.

J. Jayaraman and J. Ashraf, Dept. of Biochemistry, Madurai Kamaraj University School of Biological Sciences, India: A specific protease for processing proteins made in the mitochondria?

M. F. Jubier,[1] B. Lejeune,[2] and F. Quétier,[2] [1]Rockefeller University, New York, New York; [2]Université Paris, Orsay, France: Heterogeneity of wheat mtDNA.

E. Keyhani and J. Keyhani, Institute of Biochemistry and Biophysics, University of Tehran, Iran: Subunit I as a binding site of copper in cytochrome *c* oxidase in *oxi* mutants of the yeast *Saccharomyces cerevisiae.*

N. A. Khan, Dept. of Biology, Brooklyn College, New York, New York: Suppression of maltose-negative phenotype by a nuclear gene in rho^- cells of yeast.

G. Kidane,[1] J. Scott,[2] T. Spithill,[1] and L. Simpson[1,2] [1]Biology Dept.; [2]Molecular Biology Institute, University of California, Los Angeles: Kinetoplast DNA minicircles from *Leishmania tarentolae* can serve as autonomous replicating sequences in yeast.

C. M. Lazarus and H. Kuntzel, Abt. Chemie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany: Amplified mtDNA of *Aspergillus amstelodami* ragged mutants—Assignments on homologous and heterologous restriction maps.

A. Lustig, D. Levens, and M. Rabinowitz, Depts. of Medicine and Biochemistry, University of Chicago, Illinois: The biogenesis and regulation of mtRNA polymerase.

M. Mangin, G. Faugeron-Fonty, and G. Bernardi, Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, France: An investigation on the instability of a rearranged mitochondrial genome from a spontaneous petite mutant.

J. C. Marini and P. T. Englund, Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: Anomalous electrophoretic behavior of a kinetoplast DNA restriction fragment.

E. E. McKee, S. D. Power, G. Bellus, J. McEwen, and R. O. Poyton, Dept. of Microbiology, University of Connecticut Health Center, Farmington: In vitro synthesis and membrane insertion of mitochondrial gene products.

M. M. K. Nass and M. A. D'Agostino, Dept. of Radiation Therapy, University of Pennsylvania School of Medicine, Philadelphia: Regulation of mtDNA replication—Differential structural constraint on the mtDNA polymerase in normal and malignant cells.

Y. Pollack, J. Kasir, and G. Glaser, Dept. of Cellular Biochemistry, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Characterization of a plasmid containing mouse mtDNA origin of replication.

D. Rickwood,[1] J. A. A. Chambers,[1] L. Robson,[1] and M. Barat,[2] [1]Dept. of Biology, University of Essex, Colchester, England; [2]Université Paris, Orsay, France: Proteins associated with mtDNA.

B. A. Roe, P. W. Armstrong, J. F. H. Wong, E. Y. Chen, A. Stankiewicz, D. P. Ma, and J. McDonough, Chemistry Dept., Kent State University, Ohio: Studies on mammalian mitochondrial tRNA.

C. Schmelzer and R. J. Schweyen, Genetisch-Mikrobiologisches Institut der Universität München, Munich, Federal Republic of Germany: Inhibition of mitochondrial translation blocks processing of transcripts in yeast mitochondria.

E. P. Sena, Biology Dept., Case Western Reserve University, Cleveland, Ohio: Cytoduction of petite genomes in yeast—Effects on the suppressivity of specific mitochondrial genomes.

K. Wakabayashi and T. Mabuchi, Dept. of Biochemistry, University of Yamanashi, Japan: Cloning of yeast mtDNA.

G.-A. Wild and S. Werner, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Munich, Federal Republic of Germany: Biogenesis of cytochrome oxidase in *Neurospora crassa*—Evidence that the mitochondrially made subunit 1 is synthesized as a larger precursor in vitro.

Session 8: *Genes of Filamentous Fungi*

Chairperson: A. Kroon, State University, Groningen, The Netherlands

S. Yin and U. L. RajBhandary, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: DNA sequence of a cluster of tRNA genes and the 3′ end of the large rRNA gene in *Neurospora crassa* mitochondria.

A. M. Lambowitz,[1] H. Bertrand,[2] G. Garriga,[1] D. M. Grant,[1] R. Vincent,[1] and R. A. Collins,[1] [1]Dept. of Biochemistry, St. Louis University Medical School, Missouri; [2]Dept. of Biology, University of Regina, Saskatchewan, Canada: RNA splicing in *Neurospora* mitochondria.

H. G. Köchel and H. Küntzel, Abt. Chemie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany: Nucleotide sequence and evolution of *Aspergillus nidulans* mitochondrial genes.

H. Bertrand,[1] A. M. Lambowitz,[2] R. A. Collins,[2] R. Vincent,[2] and G. Garriga,[2] [1]Biology Dept., University of Regina, Canada; [2]St. Louis University School of Medicine, Missouri: Regulation of mitochondrial biogenesis and RNA splicing in *Neurospora*.

P. van den Boogaart, J. Samallo, S. van Dijk, and E. Agsteribbe, Laboratory of Physiological Chemistry, State University, Groningen, The Netherlands: Structural and functional analysis of *Neurospora crassa* mtDNA. I. Fine-mapping and base sequence analysis of the *oli1* region.

G. Macino, G. Morelli, and M. Melissari, Istituto di Fisiologia Generale, Università di Roma, Italy: Cloning and sequencing of *Neurospora crassa* mitochondrial structural genes.

L. Belcour, O. Begel, A. M. Keller, and C. Vierny, Centre de Ge✓ne✓tique Moléculaire du CNRS, Gif-sur-Yvette, France: Does senescence in *Podospora anserina* result from instability of the mitochondrial genome?

K. Esser, U. Kück, U. Stahl, and P. Tudzynski, Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Böchum, Federal Republic of Germany: The *Podospora* plasmid causing senescence originates from mtDNA.

G. Turner, A. Earl, and D. R. Greaves, Dept. of Bacteriology, University of Bristol, England: Does the nuclear background select for species-specific mtDNA sequences in interspecies hybrids of *Aspergillus?*

P. Van't Sant, J. F. C. Mak, J. C. de Jonge, and H. de Vries, Laboratory of Physiological Chemistry, State University, Groningen, The Netherlands: Processing and integration of mitochondrial translation products in wild-type *Neurospora crassa* and in a mitochondrial "stopper" mutant.

Session 9: *Genes of Animals, Plants, and Trypanozomes*

Chairperson: G. Attardi, California Institute of Technology, Pasadena

F.J. Castora, R. Sternglanz, and M.V. Simpson, Dept. of Biochemistry, State University of New York, Stony Brook: A new mitochondrial topoisomerase that catenates DNA and preliminary comparison with its nuclear counterpart.

W.M. Brown, Division of Biological Sciences, University of Michigan, Ann Arbor: Evolution of mitochondrial genes in humans and apes.

D.C. Wallace, H. Blanc, N. Oliver, and C.W. Adams, Dept. of Genetics, Stanford University School of Medicine, California: Human mitochondrial genes—A system for genetic and molecular analysis.

C.J. Leaver, L.K. Dixon, and B.G. Forde, Dept. of Botany, University of Edinburgh, Scotland: Mitochondrial gene products and cytoplasmically inherited variation in higher plants.

T.D. Fox[1] and C.J. Leaver,[2] [1]Biocenter, University of Basel, Switzerland; [2]Dept. of Biology, University of Edinburgh, Scotland: The *Zea maize* mitochondrial gene coding cytochrome oxidase subunit II.

P.T. Englund, Dept. of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland: The replication of kinetoplast DNA.

L. Simpson,[1,2] A. Simpson,[1] T. Spithill,[1] and L. Livingston,[1] [1]Biology Dept.; [2]Molecular Biology Institute, University of California, Los Angeles: Sequence organization of maxicircle kinetoplast DNA from *Leishmania tarentolae*.

Session 10: *Genes of Plants, Protozoa, and Mammals*

Chairperson: P. Borst, University of Amsterdam, The Netherlands

D. Grant[1] and K.-S. Chiang,[2] [1]Dept. of Biochemistry, St. Louis University School of Medicine, Missouri; [2]Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: The 5μ mtDNA molecules of the green alga *Chlamydomonas reinhardtii*—Some unexpected novel properties.

R.M.K. Dale, Dept. of Biology, Yale University, New Haven, Connecticut: Sequence homologies among plant mtDNAs.

A.J. Bendich, Depts. of Botany and Genetics, University of Washington, Seattle: The mitochondrial genome in plants—Another C-value paradox.

M.W. Gray, L. Bonen, D. Falconet, T.Y. Huh, M.N. Schnare, and D.F. Spencer, Dept. of Biochemistry, Dalhousie University, Halifax, Canada: Sequences of plant mitochondrial rRNAs and organization of their genes.

E. Bartnik, M. Kozlowski, and P.P. Stepień, Dept. of Genetics, Warsaw University, Poland: Evolution of mtDNA in the genus *Aspergillus*.

A.E. Pritchard and D.J. Cummings, Dept. of Microbiology, University of Colorado Health Sciences Center, Denver: Sequence analysis of the initiation region of mtDNA from *Paramecium aurelia*.

Y. Suyama, Dept. of Biology, University of Pennsylvania, Philadelphia: Mapping native and imported tRNAs in *Tetrahymena* mitochondria by two-dimensional urea-polyacrylamide gel electrophoresis.

A. Tait, Dept. of Genetics, University of Edinburgh, Scotland: The genetic control of mitochondrial ribosomal proteins in paramecium.

C. Saccone,[1] P. Cantatore,[1] R. Gallerani,[2] A. de Benedetto,[1] G. Gadaleta,[1] C. Quagliariello,[2] G. Pepe,[1] C. Lanave,[1] and A.M. Kroon,[3] [1]Centro di Studio sui Mitocondri e Metabolismo Energetico presso Istituto di Chimica Biologica, Università di Bari; [2]Dipartimento di Biologia Cellulare, Università della Calabria, Italy; [3]Laboratory of Physiological Chemistry, State University, Groningen, The Netherlands: Rat mtDNA—Evolutionary considerations based on the nucleotide sequence analysis.

K. Koike, M Kobayashi, K. Yaginuma, T. Seki, and M. Imai, Gene Research Laboratory, Cancer Institute, Tokyo, Japan: Structural feature of rat mitochondrial genome.

R. Sager, Division of Cancer Genetics, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Studies of methylation in mtDNAs.

J. Solus and J. Eisenstadt, Dept. of Human Genetics, Yale University, New Haven, Connecticut: Segregation of mtDNA species within mouse-Chinese hamster hybrid cell lines in response to chloramphenicol and antimycin.

C.-J. Doersen and E.J. Stanbridge, Dept. of Microbiology, College of Medicine, University of California, Irvine: Nuclear and cytoplasmic inheritance of erythromycin resistance in human cells.

Session 11: *Evolutionary Comparisons*

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

B. Greenberg,[1,2] A. Sugino,[1,2] and J.E. Newbold,[1] [1]Curriculum in Genetics, University of North Carolina, Chapel Hill; [2]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Nucleotide sequence variability surrounding the origin of replication in human placental mtDNA.

R.L. Cann,[1] W.M. Brown,[2] and A.C. Wilson,[1] [1]Dept. of Biochemistry, University of California, Berkeley; [2]Division of Biological Sciences, University of Michigan, Ann Arbor: A mitochondrial perspective on human origins.

M. George, Jr.[1] and A.C. Wilson,[2] [1]Research Dept., San Diego Zoo, California; [2]Dept. of Biochemistry, University of California, Berkeley: Genealogical behavior of mtDNA.

D.R. Wolstenholme and J.M. Goddard, Dept. of Biology, University of Utah, Salt Lake City: The distribution among genes of nucleotide differences found between mtDNA molecules of domesticated and wild *Rattus norvegicus*.

G.G. Brown and M.V. Simpson, Dept. of Biochemistry, State University of New York, Stony Brook: Sequence variability of the rat mitochondrial genome.

R.A. Lansman and J.C. Avise, Dept. of Molecular and Population Genetics, University of Georgia, Athens: Sequence diversity of mtDNA within a single mammalian species.

J.M. Goddard, C. M.-R. Fauron, and D.R. Wolstenholme, Dept. of Biology, University of Utah, Salt Lake City: Mitochondrial genomes from *Drosophila* species—Sequence studies of the A+T-rich region and adjacent genes.

S.E. Kearsey and I.W. Craig, Dept. of Biochemistry, Genetics Laboratory, Oxford, England: Nucleotide substitutions in the mitochondrial large rRNA genes from chloramphenicol-resistant mammalian cell lines.

A. Myers,[1] E. Harris,[2] D. Grant,[1] J. Boynton,[2] and N. Gillham,[1] Depts. of [1]Zoology and [2]Botany, Duke University, Durham, North Carolina: Genetics and cytogenetics of the chloroplast genome of *Chlamydomonas reinhardtii*.

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RNA Tumor Viruses, May 20–May 24

Arranged by **John M. Coffin,** *Tufts University School of Medicine,* **George F. Vande Woude,** *National Cancer Institute*

410 participants

Session 1: Provirus Synthesis, Structure, and Expression

Chairperson: H. Temin, University of Wisconsin, Madison

S.W. Mitra, M. Chow, and D. Baltimore, Massachusetts Institute of Technology, Cambridge: Replication of M-MuLV—The 5′-terminal sequences of plus-strand DNA synthesized de novo.

J.M. Taylor and T.W. Hsu, Institute for Cancer Research, Philadelphia, Pennsylvania: Studies on the integration of retroviral DNA.

L. DesGroseillers, E. Rassart, and P. Jolicoeur, Institut de Recherches Cliniques de Montréal, Université de Montréal, Québec, Canada: Molecular cloning of B- and N-tropic endogenous BALB/c MuLV circular DNA intermediates—Isolation of infectious recombinant clones.

J. Chinsky and R. Soeiro, Depts. of Medicine and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: *Fv-1* host restriction of F-MuLV—Analysis of unintegrated proviral DNA.

T.M. Shinnick, R.A. Lerner, and J.G. Sutcliffe, Research Institute of Scripps Clinic, La Jolla, California: Nucleotide sequence of M-MuLV.

C. Van Beveren, J.A. Galleshaw, P.J. van Straaten, and I.M. Verma, Tumor Virology Laboratory, Salk Institute, San Diego, California: Complete nucleotide sequence of cloned M-MuSV DNA.

P.E. Montandon,[1] Y.-C. Lin,[1] J. Price,[1] F. Montandon,[1] L.T. Bacheler,[2] and H. Fan,[1] [1]Salk Institute, San Diego, California; [2]Fels Research Institute, Philadelphia, Pennsylvania: Expression of M-MuLV proviral DNA in productively infected fibroblasts.

E.P. Reddy, M.J. Smith, and S.A. Aaronson, NCI, National Institutes of Health, Bethesda, Maryland: Complete nucleotide sequence of the M-MuSV genome.

S. Broome, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: An RSV *gag* gene product modulates mRNA levels in transfected cells.

J. Hoffmann,[1] D. Steffen,[2] and R.A. Weinberg,[1] [1]Massachusetts Institute of Technology, Dept. of Biology and Center for Cancer Research, Cambridge; [2]Worcester Foundation for Experimental Biology, Massachusetts: Methylation affecting the expression and DNA infectivity of MuLV proviruses.

C.M. Stoltzfus, R. Dane, and T. Ficht, Dept. of Microbiology, University of Iowa, Iowa City: Synthesis and processing of avian retroviral RNA in normal and S-adenosylmethionine-depleted chicken embryo fibroblasts.

R. Swanstrom,[1] P. Hackett,[2] J.M. Bishop,[1] J. Majors,[1] and H.E. Varmus,[1] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Dept. of Genetics and Cell Biology, University of Minnesota, St. Paul: Characterization of the spliced leader sequences of RSV and MMTV mRNAs.

D. Schwartz,[1] R. Tizard,[1] W. Gilbert,[1] J. Taylor,[2] and R. Guntaka,[3] [1]Harvard University, Cambridge, Massachusetts; [2]Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania; [3]Columbia University, New York, New York: Localization of the splice junctions in the subgenomic mRNAs of RSV.

Session 2: Provirus Expression and Exploitation

Chairperson: J. Taylor, Institute for Cancer Research, Fox Chase, Pennsylvania

D. Bryant, G. Gilmartin, and J.T. Parsons, Dept. of Microbiology, University of Virginia Medical School, Charlottesville: In vitro mutagenesis of the RSV genome.

T. Pugatsch, B. Cullen, and D.W. Stacey, Roche Institute of Molecular Biology, Nutley, New Jersey: Localization of biological functions on cloned ALV DNAs using site-specific mutagenesis and microinjection.

B. Mermer, M.H. Malamy, and J.M. Coffin, Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Part of the avian tumor virus genome can drive expression of the *lac* operon in *E. coli* in fusion vectors which require both transcriptional and translational signals.

S. Fuhrman,[1] F. Keppel,[1] C. Van Beveren,[2] E.P. Geiduschek,[1] and I.M. Verma,[2] [1]University of California, La Jolla; [2]Salk Institute, San Diego, California: In vitro transcription of molecularly cloned murine retroviral DNAs.

G.L. Hager and M.C. Ostrowski, Laboratory of Tumor Virus Genetics, NCI, National Institutes of

Health, Bethesda, Maryland: Murine type-C retroviruses contain transcription promoters that function in vitro.

D. S. Ucker, G. L. Firestone, and K. R. Yamamoto, Dept. of Biochemistry and Biophysics, University of California, San Francisco: Characterization of MuMTV RNAs produced in the presence and absence of glucocorticoid-stimulated transcription.

F. Lee,[1] B. Dieckmann,[1] P. Berg,[2] R. Mulligan,[2] and G. Ringold,[1] Depts. of [1]Pharmacology and [2]Biochemistry, Stanford Medical School, California: Glucocorticoid stimulated expression of dihydrofolate reductase cDNA in CHO cells—Hormonal sensitivity is imparted by MMTV DNA.

C. Dickson, R. Smith, and G. Peters, Imperial Cancer Research Fund, London, England: In vitro synthesis of proteins encoded by the LTR of MMTV DNA.

L. A. Donehower and G. L. Hager, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: Further evidence for the protein-coding potential of the MMTV LTR.

W. L. McClements,[1] D. G. Blair,[2] M. L. McGeady,[1] and G. F. Vande Woude,[1] [1]NCI, National Institutes of Health, Bethesda; [2]Laboratory of Viral Carcinogenesis, National Career Institute, Frederick, Maryland: Use of a retroviral sequence as a cloning vehicle.

M. J. Murray, D. Cowing, and R. A. Weinberg, Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: The use of a retroviral transcriptional promoter to activate a dihydrofolate reductase cDNA in mammalian cells.

K. Shimotohno and H. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Retroviral (SNV) DNA as a possible vector for delivery of foreign DNA (HSV *tk* gene) to vertebrate cells.

C.-M. Wei,[1] M. L. Gilson,[2] P. Spear,[2] and E. M. Scolnick,[3] Laboratories of [1]Viral Carcinogenesis, and [3]Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland; [2]Department of Microbiology, University of Chicago, Illinois: Construction and isolation of a transmissible retrovirus containing the *src* gene of Ha-MuSV and the *tk* gene of HSV-1.

Session 3: Poster Session: Provirus Synthesis, Structure, and Function Transforming Genes

C. Omer, E. Retzel, K. Staskus, and A. Faras, Dept. of Microbiology, University of Minnesota, Minneapolis: Nature of the early events during reverse transcription by the RSV polymerase in vitro.

J. Hillova,[1] M. Hill,[1] R. Mariage-Samson,[1] M. Marx,[1] J. Belehradek,[2] and E. Puvion,[3] [1]Equipe de Recherche du CNRS, Institut de Cancérologie et d'Immunogénétique; [2]Institut Gustave-Roussy; [3]Laboratoire de Microscopie Electronique, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Integration patterns of RSV provirus in RSV-transformed Chinese hamster cells suggesting transpositions of the provirus.

A. Richter,[1] H. Ozer,[2] and P. Jolicoeur,[1] [1]Institut de Recherches Cliniques de Montréal, Université de Montréal, Québec, Canada; [2]Dept. of Biological Sciences, Hunter College, CUNY, New York: MuLV replication in mouse fibroblasts temperature sensitive for cellular DNA synthesis.

S. Duttagupta, S. Saltzman, and R. Soeiro, Depts. of Medicine and Cell Biology, Albert Einstein College of Medicine, Bronx, New York: *Fv-1* host restriction of F-MuLV—Origin of NB-tropic host-range variants.

S. M. Molineaux and J. E. Clements, Johns Hopkins University, Baltimore, Maryland: Analysis of the genetic organization of visna virus using cloned viral DNA.

M. S. Reitz, R. Wong-Staal, and C. D. Trainor, NCI, National Institutes of Health, Bethesda, Maryland: Comparative restriction nuclease mapping of unintegrated proviruses of gibbon ape and woolly monkey type-C viruses.

M. L. Scott, K. Fry, and H. S. Kaplan, Cancer Biology Research Laboratory, Stanford University School of Medicine, California: Molecular cloning and partial nucleotide sequence characterization of GALV.

A. Habara, E. P. Reddy, T. Storch, and S. A. Aaronson, NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of integrated R-MuLV proviral DNA.

S. L. Voynow and J. M. Coffin, Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Characterization of a highly deleted, rapidly replicating variant of RSV.

J. J. O'Rear, K. Shimotohno, and H. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: High-frequency variation in retroviral DNA.

J. J. Kopchick, G. Ju, A. M. Skalka, and D. W. Stacey, Roche Institute of Molecular Biology, Nutley, New Jersey: Biological activity of cloned retroviral DNA in microinjected cells.

B. R. Cullen, J. J. Kopchick, and D. W. Stacey, Roche Institute of Molecular Biology, Nutley, New Jersey: ALV genomes containing regions of exogenous nucleic acid are functional in chicken fibroblasts.

N. Hynes,[1] B. Groner,[1] N. Kennedy,[1] U. Rahmsdorf,[1] L. Fabiani,[1] R. Michalides,[2] and R. Nusse,[2] [1]Kernforschungszentrum Karlsruhe, German Democratic Republic; [2]Dutch Cancer Institute, Amsterdam, The Netherlands: The expression of a molecularly cloned MMTV provirus is hormone-dependent following its transfer into cultured cells.

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G. W. Notani, Depts. of Oral and Medical Microbiology, University of Minnesota, Minneapolis: Expression of RSV and MuMTV nucleotide sequences in *E. coli.*

D. Stéhelin,[1] S. Saule,[1] M. B. Raes,[1] C. Lagrou,[1] H. Beug,[2] and T. Graf,[2] [1]INSERM, Institut Pasteur, Lille, France; [2]German Cancer Research Center, Heidelberg, Federal Republic of Germany: The avian cell DLV-related oncogenes are transcribed in immature hematopoietic cells.

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E. Gelmann, R. C. Gallo, and F. Wong-Staal, Laboratory of Tumor Cell Biology, NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of the SiSV, its helper (SSAV) and defective helper virus genomes.

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T. Curran and N. Teich, Dept. of Viral-mediated Differentiation, Imperial Cancer Research Fund, London, England: Characterization of FBJ murine-osteosarcoma-virus-transformed nonproducer cells and identification of transformation-specific 39,000-dalton protein.

Session 4: Poster Session: Virion Structure and Assembly; Transforming Proteins

T. D. Copeland and S. Oroszlan, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Synthetic peptide substrates for type-C-RNA-tumor-virus-associated proteolytic enzyme.

L. I. Messer,[1] J. G. Levin,[1] and S. K. Chattopadhyay,[2] [1]NICHD; [2]NCI, National Institutes of Health, Bethesda, Maryland: Differential stability of viral message and virion precursor RNA in MuLV-infected cells.

Y. Yoshinaka and R. B. Luftig, Dept. of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia: In vitro processing of Gz-MSV-associated $Pr65^{gag}$ by the partially purified M-MuLV proteolytic factor.

M. Satake and R. B. Luftig, Dept. of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia: Immunofluorescent localization of MuLV-derived gp70 as compared to p15E and $Pr65^{gag}$ antigens associated with the cell membrane of infected cells.

D. Borchelt, R. Resnick, and M. L. Perdue, Division of Experimental Pathology, University of Ken-

tucky Medical Center, Lexington: Translation products of ASV RNA as markers in hybrid-arrested translation analyses.

S. A. Whiteley and R. B. Naso, Dept. of Tumor Virology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston: Intracellular precursor polyproteins of SiSV(SSAV), GALV, and related human retroviruses.

J. Bradac, S. Chatterjee, and E. Hunter, Dept. of Microbiology, University of Alabama, Birmingham: Virus-specific protein synthesis in cells infected with M-PMV.

S. A. Maxwell and R. B. Arlinghaus, Dept. of Tumor Virology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston: In vitro proteolytic cleavage of Gz-MuSV $p65^{gag}$.

A. Tanaka and A. Kaji, University of Pennsylvania, Philadelphia: A transformation-defective RSV mutant with altered p19 of the *gag* gene—Inhibitory effect of this mutant on host cell growth.

W. S. Kloetzer and R. B. Arlinghaus, Dept. of Tumor Virology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston: Purification of a p10-associated protein kinase from M-MuLV.

E. C. Murphy, Jr., University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston: Cell-free translation and size analysis of intracellular virus-specific RNA in a polymerase-defective variant of M-MuLV.

T. Lerner and H. Hanafusa, Rockefeller University, New York, New York: The nature of the polymerase defect in BH-RSVα—Protein and nucleic acid studies.

J. H. Weiss and A. J. Faras, Dept. of Microbiology, University of Minnesota, Minneapolis: Analysis of the avian retrovirus reverse transcriptase using monoclonal antibodies and molecular cloning procedures.

J. A. Bilello and P. M. Pitha, Johns Hopkins Oncology Center, Baltimore, Maryland: Effect of mouse fibroblast interferon on the synthesis, processing, glycosylation, and shed of Friend and AKR-MCF viral proteins.

H. Niman and J. Elder, Research Institute of Scripps Clinic, La Jolla, California: Molecular dissection of Rauscher gp70 with monoclonal antibodies—A progress report.

J. Elder,[1] T. Kawakami,[2] and R. Smith,[3] [1]Research Institute of Scripps Clinic, La Jolla, California: [2]Comparative Oncology Laboratory, University of California, Davis; [3]Laboratory of Tumor Cell Biology, NCI, National Institutes of Health, Bethesda, Maryland: Peptide fingerprint comparisons of the surface glycoproteins of gibbon ape and woolly monkey retroviruses.

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A. Sen,[1] J. C. Neil,[2] J. Ghuysdael,[2] P. K. Vogt,[2] and R. A. Lerner,[1] [1]Cellular Biology and Immunopathology Departments, Research Institute of Scripps Clinic, La Jolla, California; [2]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles: A chemically synthesized peptide fragment containing the active tyrosine of RSV $pp60^{src}$ allows immunological studies on the *src* gene activities in transformed cells.

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D. J. Fujita, J. Bechberger, and I. Nedic, Cancer Research Laboratory and Dept. of Biochemistry, University of Western Ontario, London, Canada: Mutants of RSV which affect transformed-cell morphology and exhibit altered $pp60^{src}$ molecules.

J. Ghysdael, J. C. Neil, and P. K. Vogt, Dept. of Microbiology, University of Southern California, Los Angeles: Structural properties of ASV transformation-specific proteins.

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A. Lau,[1] A. Faras,[2] R. Mitchell,[2] and C. Collins,[3] [1]Dept. of Microbiology, Cancer Center, University of Hawaii, Honolulu; [2]Dept. of Microbiology, University of Minnesota, Minneapolis; [3]University of Virginia School of Medicine, Charlottesville: ASV-transformed and revertant field vole cells—$pp60^{src}$, cellular protein substrates, and arrangement of the integrated ASV genome.

T. Gilmore and G. S. Martin, Dept. of Zoology, University of California, Berkeley: Tyrosine-specific phosphorylation of the 50K polypeptide associated with $pp60^{src}$.

B. Gallis, P. Bornstein, and D. L. Brautigan, Dept. of Biochemistry, University of Washington, Seattle: Specific inhibition of phosphotyrosyl-protein phosphatase in membrane vesicles by micromolar levels of Zn^{2+}.

S. Amini and A. Kaji, Dept. of Microbiology, University of Pennsylvania School of Medicine, Philadelphia: Localization of PP 36K, the presumed target protein for the *src* protein kinase of RSV, to the membrane of chicken cells transformed by RSV.

J. G. Burr, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Phosphorylation of a 68,000-dalton protein in the cytoskeletal structures of RSV-transformed cells.

B. Sefton, T. Patschinsky, T. Hunter, J. Cooper, and F. Esch, Salk Institute, San Diego, California: Tyrosine phosphorylation and cellular transformation.

K. Beemon and B. Adkins, Salk Institute, San Diego, California: Tyrosine phosphorylation of cellular proteins implicated in transformation by four classes of retroviruses.

I. U. Ali,[1] S. Rasheed,[2] and T. Hunter,[3] [1]Meloy Laboratories, Rockville, Maryland; [2]Department of Pathology, University of Southern California, Los Angeles; [3]Salk Institute, San Diego, California: Structural analysis of fibronectins from normal and transformed cells.

W.-H. Lee,[1] K. Bister,[1] C. Moscovici,[2] and P. Duesberg,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Virus Research Laboratory, Veterans Administration Hospital, Gainesville, Florida: *ts* mutants of Fujinami sarcoma virus—Tumorigenicity and reversible phosphorylation of the transforming protein.

B. Adkins, T. Hunter, and K. Beemon, Salk Institute, San Diego, California: PRCII ASV—Comparative characterization of the in vivo and in vitro synthesized transforming protein.

G. Ramsay,[1] T. Graf,[2] and M. J. Hayman,[1] [1]Imperial Cancer Research Fund, London, England; [2]Deutsches Krebsforshungszentrum, Heidelberg, Federal Republic of Germany: Tryptic peptide mapping of the fusion proteins of MC29 virus mutants.

J. H. Chen[1] and S. M. Anderson,[2] [1]Life Sciences Biomedical Research Institute, St. Petersburg, Florida; [2]Rockefeller University, New York, New York: In vitro translation of AMV RNA.

S. Harmon,[1,3] and S. Wright,[2,3] [1]Dept. of Biology; [2]Dept. of Medicine, and Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City; [3]Viral Oncology Laboratory, Veterans Administration Medical Center, Salt Lake City, Utah: In vitro translation of AMV genomic RNA yields a 92,000-dalton protein with *gag* determinants.

M. L. Privalsky,[1] B. Vennstrom,[2] and J. M. Bishop,[1] [1]University of California Medical Center, San Francisco; [2]University of Uppsala, Sweden: Polypeptides specified by the *erb* region of AEV.

M. A. Boss, G. Dreyfuss, and D. Baltimore, Dept. of Biology, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Localization of the Ab-MuLV protein in a detergent-insoluble matrix—Architecture of the protein.

C. Machida,[1,2] and D. Kabat,[2] Depts. of [1]Microbiology and Immunology, [2]Dept. of Biochemistry, University of Oregon Health Sciences Center, Portland: Identification of an Ab-MuLV encoded protein related to p120 on the plasma membrane of transformed lymphoid cells.

J. P. Horn,[1] T. G. Wood,[2] E. C. Murphy,[1] R. L. Brown,[3] R. Junghans,[4] D. G. Blair,[3] and R. B. Arlinghaus,[1] [1]Dept. of Tumor Virology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston; [2]NCI, National Institutes of Health, Bethesda; [3]Frederick Cancer Research Center, Frederick, Maryland; [4]Roche Institute of Molecular Biology, Nutley, New Jersey: A selective ts defect in *gag-src* viral RNA expression in cells infected with a ts mutant of MuSV.

A. Sen and A. Cresse, Cellular Biology Department, Research Institute of Scripps Clinic, La Jolla, California: Purified protein kinase from MSV particles catalyzes preferential phosphorylation of certain proteins present in MSV-transformed cells.

H. A. Young,[1] C.-C. Li,[1] R. W. Ellis,[2] and S. Rasheed,[3] [1]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick Maryland; [2]Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland; [3]Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: Analysis of the transforming gene of Rasheed rat sarcoma virus.

M. E. Furth, J. Maryak, A. Papageorge, and E. M. Scolnick, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of the p21 *src* protein of HaSV and related proteins using monoclonal antibodies.

H. W. Snyder, Jr. and M. Singhal, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of protein kinase activity associated with transforming gene products of the ST and GA strains of FeSV.

A. Chen,[1] M. Kelliher,[1] F. de Noronha,[2] and M. Essex,[1] [1]Dept. of Microbiology, Harvard University School of Public Health, Boston, Massachusetts; [2]Dept. of Veterinary Pathology, Cornell University, Ithaca, New York: Expression of FeSV "*gag-fes*" in cultivated cat tumor cells.

M. Barbacid, NCI, National Institutes of Health, Bethesda, Maryland: The McDonough strain of

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H.-J. Thiel, T. J. Matthews, and E. M. Broughton, Dept. of Surgery, Duke University Medicial Center, Durham, North Carolina: Transformation by SiSV—Expression of antigens in non-producer cells.

Session 5: *Virion Structure and Assembly*

Chairperson: J. Elder, Research Institute of Scripps Clinic, La Jolla, California

E. Hunter,[1] A. Bhown,[1] A. D. Schwartz,[2] and R. Eisenman,[3] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]The Biological Laboratories, Harvard University, Cambridge, Massachusetts; [3]Hutchinson Cancer Center, Seattle, Washington: Organization and processing of the gag gene product of RSV.

L. E. Henderson,[1] C. Van Beveren,[2] T. D. Copeland,[1] R. J. Versteegen,[1] I. M. Verma,[2] and S. Oroszlan,[1] [1]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland; [2]Tumor Virology Laboratory, Salk Institute, San Diego, California: Primary structure of MuLV $Pr65^{gag}$ determined by protein and nucleic acid sequencing.

W. N. Burnette, C. van Beveren, I. M. Verma, and H. Fan, Salk Institute, San Diego, California: Biosynthesis of MuLV gag polyproteins—Implications from nucleic acid sequence studies.

J. Leis and G. Duyk, Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Nucleotide sequences of AMV p19-protein-binding sites on RSV (Pr-C) RNA.

S. Johnson,[1] M. Veigel,[1] T. Vanaman,[1] and J. Leis,[2] [1]Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina; [2]Dept. of Biochemistry, Case Western Reserve University, Cleveland, Ohio: Isolation of a cyanogen bromide peptide of AMV p19 protein that contains its RNA binding site.

Y. Yoshinaka and R. B. Luftig, Dept. of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia: Two MuLV protein kinase activities and their possible functions.

D. P. Grandgenett,[1] T. K. Misra,[1] and J. T. Parsons,[2] [1]St. Louis University Medical Center, Missouri; [2]University of Virginia, Charlottesville, Virginia: Avian retrovirus pp32 protein—Specific binding sites on retroviral DNA terminal repeats.

E. Hunter,[1] M. Hardwick,[1] G. Davis,[1] A. Brown,[1] and D. Schwartz,[2] [1]Dept. of Microbiology, University of Alabama, Birmingham; [2]The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Organization and processing of the *env* gene product of RSV.

N. Green,[1] T. M. Shinnick,[1] O. Witte,[2] A. Ponpicelli,[2] J. G. Sutcliffe,[1] and R. A. Lerner,[1] [1]Research Institute of Scripps Clinic, La Jolla; [2]University of California, Los Angeles: Sequence-specific antibodies reveal that the maturation of M-MuLV envelope polyprotein involves the removal of a carboxyterminal peptide.

A. Schultz, T. Copeland, L. Henderson, and S. Oroszlan, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Virion- and cell-associated proteins coded by the 3′ end of the *env* gene of MuLV.

R. Massey and G. Schochetman, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Monoclonal antibodies define domains on an MMTV *env* gene product involved in infectivity and oncogenicity.

N. A. Wivel,[1] J. A. Bilello,[2] and P. M. Pitha,[2] [1]National Institutes of Health, Bethesda; [2]Johns Hopkins School of Medicine, Baltimore, Maryland: Changes in the pattern of assembly of MCF virus in interferon-treated mouse cells.

R. Naso, Y.-H. Wu, and C. Edbauer, Dept. of Tumor Virology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston: Antiretroviral action of interferon.

S. Chatterjee,[1] H. C. Cheung,[2] and E. Hunter,[1] Depts. of [1]Microbiology, and [2]Biomathematics, University of Alabama, Birmingham: Interferon inhibition of primate retroviral fusion and replication—A common effect of membrane stiffening?

Session 6: *Viral and Cellular* onc *Genes*

Chairperson: D. Stéhelin, Institut Pasteur, Lille, France

D. Sheiness, T. Gonda, B. Vennstrom, G. Payne, and J. M. Bishop, Dept. of Microbiology, University of California, San Francisco: Structure and expression in various tissues of cellular homologs of viral oncogenes: c-*myc*, c-*myb*, c-*erb*, and c-*src*.

M. Shibuya, L.-H. Wang, and H. Hanafusa, Rockefeller University, New York, New York: Structure and expression of Fujinami sarcoma virus unique sequence and related cellular sequences.

T. S. Robins,[1] K. Bister,[1] P. Duesberg,[1] and T. Papas,[2] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]NCI, National Institutes of Health, Bethesda, Maryland: Molecular cloning of the chicken cellular c-*mcv* locus and partial sequence comparison to MC29 viral RNA.

D. Chiswell, G. Ramsay, and M. Hayman, Tumor Virology Laboratory, Imperial Cancer Research Fund, London, England: Cells infected by OK10 produce two RNA species containing virus- and *myc*-specific sequences.

T. G. Wood,[1] D. G. Blair,[2] W. L. Clements,[1] M. Oskarsson,[1] and G. Vande Woude,[1] Laboratories of [1]Molecular Virology and [2]Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: *mos*-specific RNA in cells transfected with recombinant cellular and viral DNAs.

S. Gattoni,[1] P. Kirschmeier,[1] W. Hsiao,[1] I. B. Weinstein,[1] J. Escobedo,[2] and D. Dina,[2] [1]Institute of Cancer Research, Columbia University, New York; [2]Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Cellular M-MuSV (*mos*) sequences are hypermethylated and transcriptionally silent in normal and carcinogen-transformed rodent cells.

D. R. Lowy,[1] R. W. Ellis,[2] D. DeFeo,[2] E. H. Chang,[1] M. A. Gonda,[3] H. A. Young,[3] N. Tsuchida,[4] T. Y. Shih,[2] and E. M. Scolnick,[2] [1]Dermatology Branch; [2]Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda; [3]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland; [4]Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: The cellular p21 *sarc* genes represent a family of divergent normal genes which have the capacity to transform mouse cells.

S. Goff,[1] C. Tabin,[1] R. Lee,[1] J. Wang,[1] P. D'Eustachio,[2] F. Ruddle,[2] and D. Baltimore,[1] [1]Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Biology, Yale University, New Haven, Connecticut: Molecular clones of biologically active Ab-MuLV DNA and the homologous cellular gene.

L. A. Fedele, L. Donner, J. Even, and C. J. Sherr, Viral Pathology Section, NCI, National Institutes of Health, Bethesda, Maryland: Comparative studies of three strains of FeSV.

K. C. Robbins, S. G. Devare, S. R. Tronick, and S. A. Aaronson, Laboratory of Cellular and Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Physical and biological analysis of molecular clones of integrated SiSV.

F. Wong-Staal, E. Gelman, R. Dalla Favera, S. Szala, S. Josephs, and R. C. Gallo, Laboratory of Tumor Cell Biology, NCI, National Institutes of Health, Bethesda, Maryland: The transforming gene of SiSV (v-*sis*)—A new *onc* gene of primate origin.

E. Stavnezer, D. S. Gerhard, R. C. Binari, and I. Balazs, Sloan-Kettering Institute for Cancer Research, New York, New York: The generation of transforming viruses in cultures of chicken fibroblasts infected with an ALV.

P. C. Balduzzi,[1] M. F. D. Notter,[1] H. R. Morgan,[1] M. Shibuya,[2] and H. Hanafusa,[2] [1]Dept. of Microbiology, University of Rochester School of Medicine and Dentistry; [2]Rockefeller University, New York, New York: ASV UR1 and UR2. I. Their biological properties and transforming inserts.

M. Linial, K. Lewison, and P. E. Neiman, Fred Hutchinson Cancer Research Center, Seattle, Washington: Generation of transforming virus by recombination between cellular and viral sequences.

Session 7: Poster Session: Endogenous Viruses; Viral Leukemogenesis

V. Erfle,[1] J. Schmidt,[1] R. Hehlmann,[2] and A. Luz,[1] [1]Abt. Pathologie, Gessellschaft für Strahlen-u. Umweltforschung, Neuherberg; [2]Medizinische Poliklinik der Universitä München, Federal Republic of Germany: Infectious endogenous retroviruses early and late in the course of radiation-induction of osteosarcomas in mice.

O. Niwa and T. Sugahara, Dept. of Experimental Radiology, Kyoto University, Japan: 5-Azacytidine activates endogenous type-C-virus genome of mouse cells in tissue culture.

R. Emanoil-Ravicovitch, F. Saal, F. Cavalieri, and J. Peries, Unité 107 INSERM, Hôpital Saint Louis, Paris, France: Nonproductive chronic infection of a human cell line by a murine xenotropic retrovirus.

R. Crowther, D. Tenney, and W. Haseltine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: A xenotropic virus of AKR origin that replicates in human cells.

J. P. Stoye, J. Jongstra, and C. Moroni, Friedrich Mieschler-Institut, Basel, Switzerland: Mitogen induction of defective retroviruses.

J. P. Kim, R. A. Grymes, H. S. Kaplan, and K. Fry, Cancer Biology Research Laboratory, Stanford University, California: Molecular cloning of two endogenous retroviruses of the c57BL/Ka mouse, RadLV and B virus.

M. Bondurant, St. Jude Children's Research Hospital, Memphis, Tennessee: Transcription of endogenous retroviral sequences in non-virus-producing embryonic cell lines from Swiss and BALB/c mice. Molecular cloning of BALB:virus-2.

F. van der Hoorn,[1] C. Onnekink,[1] H. van der Putten,[1] M. Zijlstra,[2] and H. Bloemers,[1] [1]Dept. of Biochemistry, University of Nijmegen; [2]CLB, Amsterdam, The Netherlands: Cloning and expression of noninducible xenotropic *env* sequences.

J.M. Young, R.J. Mural, R. Roblin, and J.N. Ihle, Frederick Cancer Research Center, Maryland: Molecular cloning and characterization of a deleted endogenous murine type-C virus genome.

E. Rassart and P. Jolicoeur, Institut de Recherches Clinques de Montréal, Université de Montréal, Québec, Canada: Viruslike organization of the majority of endogenous MuLV sequences in the mouse genome.

N.A. Jenkins, N.G. Copeland, B.A. Taylor, and B.K. Lee, Jackson Laboratory, Bar Harbor, Maine: Restriction enzyme analysis of endogenous ecotropic MuLV DNA sequences in 46 inbred mouse strains.

W. Quint, H. van der Putten, F. van der Hoorn, and A. Berns, Dept. of Biochemistry, University of Nijmegen, The Netherlands: Chromosomal location and expression of MuLV genomes in the germ-line and tumor tissues of AKR strains.

S.J. O'Brien[1] and J.E. Womack,[2] [1]National Cancer Institute, Frederick, Maryland; [2]Dept. of Veterinary Pathology, Texas Agricultural and Mechanical University, College Station: Evidence for the horizontal acquisition of murine AKR virogenes by recent horizontal infection of the germ line.

J.L. Moore, H. Chan, W. Rowe, and M. Martin, NIAID, National Institutes of Health, Bethesda, Maryland: Heterogeneity of endogenous ecotropic retrovirus in AKR inbred mice.

W. Drohan,[1] D. Gallahan,[2] L. D'Hoostelaere,[2] M. Potter,[2] and R. Callahan,[1] Laboratories of [1]Cellular and Molecular Biology, and [2]Clinical Biochemistry, NCI, National Institutes of Health, Bethesda, Maryland: Natural populations of breeding feral mice free of MMTV proviral DNA.

J.I. MacInnes,[1] V.L. Morris,[1] W.F. Flintoff,[1] and C.A. Kozak,[2] [1]University of Western Ontario, London, Canada; [2]NIAID, National Institutes of Health, Bethesda, Maryland: Mapping of endogenous MMTV proviral DNA sequences in GR mice.

P.R. Etkind,[1] P. Szabo,[2] and N. Sarkar,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Institut de Recherche en Biologie Moléculaire, Paris, France: Restriction endonuclease mapping of the proviral DNA of the exogenous RIII MuMTV.

D. Gray,[1] V. Morris,[1] R. Jones,[2] E. Lee Chan,[1] and C. McGrath,[2] [1]Dept. of Microbiology and Immunology, University of Western Ontario, London, Canada; [2]Dept. of Tumor Biology, Michigan Cancer Foundation, Detroit: MMTV sequences in tumor cell populations.

T. Breznik and J.C. Cohen, Dept. of Microbiology, Tulane University School of Medicine, New Orleans, Louisiana: Altered methylation of genetically transmitted MMTV during carcinogenesis.

L. Benade,[1] W. Drohan,[2] D. Graham,[2] and G. Smith,[2] [1]Meloy Laboratories, Springfield, Virginia; [2]NCI, National Institutes of Health, Bethesda, Maryland: Differential hypomethylation and lack of amplification of MMTV proviral DNA in spontaneous and chemically induced C3H/StWi mammary tumors.

N. Fasel, K. Pearson, E. Buetti, R. Klemenz, and H. Diggelmann, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: Nucleotide sequence analysis of cloned MMTV DNA.

E. Keshet, A. Itin, and G. Rotman, Dept. of Virology, Hadassah Medical School, Hebrew University, Jerusalem, Israel: The murine "retroviruslike" gene family expressing 30S RNA—Patterns of distribution, heterogeneity, and sequence conservation.

H. Mondal, J. Jendis, H.G. Münch, and P.H. Hofschneider, Max-Planck-Institut für Biochemie, Munich, Federal Republic of Germany: Expression of retrovirus-related genes during embryogenesis of some vertebrate species.

R. Callahan,[1] E.L. Kuff,[2] K.K. Lueders,[2] and G. Mark,[3] Laboratories of [1]Cellular and Molecular Biology, [2]Biochemistry, and [3]Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda, Maryland: The genetic relationship between the *M. cervicolor* M432 retrovirus and the intracisternal type-A-particle genome.

K. Stromberg and R.I. Huot, Laboratory of Viral Carcinogenesis, NCI, Bethesda, Maryland: Rapid and frequent isolation of infectious type-C virus after cocultivation of rhesus trophoblasts with feline embryo cells.

T.I. Bonner,[1] E. Birkenmeier,[1] M.A. Gonda,[2] N. Battula,[1] and G.J. Todaro,[1] [1]Laboratory of Viral Carcinogenesis, NCI, National Institutes of Health, Bethesda; [2]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: The type-C-retrovirus-related sequences of chimpanzee.

M. Cohen, C. O'Connell, R. Stephens, and N. Rice, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: DNA sequence relationship of the baboon endogenous virus genome to the genomes of other type-C and type-D retroviruses.

C. O'Connell, R. Stephens, A. Rein, and M. Cohen, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Molecular cloning and structural characterization of nondefective baboon endogenous virus genomes from DNA of a baboon cell strain.

W. H. Schubach, M. Groudine, and P. E. Neiman, Fred Hutchinson Cancer Research Center, Seattle, Wahington: Activation of c-*myc* in the vicinity of the integration site of an exogenous provirus in a cell line derived from an ALV-induced bursal lymphoma.

Y. K. T. Fung,[1] A. M. Fadly,[2] L. B. Crittenden,[2] and H. J. Kung,[1] [1]Dept. of Biochemistry, Michigan State University; [2]Regional Poultry Research Laboratory, United States Dept. of Agriculture, East Lansing, Michigan: Identification of the possible oncogenes involved in different neoplasms induced by ALV.

A. Rein,[1] D. R. Lowy,[2] A. M. Schultz,[1] M. A. Gonda,[1] B. I. Gerwin,[2] S. K. Ruscetti,[2] and R. H. Bassin,[2] [1]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick; [2]NCI, National Institutes of Health, Bethesda, Maryland: Properties of a replication-defective MuLV isolated from cultured AKR leukemia cells.

P. V. O'Donnell,[1] R. C. Nowinski,[2] and E. Stockert,[1] [1]Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington: Amplification of MuLV-related antigens on preleukemic AKR thymocytes represents expression of *env* gene products encoded by an infecting dualtropic (MCF) MuLV genome.

N. G. Famulari and P. V. O'Donnell, Memorial Sloan-Kettering Cancer Center, New York, New York: Leukemogenesis by Gross Passage A virus—Influence of a newly detected virus component.

P. Tsichlis,[1] T. Wood,[2] and L.-F. Hu,[1] Laboratories of [1]Tumor Virus Genetics and [2]Molecular Virology, NCI, National Institutes of Health, Bethesda, Maryland: M-MuLV induced thynomas in rats.

S. K. Swanson and E. F. Hays, Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles: AKR spontaneous lymphoma virus.

F. Yoshimura and M. Breda, Fred Hutchinson Cancer Research Center, Seattle, Washington: Analysis of MuLV proviruses in the AKR mouse during the development of lymphomas using cloned DNA virus-specific probes.

C. Y. Thomas, R. Khiroya, R. Schwartz, and J. M. Coffin, Tufts University School of Medicine, Boston, Massachusetts: Genetic structure of MuLVs isolated from spontaneous and induced lymphomas in HRS/J, AKR/J, and CBA/J mice.

J. Lenz, R. Crowther, A. Straceski, and W. Haseltine, Sidney Farber Cancer Institute Harvard Medical School, Boston, Massachusetts: Nucleotide sequence of the *env* gene of AKV virus.

C. A. Holland,[1] S. K. Cattopadhyay,[2] and N. Hopkins,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]NCI, National Institutes of Health, Bethesda, Maryland: A genetic analysis of the 3′ ends of MuLVs by construction of site-specific recombinants between DNA clones of MCF and AKV viruses.

D. R. Joseph, Papanicolaou Cancer Research Institute, Miami, Florida: Possible identification by restriction endonuclease mapping of the type xenotropic virus involved in generation of the recombinant AKR MCF 247 MuLV.

U. R. Rapp,[1] E. Birkenmeier,[1] and M. A. Gonda,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Genome comparison of a leukemogenic with a nonleukemogenic variant of MuLV.

U. R. Rapp,[1] E. Birkenmeier,[1] and M. A. Gonda,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Genome analysis of a lung-carcinoma-inducing virus.

M. W. Cloyd, J. L. Portis, and B. Chesebro, Rocky Mountain Laboratory, Hamilton, Montana: MCF-specific murine monoclonal antibodies made against AKR MCF 247 virus recognize a unique determinant associated with the gp70-p15(E) complex.

A. Pinter, W. J. Honnen, J.-S. Tung, P. V. O'Donnell, and U. Hammerling, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of antigenic determinants of ecotropic and recombinant murine retroviruses defined by monoclonal antibodies.

J. Elder and H. Niman, Research Institute of Scripps Clinic, La Jolla, California: Structural homologies in the substituted regions of the gp70s of AKR-, Rauscher-, and Moloney- virus-derived recombinant retroviruses.

R. Michalides,[1] J. Hilkens,[1] B. Groner,[2] and N. E. Hynes,[2] [1]Dutch Cancer Institute, Amsterdam, The Netherlands; [2]Institute for Genetics, Karlsruhe, Federal Republic of Germany: Acquisition of MMTV DNA at specific genomic locations in thymic leukemias of GR and GR/Mtv-2- mice.

G. A. Dekaban and J. K. Ball, Dept. of Biochemistry, University of Western Ontario, London, Canada: The presence of unintegrated MMTV in virus-induced primary murine thymic lymphomas.

J. Deschamps,[1] R. Kettmann,[2] and A. Burny,[2] [1]Faculty of Agronomy, Gembloux; [2]Laboratory of Biological Chemistry, University of Brussels, Belgium: Study of host-virus interaction with cloned BLV DNA.

C. Bruck, D. Portetelle, M. Mammerickx, and A. Burny, Laboratory of Biological Chemistry, University of Brussels, Belgium: Immunological importance of the BLV envelope glycoprotein gp51.

Y. Obata, A.B. DeLeo, E. Stockert, and L.J. Old, Memorial Sloan-Kettering Cancer Center, New York, New York: A new catagory of surface antigens on AKR leukemia cells—Individually distinct antigens.

E.V. Genovesi, D. Livnat, and J.J. Collins, Dept. of Surgery, Duke University Medical Center, Durham, North Carolina: Induction of immunologic memory in mice protected against FLV-induced disease by passive serum therapy.

J. Gautsch, H. Niman, and J. Elder, Research Institute of Scripps Clinic, La Jolla, California: Neutralization of MuLV with monoclonal antibodies to gp70.

S.S. VedBrat,[1] W. Prensky,[1] H. Lutz,[2] and S. Ruscetti,[3] [1]Sloan-Kettering Institute for Cancer Research, New York, New York; [2]University of California, Davis; [3]NCI, National Institutes of Health, Bethesda, Maryland: Feline oncornavirus-associated cell membrane antigen (FOCMA) expression—Current concepts from immunofluorescence and immunoprecipitation data using monoclonal antibodies.

M. Popovic,[1] A. Ridgeway,[1] I. Royston,[2] and R.C. Gallo,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Hematology/Oncology Section, Veterans Administration Hospital, San Diego, California: Induction of a new antigen (HAA) associated with T-cell activation of human "resting" lymphocytes by animal retroviruses and by HTLV.

D. Mathieu-Mahul,[1] J.M. Heard,[2] S. Fichelson,[2] S. Gisselbrecht,[1] and C.J. Larsen,[1] [1]Centre Hayem, Hôpital St. Louis, Paris, France; [2]Laboratoire Virologie et Immunologie des Tumeurs, Hôpital Cochin, Paris, France: Expression of viral proteins in long-term bone marrow myelomonocytic cells infected by Friend polycythemia virus.

L. Wolff and S. Ruscetti, Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland: Monoclonal antibodies to SFFV-encoded gp52 cross-react with recombinant MCF and xenotropic viruses.

T. Mak, S. Clark, Y. Yamamoto, C. Gamble, A. Joyner, D. Mager, T. Shibuya, and A. Bernstein, Ontario Cancer Institute, Toronto, Canada: Clonal analysis of Friend erythroleukemia and the Friend genome with molecularly cloned SFFV.

D.L. Linemeyer, J.G. Menke, S.K. Ruscetti, and E.M. Scolnick, NCI, National Institutes of Health, Bethesda, Maryland: Genetic study of SFFV DNA.

T.A. Kost,[1] W.D. Hankins,[2] and I.B. Pragnell,[3] [1]Dept. of Medicine, Vanderbilt University and Veterans Administration Medical Center, Nashville, Tennessee; [2]Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland; [3]Beatson Institute for Cancer Research, Bearsden, Glasgow, Scotland: Myeloproliferative sarcoma virus causes erythroid transformation in vitro.

J. Salles, J. Rowe, and N. Teich, Imperial Cancer Research Fund, London, England: Studies of MyLV, a virus that causes acute myelogenous leukemia in C57BL mice.

N. Teich and J. Rowe, Imperial Cancer Research Fund, London, England: Studies of BSB, a virus that causes erythroleukemia in C57BL mice.

Y. Ikawa, M. Aida, T. Matsugi, and N. Sagata, Dept. of Viral Oncology, Cancer Institute, Tokyo, Japan: Tropism to hematopoietic cells of helper components in anemic and polycythemic FLVs.

B.R. Brooks, J. Gossage, and R.T. Johnson, Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Age-dependent in vitro restriction of mouse neurotropic retrovirus replication in CNS derived cells from susceptible $Fv\text{-}1^{nn}$ mice.

E. Fleissner, E. Tress, J. Bieler, M. Boccara, E. Stavnezer, and P.V. O'Donnell, Memorial Sloan-Kettering Cancer Center, New York, New York: Analysis of viral gene expression in radiation-induced leukemias of BALB/c mice.

A. Mayer, Dept. of Pathology, New York University Medical Center, New York: Xenotropic MuLV expression in thymoma as induced by radiation or methylcholanthrene.

M. Kotler,[1] S. Bendavid,[1] and N. Haran-Gera,[2] [1]Dept. of Molecular Virology, Hebrew University-Hadassah Medical School, Jerusalem; [2]Dept. of Immunology, Weizmann Institute, Rehovot, Israel: Characterization of the viruses released by thymomas induced in C57BL mice by X-ray irradiation.

K.D. Somers and M.M. Murphey, Department of Microbiology and Immunology, Eastern Virginia Medical School, Norfolk: Uncontrolled nuclear division—A marker of altered growth control in MSV-transformed cells and several human tumor cell lines.

G.R. Anderson, Dept. of Microbiology, University of Pittsburgh School of Medicine, Pennsylvania: LDH_k—A primary or secondary event in transformation by the KSV?

W.D. Hankins and E.M. Scolnick, NCI, National Institutes of Health, Bethesda, Maryland: Transformation of hemopoietic cells in vitro by HaSV and KSV.

P.L. Kaplan and B. Ozanne, University of Texas Health Science Center, Dallas: K-MuSV-transformed cells grow in serum-free/mitogen-free medium.

T. Shibuya,[1] I. Chen,[2] A. Howatson,[1] and T. Mak,[1] [1]Ontario Cancer Institute, Toronto, Canada; [2]McArdle Laboratory for Cancer Research, Madison, Wisconsin: REV-T-transformed cells have characteristic of T lymphoblasts.

O. Jarrett,[1] D. E. Onions,[1] N. Testa,[2] S. Toth,[1] and M. Mochizuki,[1] [1]University of Glasgow, Scotland; [2]Paterson Laboratories, Christie Hospital, Manchester, England: FeLVs of subgroup-C cause erythroid aplasia.

P. Kahn,[1] S.-I. Shin,[1] and M. J. Weber,[2] [1]Dept. of Genetics, Albert Einstein College of Medicine, New York, New York; [2]Dept. of Microbiology, University of Illinois, Urbana: Tumorigenicity of partial transformation mutants of RSV.

W. Prensky and S. S. VedBrat, Memorial Sloan-Kettering Institute for Cancer Research, New York, New York: Gene expression pathways altered in virus-transformed cat cells.

D. Boettiger,[1] M. Pacifici,[2] H. Holtzer,[2] and S. Adams,[1] Depts. of [1]Microbiology, [2]Anatomy, and [3]Human Genetics, University of Pennsylvania, Philadelphia: Control of differentiated cell functions by RSV in infected chicken chondroblasts.

S. Rasheed, Dept. of Pathology, University of Southern California School of Medicine, Los Angeles: Differentiation of a spontaneous cat melanoma culture by endogenous RD-114 virus.

K. Hsia and A. Kaji, University of Pennsylvania, Dept. of Microbiology, Philadelphia: Effect of cell differentiation on expression of RSV genes.

D. J. Grunwald,[1] B. Dale,[2] J. Dudley, B. Ozanne,[2] and R. Risser,[1] [1]McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; [2]Dept. of Microbiology, University of Texas Medical Center Dallas: "Differentiation" of a clonal Abelson lymphoma line and concomitant loss of viral genome expression.

A. Laszlo and M. Bissell, Laboratory of Cell Biology, Lawrence Berkeley Laboratory, Berkeley, California: Tumor virus transforming genes and tumor promoters do not act via common pathways.

U. R. Rapp,[1] P. Borchert,[2] and H. Young,[1] [1]NCI, National Institutes of Health, Bethesda; [2]Intramural Research Support Program, Frederick Cancer Research Center, Frederick, Maryland: Transformation of cells in culture by MuLV and TPA.

G. De Petro,[1,2] S. Barlati,[2] T. Vartio,[1] and A. Vaheri,[1] [1]Dept. of Virology, University of Helsinki, Finland; [2]Laboratorio di Genetica, Biochimica ed Evoluzionistica, CNR, Pavia, Italy: RSV-induced cell transformation is promoted by small concentrations of defined proteolytic fragments of fibronectin.

F. H. Reynolds, Jr.,[1] G. J. Todaro,[2] C. Fryling,[2] and J. R. Stephenson,[2] [1]Frederick Cancer Research Center, [2]National Cancer Institute, Frederick, Maryland: Transforming growth factors specifically induce phosphorylation of tyrosine residues in epidermal growth factor membrane receptors.

P. Gupta and J. F. Ferrer, New Bolton Center, University of Pennsylvania, Kennett Square: Detection of a factor in the plasma of BLV-infected cattle that blocks the expression of the viral genome in infected lymphocytes.

P. Herbrink, F. J. van Bussel, G. N. P. van Muijen, and S. O. Warnaar, Dept. of Pathology, State University of Leiden, The Netherlands: Natural antibodies cross-reactive with type-C-virus p30 in normal and malignant human sera.

H. Schetters,[1] C. Leib,[1] V. Erfle,[2] and R. Hehlmann,[1] [1]Medizinische Poliklinik der Universität München; [2]Gessellschaft für Strahlen-und Umweltforschung, Neuherberg, Federal Republic of Germany: Characterization of human serum proteins cross-reacting with α SiSV p30 and α BaEV p30 antibody.

M. Reitz, M. Wainberg, F. Ruscetti, L. Ceccherine-Nelli, and R. C. Gallo, NCI, National Institutes of Health, Bethesda, Maryland: HTLV, a type-C virus isolated from human T-cell malignancies, is T-cell-tropic.

J. Suni[1] and A. Vaheri,[2] [1]Dept. of Virology, Municipal Bacteriological Laboratory, Helsinki; [2]Dept. of Virology, University of Helsinki, Finland: Retrovirus expression during pregnancy in humans.

R. C. Mellors, J. W. Mellors, L. Jerabek, and S. Maeda, Hospital for Special Surgery and New York Hospital-Cornell University Medical College, New York: Human normal trophoblast contains an ~25Kd protein that is antigenically related to group-specific determinants of SSAV/GALV p28.

E. Hefti, R. Tuetken, C. Raineri, and S. Panem, Kovler Viral Oncology Laboratories, University of Chicago, Illinois: Retrovirus-specific sequences in human placental DNA.

R. Kurth,[1,3] J. Lower,[1,3] R. Lower,[1] D. Wernicke,[1] H. Frank,[2] [1]Fr.-Miescher-Laboratory; [2]Max-Planck-Institute for Virus Research, Tübingen, Federal Republic of Germany: Human teratocarcinomas produce unique oncoviruslike particles.

Session 9: Transforming Proteins and Their Targets

Chairperson: N. Rosenberg, Tufts University, Boston, Massachusetts

H. Oppermann,[1] J.M. Bishop,[1] A.F. Purchio,[2] R.L. Erikson,[2] and J. Smart,[3] [1]Dept. of Microbiology and Immunology, University of California, San Francisco; [2]Dept. of Pathology, University of Colorado Medical Center, Denver; [3]Cold Spring Harbor Laboratory, New York: pp60^{src} as a tyrosine protein kinase—Characterization of potential substrate sites.

J. Brugge, D. Darrow, and J. Cutt, Dept. of Microbiology, State University of New York, Stony Brook: Analysis of cellular proteins which interact with the transforming proteins of ASV.

K.D. Nakamura, R. Martinez, and M.J. Weber, Dept. of Microbiology, University of Illinois, Urbana: Identifying targets of pp60^{src} using partial transformation mutants of RSV.

V. Rotter,[1] R. Coffman,[2] M.A. Boss,[1] and D. Baltimore,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Pathology, Stanford University School of Medicine, California: Two proteins in Abelson-virus-transformed lymphoid cells with shared antigenicity—Soluble P50 and membrane-bound P30.

J.C. Neil,[1] J. Ghysdael,[1] J.E. Smart,[2] and P.K. Vogt,[1] [1]Dept. of Microbiology, University of Southern California School of Medicine, Los Angeles; [2]Cold Spring Harbor Laboratory, New York: Transformation-specific proteins of the ASVs.

M. Spector, R.B. Pepinsky, V.M. Vogt, and E. Racker, Molecular and Cell Biology, Cornell University, Ithaca, New York: A tyrosine-specific protein kinase cascade in cells transformed by retroviruses.

K. Shriver and L. Rohrschneider, Fred Hutchinson Cancer Research Center, Seattle, Washington: Characterization of the tyrosine phosphotransferase activities associated with isolated adhesion plaques and junctions from NRK and SR-NRK cells.

J.G. Krueger, A.R. Goldberg, R. Karess, and H. Hanafusa; Rockefeller University, New York, New York: Membrane association of pp60^{src} correlates with increased in vivo tumorigenicity.

H. Abrams, L. Rohrschneider, and R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington: Intracellular location of the MC29-encoded polyproteins in MC29-transformed quail cells.

T. Pawson,[1,2] J. Guyden,[2] T.-H. Kung,[2] T. Gilmore,[2] K. Radke,[2] and G.S. Martin,[2] [1]Dept. of Microbiology, University of British Columbia, Vancouver, Canada; [2]Dept. of Zoology, University of California, Berkeley: Structure, phosphorylation, and associated kinase activity of the transforming protein of Fujinami ASV.

J. Papkoff, M.H.-T. Lai, T. Hunter, and I.M. Verma, Tumor Virology Laboratory, Salk Institute, San Diego, California: Analysis of the transforming gene product of M-MuSV.

T.Y. Shih,[1] M.O. Weeks,[1] S. Oroszlan,[2] P. Gruss,[3] G. Khoury,[3] and E.M. Scolnick,[1] Laboratories of [1]Tumor Virus Genetics, and [3]Molecular Virology, NCI, National Institutes of Health, Bethesda; [2]Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: Biosynthesis of the p21 *src* protein of Ha-MuSV.

Session 10: Viral Leukemogenesis

Chairperson: P. Neiman, Fred Hutchison Cancer Center, Seattle, Washington

B.G. Neel,[1] C.E. Rogler,[2] G.P. Gasic,[1] A.M. Skalka,[3] S.M. Astrin,[2] and W.S. Hayward,[1] [1]Rockefeller University, New York, New York; [2]Institute for Cancer Research, Philadelphia, Pennsylvania; [3]Roche Institute of Molecular Biology, Nutley, New Jersey: Molecular cloning of virus-cell junctions from ALV-induced lymphomas.

G.P. Gasic,[1] B.G. Neel,[1] C.E. Rogler,[2] S.M. Astrin,[2] and W.S. Hayward.[1] [1]Rockefeller University, New York, New York; [2]Institute for Cancer Research, Philadelphia, Pennsylvania: Nucleotide sequence analysis of molecularly cloned virus-cell junction fragments from ALV-induced lymphomas.

S.M. Astrin,[1] B.G. Neel,[2] C. Rogler,[1] J. Fang,[1] A.M. Skalka,[3] and W.S. Hayward,[1] [1]Institute for Cancer Research, Philadelphia, Pennsylvania; [2]Rockefeller University, New York, New York; [3]Roche Institute of Molecular Biology, Nutley, New Jersey: Viral oncogenesis by promoter insertion—ALV DNA is integrated next to the cellular *myc* gene in 85% of virally induced lymphomas.

G. Payne,[1] J. M. Bishop,[2] and H. E. Varmus,[2] Depts. of [1]Biochemistry and Biophysics, and [2]Microbiology, University of California, San Francisco: Oncogenesis by ALV—Evidence for a mechanism involving insertional mutagenesis.

H. Robinson,[1] B. Blais,[1] G. Gagnon,[1] P. Tsichlis,[2] and J. Coffin,[3] [1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]Tufts University School of Medicine, Boston, Massachusetts: Sequences in *U3* determine the oncogenic potential of ALV—Sequences immediately 5′ of *U3* target ALV toward lymphomogenesis.

M. R. Noori-Daloii,[1] H. J. Kung,[1] L. B. Crittenden,[2] and R. L. Witter,[2] [1]Dept. of Biochemistry, Michigan State University; [2]Regional Poultry Laboratory, United States Dept. of Agriculture, East Lansing, Michigan: The REV proviruses in avian bursal lymphomas—Amplification of the proviruses and specific integration near the proto-onc_{MCV} locus.

W. A. Haseltine,[1] F. S. Pedersen,[2] R. Crowther,[1] and D. Tenney,[1] [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Molecular Biology, University of Aarhus, Denmark: Genomic determinants of virulence in AKR retroviruses.

M. M. Kelly,[1] M. L. Lung,[1] C. A. Holland,[1] S. K. Chattopadhyay,[2] D. R. Lowy,[2] and N. Hopkins,[1] [1]Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; [2]NCI, National Institutes of Health, Bethesda, Maryland: Nucleotide sequences of the LTR and p15E coding sequences of MCF 247.

S. K. Chattopadhyay,[1,2] M. W. Cloyd,[4] D. L. Linemeyer,[3] M. R. Lander,[2] E. Rands,[1] and D. R. Lowy,[1] [1]Dermatology Branch; [2]Pediatric Oncology Branch; [3]Laboratory of Tumor Virus Genetics, NCI, National Institutes of Health, Bethesda, Maryland; [4]Rocky Mountain Laboratory, Hamilton, Montana: MCF viral genomes—Their cellular origin and role in AKR thymic lymphomas.

W. Herr and W. Gilbert, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Structure and arrangement of recombinant proviruses in leukemic thymuses of AKR mice.

M. S. McGrath and I. L. Weissman, Stanford University, California: Receptor-mediated leukemogenesis—The murine B cell lymphoma BCL_1 utilizes cell-surface immunoglobulin as an MuLV receptor.

P. Fischinger,[1] H. Thiel,[2] J. Ihle,[3] and N. Dunlop,[1] [1]National Cancer Institute; [3]Frederick Cancer Research Center, Frederick, Maryland; [2]Dept. of Surgery, Duke University, Chapel Hill, North Carolina: Presence of a recombinant MuLV-like glycoprotein in virus-free mouse lymphomas.

J. I. Mullins, J. W. Casey, M. O. Nicolson, A. Roach, J. B. Santon, K. B. Burck, and N. Davidson, Dept. of Chemistry, California Institute of Technology, Pasadena: Cat endogenous FeLV-related sequences contain unique 3′ regions in LTRs distinct from those in infectious FeLV.

Session 11: *Endogenous Viruses*

Chairperson: H. Robinson, Worcester Foundation, Shrewsbury, Massachusetts

L. B. Crittenden, E. J. Smith, and A. M. Fadly, Regional Poultry Laboratory, United States Dept. of Agriculture, East Lansing, Michigan: Influence of endogenous ALV gene expression on response to RAV-1 and REV infection.

R. Eisenman,[1] H. Weintraub,[1] K. Conklin,[2] J. Coffin,[2] H. Robinson,[3] and M. Groudine,[1] [1]Hutchinson Cancer Center, Seattle, Washington; [2]Tufts Medical School, Boston; [3]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Control of endogenous avian retrovirus gene expression by DNA methylation.

K. F. Conklin,[1] R. N. Eisenman,[2] H. L. Robinson,[3] and J. M. Coffin,[1] [1]Tufts University School of Medicine, Boston, Massachusetts; [2]Fred Hutchinson Cancer Research Center, Seattle, Washington; [3]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Spontaneous expression and phenotype of the common ev-1 endogenous provirus of chickens.

F. Hishinuma and A. M. Skalka, Roche Institute of Molecular Biology, Nutley, New Jersey: Sequence analysis of the 3′ end of the ev-1 provirus.

R. Mural,[1] J. Young,[1] R. Roblin,[1] J. Ihle,[1] D. Steffen,[2] and R. Weinberg,[3] [1]Frederick Cancer Research Center, Frederick, Maryland; [2]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [3]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The major MuLV-related sequences in mouse DNA represent a limited number of proviral classes.

A. S. Khan,[1] C. F. Garon,[1] S. P. Staal,[2] C. Buckler,[1] W. P. Rowe,[1] and M. A. Martin,[1] [1]NIAID, National Institutes of Health, Bethesda; [2]Dept. of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Characterization of endogenous MuLV-related sequences in BALB/c and AKR mice.

N. A. Jenkins, N. G. Copeland, B. A. Taylor, and B. K. Lee, Jackson Laboratory, Bar Harbor, Maine: Mapping the chromosomal location of ecotropic DNA sequences in C57BL/6J, C3H/HeJ, and DBA/2J inbred mouse strains.

J. McCubrey and R. Risser, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Genetic interactions in the induction of ecotropic MuLV.

P. Roy-Burman,[1,2] A. Mock,[1] B. G. Devi,[1] and M. B. Gardner,[1] Depts. of [1]Pathology, and [2]Biochemistry, University of Southern California School of Medicine, Los Angeles: Restricted transcription of endogenous ecotropic provirus genes by the Akvr-1^{R} allele in wild mice.

A. B. Vaidya, N. E. Taraschi, S. L. Tancin, and C. A. Long, Dept. of Microbiology, Hahnemann Medical College, Philadelphia, Pennsylvania: Control of endogenous MuMTV gene expression in lactating mammary glands of C57BL mice.

R. Jaenisch, K. Harbers, D. Jähner, P. Nobis, and H. Stuhlmann, Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany: Experimental insertion of retroviral genomes into the germ line of mice.

G. G. Lovinger and G. Schochetman, Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland: 5'-terminal nucleotide noncoding sequences of retroviruses—A model for evolutionary divergence.

G. E. Mark, E. H. Birkenmeier, and G. J. Todaro, National Cancer institute, Frederick, Maryland: Structure and function of the promoter sequences of an endogenous C-type retrovirus.

P. Kirschmeier,[1] S. Gattoni,[1] I. B. Weinstein,[1] E. Athan,[2] and D. Dina,[2] [1]Institute of Cancer Research, Columbia University, New York; [2]Dept. of Genetics, Albert Einstein College of Medicine, Bronx, New York: Transcription of sequences related to the LTRs of MSV in carcinogen-transformed mouse cell lines.

Session 12: Viruses and Tumors

Chairperson: S. Aaronson, National Cancer Institute, Bethesda, Maryland

E. C. Ward and D. P. Bolognesi,[2] Depts. of Microbiology and Immunology, and [2]Surgery, Duke University Medical Center, Durham, North Carolina: Approaches toward immunotherapy of "nonantigenic" tumors.

B. Chesebro and W. J. Britt, NIAID, Rocky Mountain Laboratory Hamilton, Montana: Monoclonal anti-gp70 antibodies cause in vivo reduction of Friend virus infectious centers in leukemic spleens.

S. Ruscetti,[1] A. Rein,[2] and R. Bassin,[1] [1]NCI, National Institutes of Health, Bethesda; [2]Frederick Cancer Research Center, Frederick, Maryland: F-MuLV-induced leukemia is mediated by MCF viruses and is blocked in strains endogenously expressing a novel MCF-related glycoprotein.

G. L. Waneck and N. Rosenberg, Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts: Ab-MuLV induction of lymphoid and erythroid colonies in fetal liver cell cultures.

F. Alt,[1] N. Rosenberg,[2] E. Thomas,[1] S. Lewis,[1] and D. Baltimore,[1] [1]Massachusetts Institute of Technology, Cambridge; Tufts University School of Medicine, Boston, Massachusetts: Abelson virus transformants usually have rearranged heavy-chain genes but not light-chain genes.

L.-C. Chen, S. A. Courtneidge, and J. M. Bishop, Dept. of Microbiology and Immunology, University of California, San Francisco: The immunological phenotype of tumors induced by ALV.

U. G. Rovigatti[1] and R. A. Weiss,[2] [1]Istituto Fisio logia Generale, Università Roma, Italy; [2]Chester Beatty Cancer Research Institute, London, England: The integrated provirus is involved in the reexpression of the transformed phenotype in revertant rat cells.

P. Sankar-Mistry, G. Lemay, and P. Jolicoeur, Dept. de Microbiologie et d'immunologie, Institut de Recherches Cliniques, University de Montreal, Quebec, Canada: The presence of 8.0- and 5.6-kb genomic RNA in MuLV isolated from primary X-ray-irradiation-induced thymomas.

M. Robert-Guroff, F. W. Ruscetti, V. S. Kalyanaraman, M. S. Reitz, and R. C. Gallo, NCI, National Institutes of Health, Bethesda, Maryland: In vitro transmission of the human retrovirus HTLV to cells of relatives of a patient with acute lymphoblastic leukemia.

V. S. Kalyanaraman,[1] M. Robert-Guroff,[1] M. G. Sarngadharan,[1] P. Bunn,[2] J. Minna,[2] and R. C. Gallo,[1] [1]Laboratory of Tumor Cell Biology, NCI, National Institutes of Health, Bethesda, Maryland; [2]NCI-VA Clinical Oncology, Washington, DC: Natural antibodies in human sera reactive against the human retrovirus HTLV.

M.-A. Lane, A. Sainten and G. M. Cooper, Dept. of Pathology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Activation of related transforming genes in mouse and human mammary carcinomas.

A. Eva,[1] K. Robbins,[1] P. Andersen,[1] A. Srinivasan,[1] T. Papas,[2] S. Tronick,[1] E. P. Reddy,[1] E. Westin,[3] F. Wong-Staal,[3] R. Gallo,[3] N. Ellmore,[1] and S. Aaronson,[1] Laboratories of [1]Cellular and Molecular Biology, [2]Tumor Virus Genetics, and [3]Tumor Cell Biology, N.C.I., National Institutes of Health, Bethesda, Maryland: Human tumor cells contain transcripts related to the transforming genes of retroviruses.

The Molecular Biology of Yeast, August 11–August 16

Arranged by **James B. Hicks, Amar Klar, Kim Nasmyth, Jeffrey N. Strathern**
Cold Spring Harbor Laboratory

416 participants

Session 1: *Mating Type*

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

D. Beach and P. Nurse, School of Biological Sciences, University of Sussex, Brighton, England: Isolation of mating-type and cell genes from *S. pombe*.

A. Klar, J. Strathern, J. Abraham, K. Nasmyth, J. Ivy, and J. Hicks, Cold Spring Harbor Laboratory, New York: Controls of directedness of switching, direction of transposition and characterization of a site essential for mating-type switching.

J. Haber, D. Rogers, and S. Stewart, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Control of expression and transposition of tandem duplications of yeast mating-type genes.

V.L. MacKay[1,2] and K. Nasmyth,[2] [1]Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey; [2]Dept. of Genetics, University of Washington, Seattle: Complementation of *ste4* mutations by the cloned *STE5* gene.

A.J. Brake,[1] H.H. Liao,[1] J. Thorner,[1,2] and K. Nasmyth,[2] [1]Dept. of Microbiology and Immunology, University of California, Berkeley; [2]Cold Spring Harbor Laboratory, New York: Analysis of the role of the *STE5*-gene product in the yeast mating response using the cloned *STE5* gene.

G. Sprague, Jr. and I. Herskowitz, Dept. of Biology, University of Oregon, Eugene: The *MATα1*-gene product is a positive regulator of synthesis of RNA from the α-specific *STE3* gene.

K. Nasmyth, Cold Spring Harbor Laboratory, New York: The regulation of mating type chromatin structure by *MAR*.

S. Harashima, K. Tanaka, A. Takagi, and Y. Oshima, Dept. of Fermentation Technology, Osaka University, Japan: Mating-potency-dependent homozygosity of *sir3* mutation by allelic transposition in *S. cerevisiae*.

T.R. Manney, Dept. of Physics, Kansas State University, Lawrence: A nonsterile allele of *MATα2* that makes reduced levels of α-factor.

R. Jensen and I. Herskowitz, Institute of Molecular Biology, University of Oregon, Eugene: Control of expression of *HO* by the mating-type locus.

Session 2: *Regulation I*

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

J. Gafner, A. Stotz, H. Eibel, T. Giller, L. Panzeri, and P. Philippsen, Dept. of Microbiology, Biocenter, University of Basel, Switzerland: Analysis of TY and δ sequences.

R.T. Elder, Dept. of Biology, University of Chicago, Illinois: Both the 5′ and 3′ ends of Ty1 RNA are in the δ sequences.

D.W. Russell,[1] M. Smith,[1] V.M. Williamson,[2] D. Cox,[2] and E.T. Young,[2] [1]Dept. of Biochemistry, University of British Columbia, Vancouver, Canada; [2]Dept. of Biochemistry, University of Washington, Seattle: DNA sequence analysis of transposable-element-induced mutations of the ADHII gene.

A. Taguchi and E. Young, Dept. of Biochemistry, University of Washington, Seattle: a/α suppression of yeast alcohol dehydrogenase constitutive mutations.

B. Errede, T. Cardillo, and F. Sherman, Dept. of Radiation Biology and Biophysics, University of Rochester Medical School, New York: Mapping functional regions of the *CYC7-H2* Ty1 insertion element.

F.G. Chumley and G.R. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Transposition of *his4-912* can restore gene expression.

L. Guarente, Dept. of Biochemistry and Molecular Biology, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Structure of the promoter of the iso-1-cytochrome c gene of *S. cerevisiae*.

J.M. Boss and R.S. Zitomer, Dept. of Biological Sciences, State University of New York, Albany: The sequences of the wild-type iso-2-cytochrome *c* mRNA and two overproducing mutants.

K.S. Zaret,[1] J. Kotval,[2] and F. Sherman,[1] [1]Dept. of Radiation Biology and Biophysics, University of Rochester; [2]Albany Medical College of Union University, New York: DNA sequence required for efficient transcription termination and polyadenylation of *CYC1* mRNA.

K. Struhl, MRC Laboratory of Molecular Biology, Cambridge, England: *his3* promoter and regulatory elements.

A.W. Murray[1,3] and J.W. Szostak[2,3] [1]Committee on Cell and Developmental Biology; [2]Dept. of Biological Chemistry; [3]Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Chromosomal location affects the activity of the *HIS3* promoter.

Session 3: Poster Session

J. Margolskee and I. Herskowitz, Institute of Molecular Biology, University of Oregon, Eugene: The effect of a translocation involving chromosome III on the efficiency of mating-type interconversion.

J. Feldman,[1] J.R. Broach,[1] and J.B. Hicks,[2] [1]Dept. of Microbiology, State University of New York, Stony Brook; [2]Cold Spring Harbor Laboratory, New York: Mutations of a cloned silent mating-type cassete, *HMLα*, that allow its constitutive expression.

B. Weiffenbach and J.E. Haber, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Homothallic conversion of mating-type alleles produces a DNA break in the *MATα1* cistron in *rad52* cells.

J. McCusker, B. Weiffenbach, P. Thorburn, and J.E. Haber, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Stability of broken chromosomes and circular chromosomes of yeast.

A. Comeau, L. Davidow, and J. Haber, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Factors affecting the direction, efficiency, and pedigree of mating-type switching.

M.N. Jagadish, D.R. Fugit, and V.L. MacKay, Waksman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey: Inhibition of *MATα2* in a *tup1* mutants.

D.E. Cabin and B.-K. Tye, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Cloning of a gene that complements a non–mating-type-specific sterility mutation in yeast.

J. Strathern, A. Klar, J. Abraham, J. Ivy, K. Nasmyth, and J. Hicks, Cold Spring Harbor Laboratory, New York: Studies on mating type gene expression and transposition.

S. Murakami and D. Livingston, Dept. of Biochemistry, University of Minnesota, Minneapolis: Yeast spheroplasts exhibit cell-division-cycle-specific inhibition.

J. Wood, Dept. of Genetics, University of Washington, Seattle: Genetic and cell cycle characterization of the mitotic block caused by methyl-benzimidazole carbamate.

T.A. Peterson and S.I. Reed, University of California, Santa Barbara: The isolation of the cell-cycle start gene *CDC36* and its relationship to *STE7*.

M.E. Katz, P. Kayne, and S.I. Reed, Dept. of Biological Sciences, University of California, Santa Barbara: The isolation of suppressors of *STE* mutations conferring cell-division-cycle-defective phenotypes.

S. Okamoto, Laboratory of Genetics, Dept. of Biology, Faculty of Science, University of Tokyo, Japan: A positive regulator for the pre–second meiotic morphogeneses of outer plaques in *S. cerevisiae*.

H. Boucherie and C.S. McLaughlin, Dept. of Biological Chemistry, University of California, Irvine: Protein synthesis during transition and stationary phases under glucose limitation in *S. cerevisiae*.

L. Alberghina, L. Popolo, and M. Vanoni, Laboratory of Comparative Biochemistry, University of Milan, Italy: Dissociation of ribosomal and protein synthesis on cell cycle controls in *S. cerevisiae*.

M.A. Resnick,[1] S. Stasiewicz,[1] J.C. Game,[2] and R. Roth,[3] [1]National Institute of Environmental and Health Sciences, Research Triangle Park, North Carolina; [2]Dept. of Genetics, University of California, Berkeley; [3]Illinois Institute of Technology, Chicago: Role of *rad52* gene function in early molecular events of meiosis.

M.J. Clancy, Microbiology and Public Health, Michigan State University, East Lansing: Purification and properties of a sporulation-specific enzyme.

B. Marian, A. Linsbauer, and U. Wintersberger, Institute for Cancer Research, University of Vienna, Austria: Sodium butyrate accelerates sporulation of *S. cerevisiae*.

P. Nurse and D. Beach, Dept. of Biological Sciences, University of Sussex, Brighton, England: Gene

transposition implicated in the transition between proliferation and conjugation/sporulation in *S. pombe*.

J.C. Game,[1] D. Schild,[2] and R.E. Esposito,[3] [1]Dept. of Genetics, [2]Dept. of Biophysics and Medical Physics, University of California, Berkeley; [3]Dept. of Biology, University of Chicago, Illinois: The meiotic phenotype of the *rad57-1* mutation.

P.R. Russell and B.D. Hall, Dept. of Genetics, University of Washington, Seattle: Structure of the *S. pombe* cytochrome-*c* gene.

S.C. Cheng, P. Mirabito, and D. Ogrydziak, Institute of Marine Resources, University of California, Davis: Are there multiple secretion pathways in *Saccharomycopis lipolytica?*

R. Schmidt,[1] M. Manolson,[2] and R.J. Poole,[2] [1]Institut für Biophysik, Freie Universität Berlin, Federal Republic of Germany; [2]Dept. of Biology, McGill University, Montreal, Canada: A specific photoaffinity label for the purine transport system in *S. cerevisiae*.

K. O'Malley, J. Saltzgaber-Müller, and M. Douglas, Dept. of Biochemistry, Health Science Center, University of Texas, San Antonio: Selection of nuclear genes coding for mitochondrial components by genetic complementation.

M.R. Lamb,[1] H.R. Mahler,[2] and P.S. Perlman,[3] [1]Dept. of Biology; [2]Dept. of Chemistry, Indiana University, Bloomington; [3]Dept. of Genetics, Ohio State University, Columbus: Transition of the mosaic gene *OXI3* in intron mutants of the *COB* gene of *S. cerevisiae*.

A.M. Colson, J. Oberto, and C. Colson, Université de Louvain, Laboratoire de Cytogénétique, Belgium: A nuclear mutation conferring diuron-resistance to the NADH-cytochrome *c* reductase in the mitochondrial respiratory chain of *S. cerevisiae*.

E.M. Dimock, D.J. Perry, and T.L. Mason, Dept. of Biochemistry, University of Massachusetts, Amherst: The biosynthesis and assembly of mitoribosomal proteins in petite mutants with deleted or amplified rRNA genes.

M. Suissa, Biocenter, University of Basel, Switzerland: mRNAs for imported mitochondrial proteins are associated with both free and mitochondria-bound cytoplasmic polysomes.

M.A. Nicholas and P.S. Perlman, Dept. of Genetics, Ohio State University, Columbus: Protein synthesis-defective mutants in the *ery1-oxi1* region of mitochondrial DNA—protein synthesis analysis and effects on RNA processing.

C. Low and B.G. Adams, Dept. of Microbiology, University of Hawaii, Honolulu: The Bertholet reaction—An assay for ammonia nitrogen produced by urea amidolyase activity in *S. cerevisiae*.

C.L. Denis and E.T. Young, Dept. of Biochemistry, University of Washington, Seattle: Isolation and characterization of a positive regulatory gene, *ADR1*.

C.L. Denis,[1] J. Ferguson,[2] and E.T. Young,[1] [1]Dept. of Biochemistry, University of Washington, Seattle; [2]Dept. of Biological Sciences, University of California, Santa Barbara: A decrease in the mRNA levels for the fermentative alcohol dehydrogenase upon growth on a nonfermentable carbon source.

L. Dröll, K.-D. Entian, and D. Mecke, Physiologisch-chemisches Institut der Universität, Tübingen, Federal Republic of Germany: Reversible and irreversible inactivation of gluconeogenetic enzymes in glycolytic mutants of *S. cerevisiae*.

A.P.G.M. van Loon,[1] J. Kreike,[1] B. Lang,[2] B.V.D. Horst,[1] R. Vaessen,[1] F. Driehuis,[1] A. Dekker,[1] and L.A. Grivell,[1] [1]Section for Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, The Netherlands; [2]Genetisches Institut, München, Federal Republic of Germany: The ubiquinol–cytochrome—c reductase complex—Regulation of the synthesis of the active enzyme complex and attempts to clone nuclear genes coding for subunits.

J. Polaina, Solar Energy Research Institute, Golden, Colorado: *STA10*—A gene present in *S. cerevisiae* that inhibits the expression of the amylolytic capability.

J.A. Bossinger, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation NAD-dependent glutamate dehydrogenase in *S. cerevisiae*.

J.M. Sedivy and D.G. Fraenkel, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: *S. cerevisiae* mutants defective in fructose bisphosphatase activity.

D.B. Finkelstein and S. Strausberg, Dept. of Biochemistry, University of Texas Health Science Center, Dallas: Alternate inducers of the yeast heat-shock response.

M.N. Beremand and C.S. McLaughlin, Dept. of Biological Chemistry, University of California, Irvine: Heat shock in *C. albicans*.

J.M. Boss,[1] S. Gillam,[1] R.S. Zitomer,[1] and M. Smith,[2] [1]Dept. of Biological Sciences, State University of New York, Albany; [2]Dept. of Biochemistry, University of British Columbia, Vancouver, Canada: Sequence of the iso-1-cytochrome *c* mRNA.

D. Beier and E.T. Young, Dept. of Biochemistry, University of Washington, Seattle: Deletions of *ADCI* that alter the level of ADHI enzyme activity.

G.B. Callega,[1] S. Levy-Rick,[1] A. Nasim,[2] and F. Moranelli,[2] [1]Bio Logicals, Toronto, Canada; [2]National Research Council of Canada, Ottawa: Development of extracellular amylolytic activity in *Schwanniocyces alluvius*—Temperature sensitivity and catabolite repression.

M. Igo and H. Greer, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: The yeast *his4* region and transposable elements.

J. Jackson and G. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Meiotic recombination between duplicated genetic elements.

D. Morisato and H. Greer, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: General control of yeast *his4* transcription.

H. Stark and D. B. Mowshowitz, Dept. of Biological Sciences, Columbia University, New York: MAL6 constitutive alleles are dominant as predicted for a positive regulatory system.

M. I. Riley and R. C. Dickson, Dept. of Biochemistry, University of Kentucky, Lexington: Mutants defective in the regulation of β-galactosidase induction in *Kluyveromyces lactis*.

A. P. Mitchell and B. Magasanik, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: A reversible inactivation of glutamine synthetase in *S. cerevisiae*.

R. Roggenkamp and C. P. Hollenberg, Institut für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Processing of bacterial β-lactamase expressed in *S. cerevisiae*.

M. Schweizer, J. Höltke, C. Lebert, and L. M. Roberts, Institut für Biochemie der Universität Erlangen-Nürnberg, Federal Republic of Germany: Identification of clones for the fatty acid synthetase complex by transformation of yeast.

M. Greenberg, L. Klig, V. Letts, B. Shicker, and S. Henry, Depts. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Inositol-overproducing mutant (*opi3*) defective in the synthesis of phosphatidylcholine.

V. A. Letts and I. W. Dawes, Microbiology Dept., Edinburgh University, Scotland; Characterization of a temperature-sensitive mutant of yeast altered in lipid biosynthesis.

M. Rose and D. Botstein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Structure and regulation of the yeast *URA3* gene studied with fusions to the *E. coli lacZ* gene.

R. Hitzeman,[1] F. Hagie,[1] A. Singh,[1] C. Chen,[1] D. Goeddel,[1] G. Ammerer,[2] and B. Hall,[2] [1]Dept. of Molecular Biology, Genentech, Inc., South San Francisco, California; [2]Dept. of Genetics, University of Washington, Seattle: Expression of a human gene in yeast.

S. Silverman and G. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Insertion mutations and regulation at the *his4* locus of *S. cerevisiae*.

Session 4: *Cell Cycle and Sporulation*

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

J. Yarger,[1] K. Bostian,[1] J. Lemire,[1] H. Halvorson,[1] and J. Hopper,[2] [1]Rosenstiel Center of Brandeis University, Waltham, Massachusetts; [2]Milton S. Hershey Medical Center, Pennsylvania: Regulation of the acid phosphatase (*PHO5*) and galactokinase (*GAL1*) genes during the cell cycle in *S. cerevisiae*.

A. Lorincz,[1] M. Miller,[1] N.-H. Xuong,[1,2,3] and E. P. Geiduschek,[1] [1]Depts. of Biology, [2]Chemistry, and [3]Physics, University of California, San Diego, La Jolla: Quantitative analysis of two-dimensional gels reveals several cell-cycle-regulated proteins in *S. cerevisiae*.

J. R. Ludwig II,[1] J. J. Foy,[2] and C. S. McLaughlin,[2] [1]Dept. of Biochemistry, Institute of Science and Technology, University of Manchester, England; [2]Dept. of Biological Chemistry, University of California, Irvine: Synthesis of periodic and nonperiodic proteins through the cell cycle of *S. cerevisiae*.

G. C. Johnston and R. A. Singer, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada: Overlapping cell cycles and the dispensable G^1 period.

S. I. Reed, J. C. Groppe, and J. Ferguson, Dept. of Biological Sciences, University of California, Santa Barbara: Preliminary characterization of the transcriptional and translational products of the cell-cycle start gene—*cdc28*.

J. R. Shuster, Dept. of Genetics, University of Washington, Seattle: Suppression of mutants defective in conjugation by cell-division-cycle mutants in *S. cerevisiae*.

S. Klapholz, J. Wagstaff, and R. E. Esposito, Dept. of Biology and Committee on Genetics, University of Chicago, Illinois: Single division meiosis in *SPO13-1* haploids.

L. Smith, M. J. Clancy, and P. T. Magee, Microbiology and Public Health, Michigan State University, East Lansing: Regulation of a sporulation-specific gene product.

D. H. Williamson,[1] L. H. Johnston,[1] D. J. Fennel,[1] A. L. Johnson,[1] and G. Simchen,[2] [1]National Institute for Medical Research, London, England; [2]Genetics Dept., Hebrew University of Jerusalem, Israel: Genomic organization and the control of origin activation in premeiotic DNA synthesis in *S. cerevisiae*.

E. Chlebowicz-Sledziewska and W. L. Fangman, Dept. of Genetics, University of Washington, Seattle: Phase lengths in the unequal cell cycles of yeast mother and daughter cells.

Session 5: Chromatin and Chromosome Structure

Chairperson: T. Petes, University of Chicago, Illinois

M. Fitzgerald-Hayes, L. Clarke, E. Yeh, and J. Carbon, Dept. of Biological Sciences, University of California, Santa Barbara: Functional and sequence analysis of the centromere regions from yeast chromosomes III and XI.

K. Bloom and J. Carbon, Dept. of Biological Sciences, University of California, Santa Barbara: Chromatin structure and DNA-protein interactions of yeast centromere DNA.

C. Mann, D.T. Stinchcomb, and R.W. Davis, Dept. of Biochemistry, Stanford University School of Medicine, California: Structure and function analysis of yeast centromeres.

J.W. Szostak, Dept. of Biological Chemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston Massachusetts: The ends of the rDNA linear plasmid of *Tetrahymena* can function as telomeres on linear plasmids in yeast.

M.C. Rykowski, J.W. Wallis, J. Choe, and M. Grunstein, Molecular Biology Institute, University of California, Los Angeles: A genetic analysis of yeast histone H2B subtype function.

J.B. Rattner,[1] B. Hamkalo,[1] C.A. Saunders,[2] J.R. Davie,[2] R. Ludwig,[3] and C.S. McLaughlin,[3] [1]Dept. of Molecular Biology and Biochemistry, University of California, Irvine; [2]Dept. of Biochemistry and Biophysics, Oregon State University, Eugene; [3]Dept. of Biochemistry, Irvine School of Medicine, University of California: The ultrastructural organization of yeast chromatin.

J.F. Scott and C.M. Brajkovich, Molecular Biology Institute, University of California, Los Angeles: Chromatin organization of Trp1-RI-Circle—A high-copy-number yeast chromosomal DNA plasmid.

C.S.M. Chan and B.-K. Tye, Dept. of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Complexity and organization of autonomously replicating sequences in *S. cerevisiae.*

J.M. Walsh, C.A. Saunders, J.R. Davie, and J.H. Proffitt, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Chromatin structure of the iso-2-cytochrome *c* and *gal10* gene regions in yeast.

A. Sledziewski and E. Young, Dept. of Biochemistry, University of Washington, Seattle: DNase-I sensitivity of ADH genes in the yeast *S. cerevisiae.*

R. Rothstein and C. Helms, Dept. of Microbiology, CMDNJ, New Jersey Medical School, Newark; Rearrangements at the *SUP4* locus.

Session 6: Poster Session

A. Stotz and P. Philippsen, Dept. of Microbiology, Biocenter, University of Basel, Switzerland: Arrangement of mobile and stable sequences in yeast strains.

H.L. Klein and T.D. Petes, Dept. of Microbiology, University of Chicago, Illinois: Genetic mapping of Ty sequences.

G. Maine and B.-K. Tye, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Cloning of a centromeric DNA sequence from yeast.

R.J. Devenish, L. Lipchitz, and C.S. Newlon, Dept. of Zoology, University of Iowa, Iowa City: Replication of a circular derivative of chromosome III.

M.V. Olson, T. Frank, and M.Y. Graham, Dept. of Genetics, Washington University School of Medicine, St. Louis, Missouri: Restriction mapping of the yeast nuclear genome—A feasibility study.

T. Nilsson-Tillgren,[1] J.G.L. Petersen,[2] S. Holmberg,[2] C. Gjermansen,[2] and M.C. Kielland-Brandt,[2] [1]Institute of Genetics, University of Copenhagen; [2]Dept. of Physiology, Carlsberg Laboratory, Copenhagen Valby, Denmark: Differences between functionally homologous chromosomes from *S. carlsbergensis.*

J.R. Davie, C.A. Saunders, J.M. Walsh, and S.C. Weber, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Histone modifications in the yeast *S. cerevisiae.*

D. Kolodrubetz, J. Choe, M. Rykowski, and M. Grunstein, Dept. of Biology, Molecular Biology Institute, University of California, Los Angeles: Sequence and function of the histone H2A genes in *S. cerevisiae.*

G.A. Bitter, NIAMDD, National Institutes of Health, Bethesda, Maryland: Heterologous in vitro transcription systems utilizing components from mammalian and yeast cells.

J.W. Wallis, M. Rykowski, and M. Grunstein, Dept. of Biology, Molecular Biology Institute, University of California, Los Angeles: An in vivo test for histone H2B function.

G.J. Ide, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis: Nucleoside 5′-[γ-S] triphosphates will initiate transcription in isolated yeast nuclei.

S.E. Celniker and J.L. Campbell, Dept. of Chemistry, California Institute of Technology, Pasadena: In vitro DNA replication in *S. cerevisiae.*

H. Singh, J.J. Bieker, J.P. Lussky, and L.B. Dumas, Dept. of Biochemistry and Molecular Biology, Northwestern University, Evanston, Illinois: Single-stranded recombinant DNA molecules as probes of replication and recombination in yeast.

K.H. Scheit[1] and P.M. Bhargava,[2] [1]Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Federal Republic of Germany; [2]Centre for Cellular and Molecular Biology, Hyderabad, India: An antimicrobial protein from bull semen inhibits transcription in yeast cells.

V.A. Zakian and D.M. Kupfer, Fred Hutchinson Cancer Research Center, Seattle, Washington: Replication, copy-number, and segregation properties of a plasmid containing the origin of replication from *X. laevis* mitochondrial DNA.

R. Hackett,[1] L. Dicaprio,[2] K. Gross,[2] and B.-K. Tye,[1] [1]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York; [2]Molecular Biology Dept., Roswell Park Memorial Institute, Buffalo, New York: Replication of heterologous DNA in *S. cerevisiae*.

E.M. McIntosh, B.J. Barclay, and R.H. Haynes, Dept. of Biology, York University, Toronto, Canada: DNA synthesis in yeast is strongly inhibited by ara-CMP but not by ara-C.

O. Landman and J.G. Little, Dept. of Biology, York University, Toronto, Canada: Unusual DNA precursor metabolism in yeast—3′ dTMP and 3′,5′ cyclic dTMP as sources of DNA-thymine.

C.M. Gaillardin,[1] P. Fournier,[1] L. de Louvencourt,[1] C. Gerbaud,[2] F. Lang,[3] and H. Heslot,[1] [1]Laboratoire Génétique, Institut National Agronomique, Paris; [2]Laboratoire Biologie Génétique et Moleculaire, Faculté des Sciences, Orsay, France; [3]Genetisches Institut der Universität München, Federal Republic of Germany: Behavior of various replicators in the yeast *S. pombe*.

C. Holm, Dept. of Genetics, University of Washington, Seattle: Lethal sectoring caused by 2μ DNA in *nib*$^-$ strains.

J.F. Lemontt and S.V. Lair, Biology Division, Oak Ridge National Laboratory, Tennessee: Hydrazine-induced mutagenesis at *CAN1* in *S. cerevisiae* requires both DNA replication and the *RAD6* function.

A.K. Beck, J.F. Lemontt, F.W. Larimer, and E.G. Bernstine, Biology Division, Oak Ridge National Laboratory, Tennessee: Gamma-ray-induced nonrevertible *ade2* mutation in *S. cerevisiae* carries a large insertion as revealed with cloned *ADE2* probe.

M.L. Carbone, M.E. Bianchi, and G. Lucchini, Istituto di Genetica, Università di Milano, Italy: Yeast mutants resistant to manganese.

B. Rockmill, J. Game, and S. Fogel, Dept. of Genetics, University of California, Berkeley: Two systems for the detection of meiotic aneuploidy.

N. Sugawara and J. Szostak, Dept. of Biological Chemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Recombination events between homologous sequences on nonhomologus chromosomes.

T.D. Petes and H.L. Klein, Dept. of Microbiology, University of Chicago, Illinois: Meiotic recombination within a tandem array of *LEU2* genes.

T.S. Cardillo, B. Errede, and F. Sherman, Dept. of Radiation Biology and Biophysics, University of Rochester Medical School, New York: Integrative transformation of yeast results in multiple insertions of DNA segments.

C. Moore, Dept. of Radiation Biology and Biophysics, University of Rochester, New York: DNA ligase is required for medium-independent DNA rejoining in yeast.

S.M. Smolik-Utlaut and T.D. Petes, Dept. of Microbiology, University of Chicago, Illinois: Small amounts of heterogeneity affect recombination of the reiterated rRNA genes in yeast.

D.B. Kaback, Dept. of Microbiology, CMDNJ, New Jersey Medical School, Newark: Saturation mapping of chromosome I.

S.C. Falco, M. Rose, and D. Botstein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Characterization and applications of recombination between plasmids and chromosomes in yeast.

M.L. Dixon and R.K. Mortimer, Dept. of Biophysics and Medical Physics, University of California, Berkeley: Chromosome loss, recombination, and mutation detection in a yeast assay system.

I.L. Calderón, D. Schild, C.R. Contopoulou, and R.K. Mortimer, Dept. of Biophysics and Medical Physics, University of California, Berkeley: Cloning of yeast genes involved in repair and recombination.

D. Schild, R.K. Mortimer, and R. Contopoulou, University of California, Berkeley: Chromosome loss in radiation-sensitive diploids of yeast.

S.W. Ruby and J.W. Szostak, Dept. of Biological Chemistry, Harvard Medical School, Sidney Farber Cancer Institute, Boston, Massachusetts: Specific genes in yeast are induced by DNA damage.

J.I. Stiles, Dept. of Life Sciences, Indiana State University, Terre Haute: Physical structure of an element involved in the production of deletions, transpositions, and other chromosomal rearrangements of the COR region of *S. cerevisiae*.

P. Munz, H. Amstutz, J. Kohli, and U. Leupold, Institute of General Microbiology, University of Bern, Switzerland: Heterologous recombination between $tRNA^{Ser}$ genes in *S. pombe*.

R. Hofbauer, B. Hamilton, F. Fessl, and H. Ruis, Institut für Allgemeine Biochemie der Universitat Wien and Ludwig Boltzmann-Forschungsstelle für Biochemie, Vienna, Austria: Studies on translational control of yeast protein synthesis in a cell-free system from *S. cerevisiae*.

L. Bisson, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Utilization of exogenous dTMP by dTMP-permeable yeast strains.

T. Huffaker and P. W. Robbins, Dept. of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Temperature-sensitive mutants deficient in protein glycosylation.

G. Thill and R. A. Kramer, NCI, National Institutes of Health, Bethesda, Maryland: Mapping of the 5' termini of yeast repressible acid phosphatase mRNA and sequence analysis of the 5'-flanking region.

L. L. McAlister and M. J. Holland, Dept. of Biological Chemistry, University of California, Davis: Expression of two yeast enolase genes.

G. McKnight and F. Sherman, Dept. of Radiation Biology and Biophysics, University of Rochester Medical School, New York: Characterization of the *CYC7-H3* mutation that causes overproduction of iso-2-cytochrome *c*.

D. L. Montgomery,[1] S. J. McAndrew,[2] L. Marr,[1] P. Jackson,[1] A. Walthall,[2] and R. S. Zitomer,[2] [1]Dept. of Biochemistry, University of Texas, Health Science Center, San Antonio; [2]Dept. of Biological Sciences, State University of New York, Albany: Molecular characterization of three mutations which alter transcription of the ISO-2-cytochrome *c* gene.

V. M. Williamson,[1] D. W. Russell,[2] M. Smith,[2] and E. T. Young,[1] [1]Dept. of Biochemistry, University of Washington, Seattle; [2]Dept. of Biochemistry, University of British Columbia, Vancouver: Analysis of *ADR2* transcripts in wild-type yeast strains and constitutive mutants.

M. Goldenthal, Dept. of Biochemistry, Albert Einstein College of Medicine, Bronx, New York: Isolation of mutants affecting maltose uptake in *S. cerevisiae*.

L. L. Spielman and D. B. Mowshowitz, Dept. of Biological Sciences, Columbia University, New York: Use of a new staining procedure to examine regulation of α glucosidases.

D. B. Wilson, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Regulation of yeast galactokinase.

R. G. Buckholz and B. G. Adams, Dept. of Microbiology, University of Hawaii, Honolulu: α-Galactosidase synthesis in *S. cerevisiae*.

A. Laughon and R. F. Gesteland, Howard Hughes Medical Institute, University of Utah, Salt Lake City: Cloning of the yeast *GAL4* gene.

S. A. Johnston and J. E. Hopper, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey: A *gal4 S. cerevisiae* transformed with a *GAL4*-bearing plasmid produces galactokinase constitutively.

C. P. Hollenberg and E. Erhart, Institut für Mikrobiologie, Universität Düsseldorf, Federal Republic of Germany: Curing of 2μ DNA by transformation.

Session 7: *Transcription/Replication*

Chairperson: R. Davis, Stanford University, California

A. Toh-e, S. Tada, and Y. Oshima, Dept. of Fermentation Technology, Osaka University, Japan: 2-DNA-like plasmid in the osmotophilic haploid yeast *S. rouxii*.

E. Di Mauro,[1] P. Ballario,[2] M. Buongiorno-Nardelli,[3] F. Carnevali,[2] and F. Pedone,[3] [1]Cattedra di Biologia Molecolare; [2]C. S. per gli Acidi Nucleici, CNR, Roma; [3]Instituto di Istologia e Embriologia, Facoltá di Scienze, Universitá di Roma, Italy: Selective in vitro transcription of cloned 2 DNA by purified yeast RNA polymerase II.

D. Lohr,[1] and G. Ide,[2] [1]Chemistry Dept., Arizona State University, Tempe; [2]Biochemistry and Biophysics, Oregon State University, Corvallis: Anaysis of in vitro initiation of transcription of ribosomal genes.

R. Koski, M. Worthington, D. Allison, and B. Hall, Dept of Genetics, University of Washington, Seattle: Transcription of mutant *SUP4* $tRNA^{Tyr}$ genes with yeast cell-free extracts.

M. Klekamp and T. Weil, Dept. of Biochemistry, University of Iowa Medical School; Iowa City: Selective and accurate transcription of eukaryotic class-III genes in yeast soluble nuclear extracts.

H. Van Keulen and D.Y. Thomas, Molecular Genetics Section, National Research Council, Ottawa, Canada: Selective transcripts of 5S DNA in a yeast in vitro transcription system.

J.R. Broach, V.R. Guarascio, Y.Y. Li, J. Makuni, and M. McLeod, Dept. of Microbiology, State University of New York, Stony Brook: Replication of and recombination within the yeast plasmid, 2μ circle.

A. Sugino,[1] H. Kojo,[1] B.D. Greenberg,[1] P.O. Brown,[2], and K.C. Kim,[1] [1]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, [2]Dept. of Biochemistry, University of Chicago, Illinois: In vitro replication of yeast 2μ plasmid DNA.

C.-I. Kuo and J.L. Campbell, Dept. of Chemistry, California Institute of Technology, Pasadena: Purification of the *cdc8* protein of *S. cerevisiae* by complementation in an aphidicolin-sensitive in vitro DNA replication system.

R.H. Hice and W.L. Fangman, Dept. of Genetics, University of Washington, Seattle: Cell-cycle regulation of the replication of an autonomous chromosomal plasmid.

V.A. Zakian, Fred Hutchinson Cancer Research Center, Seattle, Washington: Replication of Trp1-RI circle—A multiple-copy synthetic plasmid derived from yeast chromosomal DNA

M.N. Conrad and C.S. Newlon, Dept of Zoology, University of Iowa, Iowa City: DNA synthesis in *cdc2*.

Session 8: *Regulation II*

Chairperson: Y. Oshima, Osaka University, Japan

T.F. Donahue, P.J. Farabaugh, and G.R. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Regulation of *his4* expression in yeast.

H. Greer, J. Schwartz, and M. Greenberg, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Regulatory mutations linked to the yeast *his4* region.

G.S. Zubenko, F.J. Park, and E.W. Jones, Dept. of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: *PEP4* gene function is required for maturation of vacuolar hydrolases.

T.G. Cooper and R. Sumrada, University of Pittsburgh, Pennsylvania: Transcriptional control of the *CAR1* gene from *S. cerevisiae*.

F. Messenguy and E. Dubois, Institit de Recherches du CERIA, Brussels, Belgium: A posttranscriptional regulatory mechanism is involved in specific repression and induction of arginine metabolism in *S. cerevisiae*.

D.G. Fraenkel and D. Clifton, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Yeast phosphofructokinase mutants.

T. Alber,[1] G. Kawasaki,[2] and G.A. Petsko,[2] [1]Biology Dept., [3]Chemistry Dept., Massachusetts Institute of Technology, Cambridge; [2]Genetics Dept., Harvard Medical School, Boston, Massachusetts: Structural analysis of expression and function of triose phosphage isomerase.

B.G. Adams and B.D.L. Li, Dept. of Microbiology, University of Hawaii, Honolulu: Detection and characterization of a novel UDP-galactose pyrophosphorylase activity in yeast.

M. Johnston, T. St. John, and R. Davis, Dept. of Biochemistry, Stanford University School of Medicine, California: Structural and genetic analysis of the *GAL*-gene cluster.

H.J. Federoff, T.R. Eccleshall, J. Giacalone, R. Silver, and J. Marmur, Albert Einstein College of Medicine, Bronx, New York: Demonstration of two functional genes at the *MAL6* locus.

C.A. Michels, K.M. Hanenberger, and Y. Sylvestre, Queens College, City University of New York, Flushing: Pleiotropic mutations regulating resistance to glucose repression are allelic to *hxk2*.

Session 9: *Poster Session*

J.F. Ernst, J.W. Stewart, and F. Sherman, Dept. of Radiation Biology and Biophysics, University of Rochester School of Medicine, New York: The formation of composite iso-cytochromes *c* by recombination between nonallelic genes in yeast.

F. Messenguy, E. Dubois, Y. Cornélis, and N. Herickx, Institut du Recherches du CERIA, Brussels, Belgium: In vivo stability of *arg3* and *car1* mRNAs in *C. cerevisiae*.

D. Perlman and H.O. Halvorson, Brandeis University Waltham, Massasuchetts: Distinct mRNAs for different cytoplasmic and presectretory invertase polypeptides are encoded by a single *suc* gene.

S. Vissers, J.-C. Jauniaux, and J.-M. Wiame, Laboratoire de Microbiologie, Université Libre de Bruxelles, Belgium: Arginase gene homology in relation to the regulatory properties of arginase.

E. Dubois and F. Messenguy, Institut de Recherches du CERIA, Brussels, Belgium: Transcriptional control of nitrogen catabolite repression in *S. cerevisiae*.

L.M. Melnick and R.K. Chan, Dept. of Microbiology, University of Cincinnati College of Medicine, Ohio: Analysis of pheromone supersensitive mutations in yeast—Suppressors of the *sst2* mutation.

K.D. Atkinson, B.A. Leish, and J.M. Wang, Dept. of Biology, University of California, Riverside: Membrane assembly in yeast secretion-defective mutants.

W.E. Courchesne and B. Magasanik, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Nitrogen regulation of permeases in *S. cerevisiae*.

M. Nishizawa, M.N. Jagadish, and J.S. Tkacz, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey: A temperature-sensitive mutant of *S. cerevisiae* with depressed production of extracellular enzymes.

A. Yasui, U. Zimny, and W. Laskowski, Institute of Biophysics, Freie Universität Berlin, Federal Republic of Germany: Photodynamic action of thiopyronine in yeast cells.

H.M. Fried and J.R. Warner, Albert Einstein College of Medicine, Bronx, New York: Cloning of yeast gene for cycloheximide resistance and ribosomal protein L29.

L. Gritz[1,2] M. Cannon,[1] B. Littlewood,[1] and J. Davies,[1,2] [1]Biochemistry Dept., University of Wisconsin, Madison; [2]Biogen S.A., Geneva, Switzerland: Ribosomal protein alterations in mutants of *S. cerevisiae*.

V.L. Larionov,[1] A.V. Grishin,[1] A.S. Krayev,[2] K.G. Skryabin,[2] and A.A. Bayev,[2] [1]Dept. of Genetics and Selection, Leningrad State University, Leningrad; [2]Institute of Molecular Biology, Academy of Sciences of USSR, Moscow: The comparison of chromosomal and extrachromosomal rDNA in *S. cerevisiae*.

A.K. Hopper, S.L. Nolan, and A. Furukawa, Dept. of Biological Chemistry, Milton S. Hershey Medical Center, Hershey, Pennsylvania: Loss of suppression as an assay for mutants defective in tRNA biosynthesis.

C. Weeks-Levy and R. Rothstein, Dept. of Microbiology, CMDNJ, New Jersey Medical School, Newark: Expression of a yeast tRNA gene in vivo.

H. Hottinger, D. Pearson, F. Yamao, L. Cooley, and D. Söll, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: The isolation of UGA suppressor genes of *S. pombe* by transformation of *S. cerevisiae* UGA mutant strains.

C. Ball,[1] C. Cummins,[1] T. Donahue,[2] I. Edelman,[1] R. Gaber,[1] L. Mathison,[1] M. Mendenhall,[1] and M. Culbertson,[1] Laboratories of Molecular Biology and Genetics, University of Wisconsin, Madison; [2]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Nontriplet reading of the genetic code.

R.F. Gaber and M.R. Culbertson, Laboratories of Molecular Biology and Genetics, University of Wisconsin, Madison: Genetic and molecular analysis of glycine-specific frameshift suppressors.

R.Y.C. Lo,[1] J.B. Bell,[1] and K.L. Roy,[2] [1]Dept. of Genetics, [2]Dept. of Microbiology, University of Alberta, Edmonton, Canada: Dihydrouridine-deficient tRNAs in *S. cerevisiae*.

G. Merkel[1] and T.L. Helser,[2] [1]Millersville State College, Pennsylvania; [2]Chemistry Dept., State University College, Oneonta, New York: The effects of antibiotics on polyribosome structure by composite gel electrophoresis.

N.J. Pearson, H.M. Fried, and J.R. Warner, Albert Einstein College of Medicine, Bronx, New York: Expression of a ribosomal protein gene carried on an autonomously replicating plasmid.

F. del Rey, T.F. Donahue, and G.R. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Organization and expression of tRNA His genes in yeast.

J.J. Clare and S.G. Oliver, Dept. of Biochemistry, University of Manchester, Institute of Science and Technology, England: tRNA charging and the coordination of transcription with translation.

A.S. Man'kin,[1] A.M. Kopylov,[1] P.M. Rubtsov,[2] and K.G. Skryabin,[2] [1]Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University; [2]Institute of Molecular Biology, Academy of Sciences of USSR: The secondary structure model of the 18S rRNA of *S. cerevisiae*.

F. Meussdoerffer and G.R. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Isolation of genes for methionine- and isoleucine-tRNA synthetases.

K. Bostian, S. Jayachandran, and D. Tipper, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: In vivo transcripts of killer yeast DS RNAs—Size, polyadenylation, and translation products.

J.D. Welsh and M.J. Leibowitz, Dept. of Microbiology, CMDNJ, Rutgers Medical School, Piscataway, New Jersey: Localization of genes on the dsRNA killer plasmid of yeast.

S.S. Sommer and R.B. Wickner, NCI, National Institutes of Health, Bethesda, Maryland: [HOK] and [NEX] are related plasmids and further evidence that *M1* requires *L*.

S.S. Sommer and R.B. Wickner, NCI, National Institutes of Health, Bethesda, Maryland: *kob*—Recessive chromosomal mutations that bypass *kex1*.

A.M. Newman,[1] M.F. Tuite,[2] J.R. Ludwig II,[3] P.A. Bower,[1] and C.S. McLaughlin,[1] [1]Dept. of Biological Chemistry, University of California, Irvine; [2]Dept. of Biochemistry, University of Oxford;

[3]Dept. of Biochemistry, Institute of Science and Technology, University of Manchester, England: Protein and dsRNA synthesis associated with the virus-like particles of yeast—In vivo and in vitro studies.

F. Messenguy,[1] M. Crabeel,[2] C. Verschuren,[2] R. Huygen,[2] and N. Glansdorff,[2] [1]Institut de Recherches du CERIA; [2]Dept. of Microbiologie, Vrije Universiteit Brussel, Belgium: Fine-structure analysis of *cis*-dominant mutations leading to constitutive expression of the *arg3* gene in *S. cerevisiae*.

J.C. Jauniaux,[1] E. Dubois,[2] M. Crabeel,[3] and J.M. Wiame,[1] [1]Laboratoire de Microbiologie, Université Libre de Bruxelles; [2]Institut de Recherches du CERIA; [3]Laboratorium voor Microbiologie, Vrije Universiteit Brussel, Belgium: DNA and RNA analysis of arginase regulatory mutants in *S. cerevisiae*.

F. Genbauffe, H.-S. Yoo, and T.G. Cooper, University of Pittsburgh, Pennsylvania: Transcriptional control of the allantoin degradative system in wild-type and mutant strains of *S. cerevisiae*.

R. Sumrada and T.G. Cooper, University of Pittsburgh, Pennsylvania. Posttranslational processing of urea amidolyase in *S. cerevisiae*.

T.G. Cooper and R. Sumrada, University of Pittsburgh, Pennsylvania: What is the inducer of arginase and ornithine transaminase?

B.C. Hyman, J.H. Cramer, and R.H. Rownd, Dept. of Biochemistry, Laboratory of Molecular Biology, University of Wisconsin, Madison: Properties of a yeast mitochondrial DNA segment conferring autonomously replicating function.

V. Turoscy, G. Chisholm, and T.G. Cooper, University of Pittsburgh, Pennsylvania: Pleiotropic control of five genes by both positive and negative control elements in *S. cerevisiae*.

T.D. Fox and S. Staempfli, Biocenter, University of Basel, Switzerland: Suppression of a mitochondrial ochre mutation by a second-site mitochondrial mutation in or near the 15S rRNA gene.

K.A. Bostian, J.M. Lemire, and D.T. Rogers, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Physical identification of multiple genes coding for variant acid phosphatase polypeptides in *S. cerevisiae*.

R.A. Kramer, G. Thill, and N. Anderson, NCI, National Institutes of Health, Bethesda, Maryland: Structural analysis of cloned restriction fragments carrying yeast repressible acid phosphatase genes.

B. Meyhack and A. Hinnen, Friedrich Miescher-Institut, Basel, Switzerland: Cloning of two structural genes for yeast acid phosphatase.

G. Kawasaki, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Isolation of glycolytic genes in *S. cerevisiae*.

A.M. Musti and R.A. Kramer, NCI, National Institutes of Health, Bethesda, Maryland: The chicken glyceraldehyde-3-phosphate dehydrogenase gene has detectable homology to the yeast gene.

E. Abrams, J. Cohen, B. Buchferer, A. Reichel, and J. Marmur, Albert Einstein College of Medicine, Bronx, New York: Characterization of plasmid mutations resulting in increased expression in yeast of the *E. coli cam*^{r} gene.

M. Yamamoto,[1] J. Sakaguchi,[1] Y. Asakura,[2] and M. Yanagida,[2] [1]Institute of Medical Science, University of Tokyo; [2]Dept. of Biophysics, Faculty of Science, Kyoto University, Japan: Cloning of a gene for aspartate transcarbamylase from *S. pombe* by complementation of *pyr*B mutations in *E. coli*.

G.R. Taylor,[1] B.J. Barclay,[1] R.K. Storms,[2] and R.H. Haynes,[1] [1]Dept. of Biology, York University, Toronto; [2]Dept. of Biology, Concordia University, Montreal, Canada: Isolation of the thymidylate synthetase gene (*TMP1*) by complementation in *S. cerevisiae*.

J. Ferguson, J.C. Groppe, and S.I. Reed, Dept. of Biological Sciences, University of California, Santa Barbara: Three new shuttle vectors that allow easy, rapid subcloning of yeast genes on small DNA fragments.

A.G. Hinnebusch and G.R. Fink, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Molecular cloning of the *HIS1* gene in yeast.

F. Hagie and R. Hitzeman, Dept. of Molecular Biology, Genentech, Inc. South San Francisco, California: Primary structure of the yeast 3-phosphoglycerate kinase gene.

M.A. Eldarov, V.E. Ivanov, and K.G. Skryabin, Institute of Molecular Biology, Academy of Sciences of USSR, Moscow: The structural organization of the *ARG4* gene from *S. cerevisiae*.

R. Losson and F. Lacroute, Institut de Biologie Moléculaire and Cellulaire, Laboratoire de Génétique Physiologique, Strasbourg, France: Cloning and expression of *ppr1* the regulatory gene acting on *URA1* and *URA3* structural genes.

Q. Ju, Q. Gong, F. Huang, H. Rong, J. He, and D. Kuang, Shanghai Institute of Cell Biology, Academia Sinica, China: Characterization and cloning of a yeast plasmid.

F.W. Larimer,[1] A.K. Beck,[1] and F.H. Gaertner,[1,2] [1]Biology Division, Oak Ridge National Laboratory; [2]University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences: Isolation of the *ARO1* gene of *S. cerevisiae*.

A. Martinez-Arias and M.J. Casadaban, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Regulated expression of yeast *leu2-E. coli β*-galactosidase gene fusion in yeast.

J. B. McNeil and J. D. Friesen, Dept. of Biology, York University, Toronto, Canada: *S. cerevisiae* DNA responsible for the expression of the herpes simplex virus thymidine kinase gene in yeast.

G. Ammerer,[1] G. Faye,[1] A. Barta,[2] J. Bennetzen,[1] and B. D. Hall,[1] [1]Dept. of Genetics, University of Washington, Seattle; [2]Endocrine Research Division, University of California, San Francisco: In vivo function of genes fused to the yeast ADHI 5′ flanking sequence.

Workshop on Cloning and Expression Vectors

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

Session 10: *Recombination and Repair*

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

P. Brown and J. Szostak, Dept. of Biological Chemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Isolation of mutations in mitotic recombination.

S. Klapholz and R. E. Esposito, Biology Dept., University of Chicago, Illinois: A new rapid mapping procedure employing a meiotic Rec⁻ mutant.

M. S. Esposito,[1] C. V. Bruschi,[1] D. T. Maleas,[1] K. A. Bjornstad,[1] and J. E. Golin,[2] [1]Lawrence Berkeley Laboratory, Berkeley, California; [2]University of Oregon, Eugene: Comparative analysis of mitotic meiotic recombination.

M. D. Mikus and T. D. Petes, Dept. of Microbiology, University of Chicago, Illinois: Gene conversion and excision of single-copy genes inserted within yeast rDNA.

G. S. Roeder,[1] and G. R. Fink,[2] [1]Dept. of Biology, Yale University, New Haven, Connecticut; [2]Dept. of Biochemistry, Cornell University, Ithaca, New York: Recombination of yeast transposable elements.

S. Fogel and D. B. Kilgore, Dept. of Genetics, University of California, Berkeley: Mitotic and meiotic gene conversion in nontandem duplications.

R. E. Malone, Dept. of Microbiology, Stritch School of Medicine, Loyola University of Chicago, Maywood, Illinois: Multiple mutant analysis of recombination during meiosis and mitosis in yeast.

M. Frankenberg-Schwager, D. Frankenberg, M. Nüsse, D. Blöcher, and R. Harbich, Gesellschaft für Strahlenund Umweltforschung mbH, Biophysikalische Strahlenforschung, Frankfurt, Federal Republic of Germany: Effect of the *rad52* gene on the cell-cycle-dependent variation of radiosensitivity in yeast.

M. A. Resnick,[1] S. Stasiewicz,[1] and J. C. Game,[2] [1]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Dept. of Genetics, University of California, Berkeley: DNA metabolism during meiosis in excision-deficient and normal yeast following UV exposure.

M. Budd and R. Mortimer, Dept. of Biophysics and Medical Physics, University of California, Berkeley: Inducible repair of X-ray damage.

L. Bell and B. Byers, Dept. of Genetics, University of Washington, Seattle: Homologous association of chromosomal DNA in meiosis.

T. L. Orr-Weaver,[1] R. J. Rothstein,[2] and J. W. Szostak,[1] [1]Dept. of Biological Chemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [2]Dept. of Microbiology, New Jersey Medical School, Newark: Plasmid integration as a model system for recombination—DNA ends are recombinogenic.

Session 11: *Cell Surface, Structure and Transport*

Chairperson: R. Mortimer, University of California, Berkeley

R. K. Chan and C. A. Otte, Dept. of Microbiology, University of Cincinnati College of Medicine, Ohio: Defect in yeast mutants supersensitive to G_1 arrest by **a**-factor and α-factor pheromones.

J. Kurjan and I. Herskowitz, Institute of Molecular Biology, University of Oregon, Eugene: A putative α-factor precursor containing four tandem repeats of mature α-factor.

L. C. Blair, A. J. Brake, D. J. Julius, J. M. Lugovoy, and J. Thorner, Dept. of Microbiology and Immunology, University of California, Berkeley: Synthesis and processing of yeast pheromones—Identification and characterization of mutants that produce altered α-factors.

P. N. Lipke and K. Terrance, Dept. of Biological Sciences, Hunter College, City University of New York, New York: Evidence for a single interacting system in *S. cerevisiae* agglutination.

P. Novick, S. Ferro, B. Esmon, and R. Schekman, Dept. of Biochemistry, University of California, Berkeley: Order of events in the yeast secretory pathway.

N. F. Neff, J. H. Thomas, and D. Botstein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetics of tubulin in yeast—A gene that mutates to benomyl resistance is tightly linked to sequences homologous to a chick β-butulin cDNA.

D. Shortle, N.F. Neff, and D. Botstein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Genetics of actin in yeast—Analysis of actin function by transformation of yeast with actin genes modified in vitro.

Session 12: *Killer*

Chairperson: C. McLaughlin, University of California, Irvine

D.J. Thiele, R.W. Wang, and M.J. Leibowitz, Dept. of Microbiology, CMDNJ, Rutgers Medical School, Piscataway, New Jersey: Structural analysis of killer dsRNA of yeast.

J. Bruenn,[1] L. Bobek,[1] L. Field,[1] D. Reilly,[1] V. Brennan,[1] and K. Gross,[2] [1]Division of Cell and Molecular Biology, State University of New York, Buffalo; [2]Molecular Biology Dept., Roswell Park Memorial Institute, Buffalo, New York: Structure and replication of yeast viral dsRNAs.

B.L. Brizzard and S.R. de Kloet, Institute of Molecular Biophysics, Florida State University, Tallahassee: Superkiller yeast strain exhibits additional species of dsRNA.

R.B. Wickner, NIAMDD, National Institutes of Health, Bethesda, Maryland: Three genetically distinct *L* dsRNA species.

K. Bostian, S. Jayachandran, V. Burn, and D. Tipper, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Functional sequence of the structural gene of killer M_1 dsRNA and maturation of the product, a precursor of toxin.

Session 13: *tRNA, Ribosomes and Translation*

Chairperson: D. Hawthorne, University of Washington, Seattle

M.F. Tuite[1] and C.S. McLaughlin,[2] [1]Dept. of Biochemistry, University of Oxford, England; [2]Dept. of Biological Chemistry, University of California, Irvine: Termination readthrough in [psi$^+$] and [psi$^-$] cell-free lysates of *S. cerevisiae*.

C. Cummins,[1] T. Donahue,[2] and M. Culbertson,[1] [1]Laboratories of Molecular Biology and Genetics, University of Wisconsin, Madison; [2]Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: The *SUF2* frameshift suppressor gene encodes an altered prolyl-tRNA with a nucleotide addition in the anticodon.

A.K. Hopper, A. Furukawa, L. Schultz, and J. Kurjan, Dept. of Biological Chemistry, Milton S. Hershey Medical Center, Hershey, Pennsylvania: Genetic and biochemical approaches to studying tRNA processing and modification in yeast.

P. Tekamp, D.N. Standring, and W.J. Rutter, Dept. of Biochemistry and Biophysics, University of California, San Francisco: RNA-processing enzymes specific for yeast 5S and tRNA.

J.L. Teem, J. Rodriguez, and M. Rosbash, Dept. of Biology, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Posttranscriptional regulation of yeast ribosomal protein gene expression.

C.H. Kim and J.R. Warner, Albert Einstein College of Medicine, Bronx, New York: The mRNAs for ribosomal proteins.

T.J. Zamb and T.D. Petes, Dept. of Microbiology, University of Chicago, Illinois: Orientation of the rRNA gene cluster—Polarity of transcription relative to other genes on chromosome XII.

K.G. Skryabin, Institute of Molecular Biology, Academy of Sciences of USSR, Moscow: rRNA genes of *S. cerevisiae*.

J.T. Barnitz, J.H. Cramer, and R.H. Rownd, Laboratory of Molecular Biology and Dept. of Biochemistry, University of Wisconsin, Madison: Organization of *S. pombe* ribosomal DNA.

R.J. Planta, J. Klootwijk, and G.M. Veldman, Biochemisch Laboratorium, Vrije Universiteit, Amsterdam, The Netherlands: Transcription and processing of yeast ribosomal precursor RNA.

Session 14: *Regulation III*

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

B. Shicker, V. Letts, and S. Henry, Dept. of Genetics and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Regulation of phospholipid biosynthesis in yeast.

Y.-P. Hsu, M. Hampsey, and G.B. Kohlhaw, Dept. of Biochemistry, Purdue University, West Lafayette, Indiana: Regulation of leucine biosynthesis in *S. cerevisiae*.

L.M. Hereford, M.A. Osley, and S.E. Bromley, Rosenstiel Center of Brandeis University, Waltham, Massachusetts: Transcriptional regulation of yeast histone gene expression.

M.R. Chevallier, J.C. Hubert, and F. Lacroute, Laboratoire de Génétique Physiologique, Institut de Biologie Moléculaire and Cellulaire Strasbourg, France: Transcription of the uracil permease gene in the wild type and in a regulatory mutant.

M. Carlson,[1,2] B. Osmond,[1] and D. Botstein,[1] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]Dept. of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, New York: Structure and regulation of the *SUC* genes.

Session 15: *Mitochondria*

Chairperson: B. D. Hall, University of Washington, Seattle

D. K. Hanson,[1] H. R. Mahler,[1] and P. S. Perlman,[2] [1]Dept. Chemistry, Indiana University, Bloomington; [2]Dept. Genetics, Ohio State University, Columbus: Intron 4 of the cytochrome *b* gene encodes a polypeptide necessary for RNA processing of two mitochondrial genes.

P. Q. Anziano, K. R. Glaus, K. Jarrel, and P. S. Perlman, Genetics Dept., Ohio State University, Columbus: Functional domains of a mitochondrial intron—Intron 4 of the cytochrome *b* gene.

S. Gasser, G. Daum, P. Böhni, and G. Schatz, Dept. of Biochemistry, Biocenter, University of Basel, Switzerland: In vitro import of proteins into mitochondria.

This meeting was supported in part by the National Institutes of Health, Cetus Corporation, and the Upjohn Company.

Nucleases, August 19–August 23

Arranged by **Stuart M. Linn,** *University of California, Berkeley,* **Richard J. Roberts,** *Cold Spring Harbor Laboratory*

101 participants

Opening Address: *Tribute to M. Laskowski*
D. Kowalski, Roswell Park Memorial Institute, Buffalo, New York

Session 1: Replication Nucleases

Chairperson: C.M. Radding, Yale University School of Medicine, New Haven, Connecticut

K. Geider, T.F. Meyer, I. Bäumel, and G. Harth, Dept. of Molecular Biology, Max-Planck-Institut für Medizinscheforschung, Heidelberg, Federal Republic of Germany: Bacteriophage fd gene-2 protein—A specific endonuclease with DNA ligase activity in replication of phage viral strands.

H.S. Jansz,[1] P.D. Baas,[1] A.D.M. van Mansfeld,[1] F. Heidekamp,[1] G.H. Veeneman,[2] and J.H. van Boom,[2] [1]Institute of Molecular Biology and Laboratory for Physiological Chemistry, State University of Utrecht; [2]Dept. of Organic Chemistry, State University of Leiden, The Netherlands: Gene-*A* protein, a site-specific nuclease involved in initiation and termination of bacteriophage ϕX174 DNA replication.

D. Reinberg, S.L. Zipursky, D. Brown, and J. Hurwitz, Albert Einstein College of Medicine, Bronx, New York: Studies on the mechanism of initiation, elongation, and termination of ϕX RFI DNA replication.

R. Low, P. Burgers, J. Kobori, K. Arai, N. Arai, L. Bertsch, and A. Kornberg, Dept. of Biochemistry, Stanford University School of Medicine, California: Primosome in DNA replication.

R.J. Crouch, NICHH, National Institutes of Health, Bethesda, Maryland: Mechanism of *E. coli* RNase H.

Session 2: Repair Nucleases

Chairperson: P. Sadowski, University of Toronto, Canada

S. Linn, D.W. Mosbaugh, and M. LaBelle, Dept. of Biochemistry, University of California, Berkeley: Studies of base-excision repair.

R.H. Grafstrom, L.P. Park, and L. Grossman, Dept. of Biochemistry, Johns Hopkins University, Baltimore, Maryland: Incision mechanism of dimer-containing DNA in *M. luteus.*

G.L. Chan, L.K. Gordon, C.P. Lindan, and W.A. Haseltine, Dept. of Pathology, Harvard Medical School, and Sidney Farber Cancer Institute, Boston, Massachusetts: Repair endonucleases for UV-irradiated DNA studied by DNA sequencing gels.

R.S. Lloyd, P.C. Seawell, C.A. Smith, A.K. Ganesan, and P.C. Hanawalt, Dept. of Biological Sciences, Stanford University, California: Properties of endonuclease V of bacteriophage T4.

W.D. Rupp, A. Sancer, and B. Kacinski, Yale University School of Medicine, New Haven, Connecticut: Identification and characterization of the *E. coli uvr* gene products.

E. Seeberg, N.D. Clarke, G.B. Evensen, and M. Kvall, Toxicology Division, Norwegian Defense Research Establishment, Kjeller, Norway: Genetic control, gene cloning, and properties of two distinct 3-meA DNA glycosylase activities in *E. coli.*

Session 3: Poster Session

J. Arendes,[1] P.L. Carl,[2] K.C. Kim,[1] and A. Sugino,[1] [1]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina; [2]Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill: Studies of RNase H mutants—Comparison of the enzyme from *E. coli* PC 412 (*wt*) and FB 2 (*rnh*$^-$).

S. Benchimol, M. Sumner-Smith, and A. Becker, Dept. of Medical Genetics, University of Toronto, Canada: PBEN—A single-stranded-DNA-dependent, ATP-dependent endodeoxyribonuclease is the product of gene *bEa59* of phage lambda.

J.E. Brooks and R.J. Roberts, Cold Spring Harbor Laboratory, New York: Modification profile of bacterial genomes.

J.G. Chirikjian,[1] J. George,[1] Y.H. Hamada,[1] P. Hensley,[1] and R. Blakesley,[2] [1]Dept. of Biochemistry, Georgetown Medical Center, Washington, D.C.; [2]Bethesda Research Laboratories Inc., Gaithersburg, Maryland: Studies on the sequence-specific endonuclease *Bam*HI.

G. DasGupta, A.M. Wu, R. Kahn, R.P. Cunningham, and C.M. Radding, Dept. of Human Genetics,

Yale University School of Medicine, New Haven, Connecticut: Concerted strand exchange and formation of Holliday structures by *E. coli recA* protein.

T. K. Dziegielewski and M. Laskowski, Sr., Laboratory of Enzymology, Roswell Park Memorial Institute, Buffalo, New York: Nucleosomes prepared from chicken erythrocyte nuclei with the aid of micrococcal nuclease contain poly(ADP-Rib).

A. Goldfarb and V. Daniel, Biochemistry Dept., Weizmann Institute of Science, Rehovot, Israel: An *E. coli* endonuclease responsible for primary cleavage of in vitro transcripts of bacteriophage T4 tRNA gene cluster.

N. Hasan and A. Landy, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Characterization of an Int fragment produced by limited proteolysis.

W. Hollomon, J. Rusche, T. Rowe, and M. Brougham, Dept. of Immunology and Medical Microbiology, University of Florida, Gainesville: DNA enzymes from *Ustilago*.

S. Iida, J. Meyer, S. Hadi, B. Bächi, T. Bickle, and W. Arber, Dept. of Microbiology, Biocenter, Basel, Switzerland: Organization of the type-III modification and restriction genes of phages P1 and P1-15.

C. M. Joyce,[1] N. D. F. Grindley,[1] and J. M. Rosenberg,[2] [1]Dept. of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, Connecticut; [2]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Reduced site specificity of endonucleases *Eco*RI and *Bst*I.

A. E. Karu and M. Urbauer, Dept. of Biochemistry, University of California, Riverside: Alteration of *E. coli recBC* enzyme activities by monoclonal antibodies.

D. Kowalski, J. P. Sanford, and T. J. Foels, Laboratory of Enzymology, Roswell Park Memorial Institute, Buffalo, New York: Mung bean and P_1 nucleases—Zn^{++} metalloenzyme properties and action on supercoiled PM2 DNA.

J. Langdale, B. D. Jiang, P. Myers, and R. J. Roberts, Cold Spring Harbor Laboratory, New York: Isolation and characterization of restriction endonucleases.

M. Lauth, R. Reed, and N. Grindley, Dept. of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, Connecticut: Location of resolvase binding sites at the resolution site of $\gamma\delta$.

D. Lee and P. Sadowski, Dept. of Medical Genetics, University of Toronto, Canada: Site-specific cutting of intracellular DNA of bacteriophage T7 in vivo.

A. D. B. Malcolm and J. R. Moffatt, Dept. of Biochemistry, St. Mary's Hospital Medical School, London, England: Differential reactivities at restriction enzyme sites.

A. MarSchel, E. Hatt, S. Colvin, L. Markowitz, and D. Hendrick, Bethesda Research Laboratories Inc., Gaithersburg, Maryland: Strains of *Caryophanon latum* differ with respect to their expression of restriction endonucleases.

H. Motamedi, L. Nichols, L. Kangryul, and F. J. Schmidt, Dept. of Biochemistry, University of Missouri, Columbia: Molecular cloning of the *E. coli rnpA* gene.

S. Nishikawa, D. Söll, and D. Frendewey, Yale University, New Haven, Connecticut: Purification of tRNA processing nucleases from *S. pombe*.

S. Riazuddin,[1] Z. Ahmed,[1] and L. Grossman,[2] [1]Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan; [2]School of Hygiene and Public Health, John Hopkins University, Baltimore, Maryland: Isolation, purification, and characterization of a gamma-radiation-damage-specific enzyme.

S. Riazuddin,[1] A. Ather,[1] and L. Grossman,[2] [1]Nuclear Institute for Agrigulture and Biology, Faisalabad, Pakistan; [2]School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland: Studies on mutants of *M. luteus* parent cell having increased resistance against the damaging effects of alkylating chemicals.

J. Rosamond, Dept. of Chemistry, University of Oxford, England: An endonuclease from the mitochondrion of the yeast *S. cerevisiae*.

P. Sadowski,[1] H. Murialdo,[1] G. Wu,[1] and N. Hozumi,[2,3] Depts. of [1]Medical Genetics and [2]Medical Biophysics, University of Toronto; [3]Ontario Cancer Institute, Canada: An immunoglobulin light-chain gene with an abnormal V-J junction and a mutated donor RNA splicing site.

B.M. Sahai and D. Kowalski, Laboratory of Enzymology, Roswell Park Memorial Institute, Buffalo, New York: Eukaryotic DNA topoisomerase II activity correlates with cell growth.

I. Schildkraut, L. Greenough, G. Wilson, R. Grandoni, R. Borsetti, D. Wise, and D. Comb, New England Biolabs, Beverly, Massachusetts: Identification and characterization of restriction endonucleases.

K. Shishido and T. Ando, Dept. of Microbiology, Institute of Physical and Chemical Research, Wako-shi, Saitama, Japan: The use of prokaryotic topoisomerase activity for the study on tertiary structure of superhelical DNA and for the screening of DNA-binding substance.

D.A. Stahl and N.R. Pace, National Jewish Hospital and Research Center and Dept. of Biophysics and Genetics, University of Colorado School of Medicine, Denver: The purification and properties of the β component of *B. subtilis* RNase M5.

J.G.K. Williams, T. Shibata, R. Kahn, and C.M. Radding, Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: *E. coli recA* protein protects single-stranded DNA or gapped duplex DNA from degradation by *recBC* DNase.

J.L. Woodhead and A.D.B. Malcolm, Dept. of Biochemistry, St. Mary's Hospital Medical School, London, England: The essential carboxyl group in restriction endonuclease *Eco*.

J.L. Woodhead and A.D.B. Malcolm, Dept. of Biochemistry, St. Mary's Hospital Medical School, London, England: Nonspecific binding of restriction endonuclease *Eco*RI to DNA.

Session 4: *Topoisomerases*

Chairperson: P. Modrich, Duke University, Durham, North Carolina

T. Goto, Y.-C. Tse, I.S. Sigal, D.S. Horowitz, L.A. Zumstein, S.L. Swanberg, K. Kirkegaard, K. Becherer, A. Shaw, and J.C. Wang, Harvard University, Cambridge, Massachusetts: Recent studies on DNA topoisomerases of *E. coli* and *S. cerevisiae*.

K.N. Kreuzer, D. Coit, and B.M. Alberts, Dept. of Biochemistry and Biophysics, University of California, San Francisco: DNA cleavage reactions of the bacteriophage-T4-induced topoisomerase.

L.M. Fisher, K. Mizuuchi, M.H. O'Dea, and M. Gellert, NIAMDD, National Institutes of Health, Bethesda, Maryland: DNA protection and double-stranded DNA breakage by *E. coli* DNA gyrase.

T.-S. Hsieh, Dept. of Biochemistry, Duke University Medical Center, Durham, North Carolina: Mechanism of the type-2 DNA topoisomerase from *D. melanogaster*.

B. Halligan, J.L. Davis, and L.F. Liu, Dept. of Physiological Chemistry, Johns Hopkins Medical School, Baltimore, Maryland: Intra-and interstrand transfer by eukaryotic DNA topoisomerase.

M.D. Been and J.J. Champoux, Dept. of Microbiology and Immunology, University of Washington, Seattle: Breakage of single- and double-stranded DNA by eukaryotic type-1 topoisomerases.

Session 5: *Restriction Endonucleases*

Chairperson: R.J. Roberts, Cold Spring Harbor Laboratory, New York

J. Burckhardt, J. Weisemann, D.L. Hamilton, and R. Yuan, NCI, Frederick Cancer Research Center, Frederick, Maryland: The mechanism of DNA methylation by the restriction endonuclease from *E. coli* K.

T.A. Bickle, S. Iida, S. Hadi, and B. Suri, Dept. of Microbiology, Biocenter, University of Basel, Switzerland: A comparison of the properties of type-I and type-III restriction endonucleases with their corresponding modification methylases.

A. Piekarowicz, Institute of Microbiology, Warsaw University, Poland: Cleavage and methylation of DNA by the *Hin*fIII and *Hin*eI restriction enzymes.

A. Kiss, É. Szomolányi, W. Reichardt, and P. Venetianer, Institute of Biochemistry, Biological Research Center, Szeged, Hungary: Molecular cloning of the modification methylase genes of *B. sphaericus* and *B. subtilis*.

K.L. Agarwal, Depts. of Biochemistry and Chemistry, University of Chicago, Illinois: Sequence recognition by *Hpa*I, *Hpa*II, and *Msp*I endonucleases.

W.E. Jack, A.-L. Lu, R. Rubin, S.-C. Cheng, A. Newman, and P. Modrich, Duke University Medical Center, Durham, North Carolina: DNA contacts, mode of specific sequence location, and structures of *Eco*RI restriction and modification enzymes.

P. Greene, H.W. Boyer, and J. Rosenberg, University of California, San Francisco: Genetic and molecular analysis of the *Eco*RI restriction enzyme.

Session 6: *Recombination Nucleases I*

Chairperson: S.M. Linn, University of California, Berkeley

P.D. Sadowski, D. Lee, L. Roberts, and W. Bradley, Dept. of Medical Genetics, University of Toronto, Canada: Recombination of bacteriophage T7 DNA in vivo and in vitro.

R. Kolodner, K. Davies, M.J. Doherty, R.A. Fishel, A.A. James, J. Joseph, and P.M. Morrison, Sidney Farber Cancer Institute and Dept. of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Genetic recombination of plasmid DNA.

H.A. Nash, N.L. Craig, and T.J. Pollock, NIMH, National Institutes of Health, Bethesda, Maryland: Protein-DNA interactions in site-specific recombination of phage lambda.

R. Kahn, R.P. Cunningham, C. DasGupta, and C.M. Radding, Dept. of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: The polarity of strand transfer promoted by *E. coli recA* protein.

A. Taylor, D.W. Schultz, and G.R. Smith, Institute of Molecular Biology, University of Oregon, Eugene: Exonuclease V, Chi sites and recombination.

R.R. Reed, N.D.F. Grindley, and J.A. Steitz, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: In vitro site-specific recombination mediated by the transposon $\gamma\delta$.

Session 7: *Recombination Nucleases II. RNA-processing Enzymes*

Chairperson: U.L. RajBhandary, Massachusetts Institute of Technology, Cambridge

M.J. Fraser, T.Y.-K. Chow, and L.M. Hunt, Dept. of Biochemistry, McGill University, Montreal, Canada: A putative recombinase of *Neurospora*, endo-exonuclease.

M. Gold, A. Becker, and W. Parris, Dept. of Medical Genetics, University of Toronto, Canada: Bacteriophage lambda DNA terminase.

G.A. Alianell, C.-F. Wei, and H.B. Gray, Jr., Dept. of Biophysical Sciences, University of Houston, Texas: The extracellular nuclease of *Alteromonas espejiana* as a tool in nucleic acid research.

S. Altman, M. Baer, C. Guerrier-Takada, and R. Kole, Dept. of Biology, Yale University, New Haven, Connecticut: Properties of RNase P—An endoribonuclease essential for tRNA biosynthesis.

M.P. Deutscher, H. Cudny, R. Zaniewski, P. Roy, and A. Solari, Dept. of Biochemistry, University of Connecticut Health Center, Farmington: Nucleases acting at the 3′ terminus of tRNA precursors.

H.D. Robertson, Laboratory of Genetics, Rockefeller University, New York, New York: Enzymes and mechanisms in mRNA processing.

R.J. Crouch, S. Kanaya, S. Seidman, R.J. Feldmann, and P. Earl, National Institutes of Health, Bethesda, Maryland: Processing of chicken rRNA.

Session 8: *Splicing Enzymes*

Chairperson: S. Altman, Yale University, New Haven, Connecticut

C.L. Peebles and J. Albelson, Dept. of Chemistry, University of California, San Diego: Pre-tRNA splicing in yeast.

P. Grabowski, A. Zaug, S. Brehm, and T. Cech, Dept. of Chemistry, University of Colorado, Boulder: In vitro splicing of the ribosomal RNA precursor of *Tetrahymena*.

Session 9: *Open Discussion*

Summary: S.M. Linn, University of California, Berkeley

This meeting was supported in part by grants from the National Institutes of Health and the National Science Foundation. Contributions from the following companies are gratefully acknowledged: Enzo Biochem; Bethesda Research Laboratories; New England Biolabs; New England Nuclear; PL Biochemicals.

Bacteriophage, August 25–August 30

Arranged by **Ahmad I. Bukhari,** *Cold Spring Harbor Laboratory*

170 participants

Session 1: T4 Genes and Their Functions

B. Kemper,[1] J. Hays,[2] K. Mizuuchi,[3] and R. Weisberg,[3] [1]Institut für Genetik, University of Cologne, Federal Republic of Germany; [2]University of Maryland, Catonsville; [3]NICHHD, National Institutes of Health, Bethesda, Maryland: Cleavage of branched DNA by purified T4 49+-enzyme.

G. Mosig and S. Bock, Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee: On the roles of topoisomerase genes and recombination genes in primary and secondary initiation of T4 DNA replication.

B. Kemper, Institut für Genetik, University of Cologne, Federal Republic of Germany: T4-induced endonuclease VII resolves branched DNA molecules in vitro.

V.I. Tanyashin and A.A. Bayev, Institute of Biochemistry and Physiology of Microorganisms, USSR Accademy of Sciences, Pushchino: T4 DNA structure and function—Gene regions 50–30; 31–38, and 42–46.

M.A. Jabber, J.M. Runnels, and L.R. Snyder, Dept. of Microbiology and Public Health, Michigan State University, East Lansing: Genetic mapping of a host gene whose product may substitute for the T4-induced polynucleotide kinase and RNA ligase enzymes.

A. Baxter[1] and C.G. Goff,[2] [1]Dept. of Cell and Molecular Biology, University of Michigan, Ann Arbor; [2]Dept. of Biology, Haverford College, Pennsylvania: Cloning and mapping the T4 gene-60 region.

W.M. Huang and Lian S. Ngai, Dept. of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City: Expression of cloned T4 gene 39 in *E. coli* cells.

K. Dharmalingam, H.R. Revel, and E.B. Goldberg, Dept. of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Deletion mapping and cloning of the T4 anti-restriction endonuclease gene, *arn*.

B. Pragai and D. Apirion, Dept. of Microbiology, Washington University, St. Louis, Missouri: Processing of RNA from the T4 tRNA cluster by host enzymes.

N.J. Casna and D.A. Shub, State University of New York, Albany: Bacteriophage T4 as a generalized cloning vector.

G.G. Wilson, Dept. of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, Connecticut: Plasmid transduction by phage T4.

Session 2: Regulation of Gene Expression: T Phages and N4

J.N. Bailey and W.T. McAllister, Dept. of Microbiology, CMDNJ-Rutgers Medical School, Piscataway New Jersey: Promoter sequences for *E. coli* and T3 RNA polymerases in T3 DNA.

A.D. Carter,[1] L.K. Jolliffe,[2] and W.T. McAllister,[2] [1]National Institutes of Health, Bethesda, Maryland; [2]Dept. of Microgiology, Rutgers Medical School, Piscataway, New Jersey: Identification of a region in T7 late promoters which may be involved in promoter selection in vivo and in vitro.

C.E. Morris and W.T. McAllister, Dept. of Microbiology, CMDNJ-Rutgers Medical School, Piscataway, New Jersey: Expression of foreign genes in recombinant plasmids under the control of T7 and T3 late promoters.

S. Shanblatt and D. Nakada, Dept. of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: T7 interactions with *E. coli* RNA polymerase.

J.R. Christensen and P. Ooi, Dept. of Microbiology, University of Rochester, New York: The transcription program of phage T1—A beginning study.

C. Malone, S. Spellman, and L.B. Rothman-Denes, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Cloning and construction of a genetic map of bacteriophage N4.

L. Haynes, D. Marchetti, and L.B. Rothman-Denes, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: Specificity of transcription of the N4 virion-associated RNA polymerase.

D. Marchetti, C. Malone, and L.B. Rothman-Denes, Dept. of Biophysics and Theoretical Biology, University of Chicago, Illinois: N4 virion RNA polymerase has an intrinsic ATPase that is required for transcription.

W. Zehring and L.B. Rothman-Denes, Dept. of Microbiology and of Biophysics and Theoretical Biology, University of Chicago, Illinois: Extensive purification and template properties of bacteriophage N4 activity-II RNA polymerase and analysis of its in vitro and in vivo products.

J.K. Rist,[1] A. Sugino,[2] and L.B. Rothman-Denes,[1] [1]Dept. of Biochemistry and of Biophysics and Theoretical Biology, University of Chicago, Illinois; [2]Laboratory of Molecular Genetics, National

Institute of Enviromental Health Sciences, Research Triangle Park, North Carolina: N4 DNA replication.

Session 3: Transposable Elements

D. Roberts and N. Kleckner, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Isolation of host mutants affected for transposition of Tn*10*.

V. Lundblad and N. Kleckner, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Host mutants of *E. coli* K12 that affect excision of transposon Tn*10*.

M. A. Davis and N. Kleckner, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Mutants of Tn*10* that are increased for transposition.

R. Isderg and M. Syvanen, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: A supercoiled substrate is required for transposition of Tn5.

D. Hinton, K. Bidwell, and R. Musso, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Transcription and translation of IS2.

M. P. van Bree and J. A. Engler, Cold Spring Harbor Laboratory, New York: The nucleotide sequence of the transposable element IS5.

R. M. Harshey and A. I. Bukhari, Cold Spring Harbor Laboratory, New York: Integration precursor of Mu.

R. M. Harshey, G. McGuinness, and A. I. Bukhari, Cold Spring Harbor Laboratory, New York: Expression of the *mom* gene of bacteriophage Mu.

Session 4: Site-specific Recombination

N. L. Craig and H. A. Nash, NIMH, National Institutes of Health, Bethesda, Maryland: Protein-DNA interactions in λ site-specific recombination.

K. Abremski and J. Davidson, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: *E. coli* host-factor requirements for bacteriophage λ excisive recombination.

H. I. Miller, and H. Echols, Dept. of Molecular Biology, University of California, Berkeley: SOS induction and autoregulation of the *himA* gene for site-specific recombination in *E. coli*.

L. C. Plantefaber,[1] M. Gellert,[2] and D. I. Friedman,[1] [1]Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor; [2]NIAMDD, National Institutes of Health, Bethesda, Maryland: Isolation of temperature-sensitive *himB* mutants.

A. Winoto, S. Chung, J. Abraham, H. Begusch, and H. Echols, Dept. of Molecular Biology, University of California, Berkeley: Directional control of the intergration-excision reaction by phage λ—A point mutation in the P′ region that blocks integration but not excision.

R. A. Weisberg,[1] C. Foeller,[2] L. Enquist,[1] A. Landy,[2] and M. E. Gottesman,[1] [1]NICHHD, National Institutes of Health, Bethesda, Maryland; [2]Brown University, Providence, Rhode Island: The role of homology in site-specific recombination of phage λ.

R. H. Hoess and N. Sternberg, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: P1 site-specific recombination—Analysis of the recombining sites.

K. Abremski and N. Sternberg, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: An in vitro system for the study of bacteriophage P1 site-specific recombination.

A. Abeles, S. Austin, D. Chattoraj, F. Hart, and M. Yarmolinsky, Cancer Biology Program, Frederick Cancer Research Center, Maryland: Localization and characterization of elements involved in the replication and partition of P1 plasmid prophage.

M. Better and D. Freifelder, Dept. of Biochemistry, Brandeis University, Waltham, Massachusetts: Electron microscopic examination of the intracellular population of bacteriophage λ DNA.

R. Calendar,[1] L. Liu,[2] J. C. Wang,[3] J. L. Davis,[2] and L. Perkocha,[1] [1]Molecular Biology Dept., University of California, Berkeley; [2]Physiological Chemistry Dept., Johns Hopkins Medical School, Baltimore, Maryland; [3]The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Knotted DNA from P2 and P4 capsids and its use in the assay of type-II topoisomerases.

M. Sumner-Smith, S. Benchimol, A. Becker, and H. Murialdo, Dept. of Medical Genetics, University of Toronto, Canada: The λ *b*-region gene bEa59 encodes a single-stranded-DNA-dependent, ATP-dependent endodeoxyribonuclease.

Session 5: Poster Session

C. Bauer and J. Gardner, Dept. of Microbiology, University of Illinois, Urbana: The secondary λ attachement site in the threonine operon attenuator—Mutations in theΔ OP' attachment site.

G. E. Christie and R. Calendar, Dept. of Molecular Biology, University of California, Berkeley: Sequence analysis of a bacteriophage P2 late gene promoter region.

F. J. Grundy and M. M. Howe, Dept. of Bacteriology, University of Wisconsin, Madison: Mutants of bacteriophage Mu defective in *S* or *U* make particles lacking tail fibers.

D. H. Hall and R. G. Sargent, School of Biology, Georgia Institute of Technology, Atlanta: Cloning and characterization of the dihydrofolate reductase gene of bacteriophage T4.

K. Kaiser[1] and K. Cartwright,[2] [1]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; [2]Western General Hospital, Edinburgh, Scotland: The role of defective prophages in phage evolution.

A. Liss and R. A. Heiland, Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana: Carrier-state stability in SV1-infected spiroplasmas.

N. E. Murray, J. Gough, and H. Senior, European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Host specificity genes of *E. coli* strains.

M. P. Ontell and D. Nakada, Dept. of Biochemistry, University of Pittsburgh School of Medicine, Pennsylvania: T7 2^- infection of *E. coli* K—Loss of concatemer processing specificity during packaging.

M. M. Susskind, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: *mys*, another new gene in the P22 *immI* region.

M. Yarmolinsky, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Antagonism between gp*ant* of phage P1 and the SOS repressor, gp*lexA* of *E. coli*.

Session 6: Regulation of Gene Expression in Temperate Phages I

R. T. Sauer,[1] W. Krovatin,[1] A. Jeffrey,[2] and C. O. Pabo,[2] [1]Dept. of Biology, Massachusetts Institute of Technology, Cambridge; [2]The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Interaction of the λ repressor with operator DNA.

Y. Takeda, C. G. Caday, and D. David, Chemistry Dept., University of Maryland, Catonsville: Interaction of λ Cro repressor with operator DNA—Mapping functional groups of Cro repressor by chemical modification.

M. A. Hoyt,[1] A. Das,[2] and H. Echols,[1] [1]Dept. of Molecular Biology, University of California, Berkeley; [2]Dept. of Microbiology, University of Connecticut Health Center, Farmington: Regulation of lysogenization by phage λ—Host controls on the quantity of CII.

Y. Ho and M. Rosenberg, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of the phage λ regulatory protein CII.

A. Rattray and G. Guarneros, Dept. of Genetics and Molecular Biology, Centro de Investigacioδn y Estudios Avanzados del I.P.M., Mexico City, Mexico: Regulation of integrative recombination—The role of the CIII function.

D. Schindler and H. Echols, Dept. of Molecular Biology, University of California, Berkeley: Retroregulation of the *int* gene λ—Control of translation completion.

A. Oppenheim, S. Gottesman, and M. Gottesman, NCI, National Institutes of Health, Bethesda, Maryland: The terminator of the λ P^{int} transcript participate in *int* gene regulation.

D. Court, T. F. Huang, and A. Oppenheim, NCI, National Institutes of Health, Bethesda, Maryland: Deletion analysis of a transcription termination site and RNase III processing site within the *sib* region of λ.

U. Schmeissner, K. McKenney, D. Court, and M. Rosenberg, NCI, National Institutes of Health, Bethesda, Maryland: Removal of a terminator structure by RNase III regulates *int* gene expression of phage λ.

C. Georgopoulos[1] and Harvey Eisen,[2] [1]Dept. of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City; [2]Institut Pasteur, Paris, France: The bacteriophage λ Hyp phenotype.

Session 7: Regulation of Gene Expression II

D. Ward and M. Gottesman, NCI, National Institutes of Health, Bethesda, Maryland: The role of translation in transcription antitermination by the λ *N* product.

N. C. Franklin, Howard Hughes Medical Institute, University of Utah, Salt Lake City: Comparison of DNA sequence in the *N-24* regions of λ and P22.

A. Das, K. Wolska, J. Wardwell, and S. Han, Dept. of Microbiology, University of Connecticut Health Center, Farmington: Transcription antitermination by *N* protein in well-defined plasmids requires λ *nut* locus and host *nus ABDE* proteins.

L. J. Mashni, L. C. Plantefaber, M. Baumann, and D. I. Friedman, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: Identification of the *nus*C-60 mutation in *E. coli.*

J. Wardwell, D. Burnham, and A. Das, Dept. of Microbiology, University of Connecticut Health Center, Farmington: Interaction of *N* and *nus* proteins with the *nut* locus in a multicopy plasmid is lethal to *E. coli.*

E. J. Grayhack and J. W. Roberts, Dept. of Biochemistry, Cornell University, Ithaca, New York: The λ *Q* protein promotes increased transcription of the λ late genes in vitro.

C. Debouck and C. Dambly, Genetique, University Libre de Bruxelles, Rhode-Saint-Genese, Belgium: Transcription termination in the P_R-t_{R1} region of bacteriophage λ.

D. L. Daniels and F. R. Blattner, Dept. of Genetics, University of Wisconsin, Madison: Nucleotide sequence of the *Q* gene and the*Q* to *S* intergenic region of bacteriophage λ.

M. A. Mozola and D. I. Friedman, Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor: A φ80 function inhibitory for phage growth in HimA mutants of *E. coli.*

J. Garrett and R. Young, Dept. of Medical Biochemistry, Texas A&M University, College Station: Induction of the λ *S* killing activity.

D. B. Wilson and A. Okabe, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Identification of the active form of the *S*-gene product.

Session 8: Regulation of Gene Expression III

S.-J. Lee, S. Mango, V. Cryns, and R. Goldstein, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: A possible role for antitermination in the transcriptional control of plasmid maintenance by satellite phage P4.

S.-J. Lee, S. Mango, and R. Goldstein, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Kinetic analysis of gene products encoded by satellite bacteriophage P4.

V. Cryns and R. Goldstein, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Mutants of helper bacteriophage P2 insensitive to morphogenic interference by satellite phage P4.

E. W. Six, J. A. Williams, and M. G. Sunshine, Dept. of Microbiology, University of Iowa, Iowa City: A phage P2 mutant insensitive to interference by its satellite P4.

L. Korsnes and B. H. Lindqvist, Institute of Medical Biology, University of Tromsø, Norway: The *ogr* gene and control of late transcription in bacteriophage P2.

U. Skreslett, L. Korsnes, and B. H. Lindqvist, Institute of Medical Biology, University of Tromsø, Norway: Purification and analysis of *E. coli* RNA polymerase from bacteriophage P2-and P4-infected cells.

E. Gulletta,[1] S. Garges,[1] A. Das,[2] and S. Adhya,[1] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]University of Connecticut, Farmington: Characterization of the *rho* gene of *E. coli.*

S. Gottesman,[1] M. Gottesman,[1] M. Pearson,[2] J. Shaw,[3] and A. Das,[4] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]NCI, Frederick Cancer Research Center, Frederick, Maryland; [3]Dept. of Medical Genetics, University of Toronto, Canada; [4]Dept. of Microbiology, University of Connecticut Health Center, Farmington: Properties of A_1 *nus* mutation in the *rho* gene—*rho*HDF026.

J. L. Pinkham and T. Platt, Yale University, New Haven, Connecticut: Nucleotide sequence of the *rho* gene in *E. coli* K12.

J. I. Auerbach[1] and P. Howard-Flanders,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Dept. of Therapeutic Radiology, Yale University, New Haven, Connecticut: Physiological significance of the role of the *lexA* gene in the repression of the *uvr* genes of *E. coli.*

H. Aiba and S. Fujimoto, Radioisotope Laboratory, Faculty of Medicine, Kyoto University, Japan: Cloning and DNA sequence of the cAMP receptor protein gene of *E. coli.*

Session 9: Regulation of Gene Expression IV Morphogensis I

P. Youderian and M. M. Susskind, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Genetic analysis of the P22 O^{ant} operator.

P. Youderian, S. Bouvier, D. Graña, and M. M. Susskind, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Fine-structure genetic analysis of the P22 P^{ant} promoter.

M.M. Susskind, S. Bouvier, P. Youderian, and S.J. Chadwick, Dept. of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester: Posttranscriptional control of P22 *ant* gene expression.

P. Riggs and D. Bostein, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Regulation of the late operon of phage P22.

D.F. Cully and A.J. Garro, City College of New York and Mt. Sinai School of Medicine, City University of New York, New York: Nucleotide sequence analysis of the *B. subtilis* phage ϕ105 immunity region.

S.H. Shore and M.M. Howe, Dept. of Bacteriology, University of Wisconsin, Madison: Gene *T* encodes the major head polypeptide of bacteriophage Mu.

M. George and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: Packaging of host sequences attached to the ends of bacteriophage Mu DNA.

S. Casjens and W.M. Huang, Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: Initiation of P22 DNA packaging.

M. Feiss,[1] W. Widner,[1] and I. Kobayashi,[2] [1]Dept. of Microbiology, University of Iowa, Iowa City; [2]Institute of Molecular Biology, University of Oregon, Eugene: λ terminase-cos interactions—Evidence for separate binding and cutting sites.

S. Frackman, D. Siegele, and M. Feiss, Dept. of microbiology, University of Iowa, Iowa City: Direct interaction of λ terminase with proheads in complex II.

Session 10: Morphogensis II

E. Wyckoff and S. Casjens, Dept. of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City: Regulation of phage P22 scaffolding protein synthesis.

D.H. Smith, D.P. Goldenberg, and J. King, Massachusetts Institute of Technology, Cambridge: Genetic analysis of protein folding in the tail spike protein of P22.

R.W. Hendrix and M. Popa, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Tail length in lambdoid phages—Correlation with size of a minor tail protein.

K. Tilly and C. Georgopoulos, Dept. of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City: Evidence that the two *E. coli groE* morphogenetic gene products interact in vivo.

J.L. Carrascosa,[1] E. Viñuela,[2] N. Garcia,[2] and A. Santisteban,[2] [1]Centro de Biologia Molecular (CSIC-UAM), Madrid; [2]Centro de Investigacion UAM-IBM, Madrid, Spain: Head-tail connecting region of bacteriophage ϕ29.

P.B. Berget, M. Chidambaram, and M.F. Plishker, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: In vitro assembly and analysis of the first assembly intermediate in T4 baseplate morphogenesis.

B. Stitt and L. Mindich, Public Health Research Institute of the City of New York, Inc., New York: Membrane assembly in *Pseudomanas* bacteriophage ϕ6.

L. Mindich, C. Goldthwaite, and T. McGraw, Dept. of Microbiology, Public Health Research Institute of the City of New York, Inc., New York: Morphogenesis of the lipid-containing bacteriophage PRD1.

Session 11: Replication and Recombination (general)

S. Reinberg, S.L. Zipursky, D. Brown, and J. Hurwitz, Albert Einstein College of Medicine, Bronx, New York: Studies on the requirements for leading and lagging strand DNA synthesis during ≥X A protein-dependent RF→RF DNA replication in vitro.

T.S.B. Yen and R.E. Webster, Dept. of Biochemistry, Duke University, Durham, North Carolina: Control of the intracellular concentrations of F1 gene-II and gene-X proteins by gene-V protein.

K. Geider, T.F. Meyer, and I. Bäumel, Dept. of Molecular Biology, Max-Planck-Institut für Medizinischeforschung, Heidelberg, Federal Republic of Germany: Strand unwinding by phage fd gene-2 protein and *E. coli rep* protein.

T.F. Meyer and K. Geider, Dept. of Molecular Biology, Max-Planck-Institut für Medizinischeforschung, Heidelberg, Federal Republic of Germany: Maintenance of a replicon with the phage fd replication origin by cloned fd gene 2.

K. Horiuchi and G.P. Dotto, Rockefeller University, New York, New York: Replication of a plasmid that contains a duplicated origin of bacteriophage f1 DNA replication.

M. Lichten and M.S. Fox, Dept. of Biology, Massachusetts Institute of Technology, Cambridge: The effect of nonhomologies on bacteriophage λ recombination.

L. Orosz, Dept. of Genetics, Attila Jozsef University, Szeged, Hungary: Mismatch repair and recombination pattern of temperate phage 16-3 of *R. meliloti.*

R. Maurer, B.C. Osmond, and D. Botstein, Massachusetts Institute of Technology, Cambridge: Complementation and pseudoreversion analysis of essential *Salmonella* genes cloned in λ.

R. Mosesson, R.M. Harshey, and A.I. Bukhari, Cold Spring Harbor Laboratory, New York: Shotgunning of *E. coli* chromosome without restriction enzymes.

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9th Cold Spring Harbor Conference on Cell Proliferation
Growth of Cells in Hormonally Defined Media, September 1–September 6

Arranged by **David A. Sirbasku,** *University of Texas Health Science Center,* **Gordon H. Sato,** *University of California, San Diego,* **Arthur B. Pardee,** *Sidney Farber Cancer Institute, Harvard Medical School*

173 participants

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

Opening Address: *Jacob Furth Dedication*
K.H. Clifton, Radiation Effects Research Foundation, Hiroshima, Japan

Session 1: *Hormone-dependent Tumor Growth: History and Current Topics*

Chairperson: G.H. Sato, University of California, San Diego

H. Kirkman, Dept. of Structural Biology, Stanford University School of Medicine, California: Estrogen- and androgen/estrogen-induced and -dependent tumors in the Syrian hamster.
R. Ross, University of Washington School of Medicine, Seattle: Growth factors—Current concepts.
R. Holley, Salk Institute, San Diego, California: Control of cell growth.
S. Cohen, Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee: Some recent and historical aspects of EGF and NGF.
G.H. Sato, Dept. of Biology, University of California, San Diego: Defined media.

Session 2: *Nutrition and Cells in Defined Media*

Chairperson: C. Waymouth, Jackson Laboratory, Bar Harbor, Maine

C. Waymouth, P. Ward, and S. Blake, Jackson Laboratory, Bar Harbor, Maine: Mouse prostatic epithelial cells in defined culture media.
R.G. Ham, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Importance of the basal nutrient medium in the design of hormonally defined media.
W.L. McKeehan, W. Alton Jones Cell Science Center, Lake Placid, New York: Growth factor–nutrient interrelationships in control of normal and transformed cell proliferation.
D. McClure,[1] G. Sato,[1] M. Hightower,[2] and W. Topp,[2] [1]Dept. of Biology, University of California, San Diego; [2]Cold Spring Harbor Laboratory, New York: Development of a defined medium for the growth and maintenance of the rat embryo cell line REF52 and its application to the study of SV40 transformation.

Session 3: *Role of Cell Matrix*

Chairperson: L. Reid, Albert Einstein College of Medicine, Bronx, New York

L. Reid, Dept. of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York: Synergistic regulation of growth and differentiation by hormones and extracellular matrix.
D. Gospodarowicz, Cancer Research Institute, University of Calfornia Medical Center, San Francisco: Extracellular matrices and the establishment in tissue culture of normal diploid cells.
G.R. Grotendorst, National Institutes of Health, Bethesda, Maryland: Role of attachment factors.
K. Yamada, S.K. Adiyama, and M. Hiyashi, NCI, National Institutes of Health, Bethesda, Maryland: Fibronectin.

Session 4: *Differentiation in Defined Media*

Chairperson: J.E. Lever, University of Texas Medical School, Houston

J.E. Lever, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Cell differentiation and dome formation in polarized epithelial monolayers.
P. Boerner and M.H. Saier, Jr., Dept. of Biology, University of California, San Diego: Nutrient transport and growth regulation in kidney epithelial cells cultured in a defined medium.
J.D. Valentich, Dept. of Physiology and Cell Biology, University of Texas School of Medicine, Houston: Basal lamina assembly by the dog kidney epithelial cell line MDCK.

N. Alavi, D. Livingston, and M. Taub, Dept. of Biochemistry, School of Medicine, State University of New York, Buffalo: Growth and functional properties of proximal tubule cells from the rabbit kidney in defined medium.

G. Erickson, R. Casper, and C. Hofeditz, Dept. of Reproductive Medicine, University of California, San Diego: The obligatory role of defined medium in the hormone-dependent differentiation of rat granulosa cells.

J. P. Mather,[1] J. M. Saez,[2] F. Haour,[2] [1]Population Council, New York, New York; [2]INSERM, Lyon, France: Hormone–hormone and hormone–vitamin interactions in the control of growth and function of testicular cells in vitro.

A. Rizzino, NCI, National Institutes of Health, Frederick, Maryland: The effect of the substratum on the differentiation of embryonal carcinoma cells.

Session 5: *Expression of Differentiated Function in Defined Media*

Chairperson: H. G. Coon, NCI, National Institutes of Health, Bethesda, Maryland

H. L. Leffert and K. S. Koch, Dept. of Medicine, University of California, San Diego: Hepatocyte growth regulation by hormones in chemically defined media—A two-signal hypothesis.

G. N. Gill, J. F. Crivello, P. J. Hornsby, and M. H. Simonian, Dept. of Medicine, University of California School of Medicine, San Diego: Cultured adrenocortical cells.

D. N. Carney, A. F. Gazdar, P. A. Bunn, H. Oie, and J. D. Minna, NCI, National Institutes of Health, Bethesda, Maryland: Growth and cloning of human small-cell lung cancer in serum-free, defined media.

J. Folkman,[1] C. Haudenschild,[2] and B. Zetter,[2] [1]Dept. of Surgery, Children's Hospital Medical Center; [2]Depts. of Surgery, Anatomy, and Physiology, Harvard Medical School, Boston, Massachusetts: Capillary endothelial cells—Growth control and differentiation.

T. Maciag,[1] G. A. Hoover,[2] M. B. Stemerman,[2] and R. Weinstein,[2] Depts. of Pathology and [2]Medicine, Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts: Human umbilical vein endothelial cell mitogens.

G. R. Serrero, Dept. of Biology, University of California, San Diego: Growth and differentiation of a fibro-adipogenic cell line in serum-free medium.

R. Wu, Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Effects of hormones on the multiplication and differentiation of tracheal epithelial cells cultured in defined media.

F. S. Ambesi-Impiombato and D. Tramontano, Centro di Endocrinologia ed Oncologia Sperimentale del CNR, Naples, Italy: The influence of hormones and serum on growth and differentiation of thyroid cell strain FRT1.

Session 6: *Cell Attachment Factors and Melanocytes*

Chairperson: D. Barnes, University of Pittsburgh, Pennsylvania

D. W. Barnes,[1] M. Darmon,[2] and J. Orly,[3] [1]Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania; [2]Dept. of Molecular Biology, Pasteur Institute, Paris, France; [3]Dept. of Biological Chemistry, Hebrew University, Jerusalem, Israel: Serum spreading factor—Effects on RF1 rat ovary cells and 1003 mouse embryonal carcinoma cells in serum-free media.

G. Brunner and R. Wieser, Institute for Immunology, University of Mainz, Federal Republic of Germany: Importance of the "contact environment" on the behavior of mammalian cells.

J. M. Varga,[1] D. T. Lambert,[1] L. Airoldi,[1] G. Moellmann,[1] J. C. Bartholomew,[2] and A. B. Lerner,[1] [1]Dept. of Dermatology, Yale University School of Medicine, New Haven, Connecticut; [2]Laboratory of Chemical Biodynamics, University of California, Berkeley: Characterization of murine melanoma cells grown in serum-containing and serum- and hormone-free media.

M. Murray, R. Fleischmann, and J. Pawelek, Dept. of Dermatology, Yale University School of Medicine, New Haven, Connecticut: A genetic approach to studies of insulin action in Cloudman S91 melanoma cells.

Session 7: *Receptor-mediated Effects on Cell Growth*

Chairperson: S. Hauschka, University of Washington, Seattle

I. Hayashi, City of Hope National Medical Center, Duarte, California: Growth of myoblasts in hormone-supplemented serum-free medium.

T. Linkhart, B. Lim, and S. Hauschka, Dept. of Biochemistry, University of Washington, Seattle: Regulation of normal and variant mouse myoblast proliferation and differentiation by specific growth factors.

H.R. Herschman, D.B. Cawley, and D.L. Simpson, University of California School of Medicine, Los Angeles: Toxic conjugates of epidermal growth factor and the enzymic chains of ricin toxin or diptheria toxin.

N. Savion and D. Gospodarowicz, Cancer Research Institute, University of California, San Francisco: Role of hormones, growth factors, and lipoproteins in the control of proliferation and differentiation of cultured bovine granulosa cells.

Session 8: *Lymphocytes and Granulocytes in Defined Media*

Chairperson: I. Hayashi, City of Hope National Medical Center, Duarte, California

J. Hayashi and I. Goldschneider, Dept. of Pathology, University of Connecticut Health Center, Farmington: In vitro culture of terminal-deoxynucleotidyl-transferase-positive rat bone marrow cells.

J. Mendelsohn, A. Caviles, Jr., and J. Castagnola, Dept. of Medicine and Cancer Center, University of California, San Diego: Proliferation of normal human lymphocytes in hormonally defined, serum-free medium.

H. Murakami,[1] H. Masui,[2] and G. Sato,[2] [1]Food Science and Technology Institute, Kyushu University, Fukuoka, Japan; [2]Dept. of Biology, University of California, San Diego: Cultivation of hybridoma cells in serum-free medium.

T.R. Breitman, NCI, National Institutes of Health, Bethesda, Maryland: Induction of terminal differentiation of HL-60 and fresh leukemic cells by retinoic acid.

J.-J. Mermod, R.N. Newby, and S. Bourgeois, Regulatory Biology Laboratory, Salk Institute, San Diego, California: Effect of glucocorticoids on the growth of lymphoid cell lines.

Session 9: *Growth Control in 3T3 Cells*

Chairperson: C.D. Scher, Sidney Farber Cancer Center, Harvard University, Boston, Massachusetts

J.C. Smith, B.J. Bockus, J.P. Singh, and C.D. Stiles, Sidney Farber Cancer Institute, and Harvard Medical School, Boston, Massachusetts: Molecular action of platelet-derived growth factor—Analysis by somatic cell fusion techniques.

C.D. Scher,[1] S.L. Hendrickson,[1] A.P. Whipple,[1] M.M. Gottesman,[2] and W.J. Pledger,[3] [1]Sidney Farber Cancer Institute, Boston, Massachusetts; [2]NCI, National Institutes of Health, Bethesda, Maryland; [3]University of North Carolina, Chapel Hill: Platelet-derived-growth-factor-modulated proteins—Constitutive synthesis by a transformed cell line.

J.L. Wang, P.A. Steck, and J.W. Kurtz, Dept. of Biochemistry, Michigan State University, East Lansing: Isolation and characterization of a growth regulatory factor from 3T3 cells.

Session 10: *Poster Session: Cell Function and Defined Media*

Chairperson: D. Sirbasku, University of Texas Medical School, Houston

B.B. Asch,[1] H.F. Dvorak,[2] and D.R. Senger,[2] [1]Dept. of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York; [2]Dept. of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts: A major phosphoprotein marker for neoplastic transformation of fibroblasts and epithelial cells.

D.W. Barnes, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: A serum-free medium for A431 human epidermoid carcinoma cells; inhibition of growth in culture by mouse epidermal growth factor.

W. J. Bettger[1] and R. G. Ham[2] [1]Dept. of Biochemistry, St. Louis University Medical School, Missouri; [2]Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Clonal growth of human diploid fibroblasts in a defined medium—The critical role of nutrients.

D. Bowen-Pope, K. Glenn, E. Raines, and R. Ross, Depts. of Pathology and Biochemistry, University of Washington School of Medicine, Seattle: Binding of radiolabeled platelet-derived growth factor to its receptor.

N. Brunet,[1] D. Gourdji,[1] A. Rizzino,[2] and A. Tixier-Vidal,[1] [1]Collège de France, Paris; [2]NCI, Frederick, Maryland: Role of attachment and spreading factors—Effect of fetuin on proliferation and prolactin secretion by GH3 cells—An adaptation to primary cultures of normal rat prolactin cells.

G. Brunner, G. Tschank, and P. Stasiecki, Institute for Immunology, University of Mainz, Federal Republic of Germany: Are different molecular pathways the reason for proliferation differences caused by hormones used in serum-free media?

R. L. Buzard and D. Horn, Molecular Biology Division, Unviersity of Southern California, Los Angeles: Growth inhibition by dexamethasone in clonal variants of hamster melanoma cells—Characterization of biological response and role of medium components.

H. Chang and O. W. Jones, Dept. of Medicine, University of California, San Diego: Human amniotic fluid cells in a hormonal supplement—Importance in amniocentesis.

D. Chaproniere-Rickenberg and M. M. Webber, Division of Urology, University of Colorado Health Sciences Center, Denver: A chemically defined medium for the growth of adult human prostatic epithelium.

D. L. Coppock and D. S. Straus, Division of Biomedical Sciences, University of California, Riverside: Growth response to insulin and MSA in mouse melanoma cells and fibroblast × melanoma hybrids.

F. J. Darfler and P. A. Insel, Pharmacology Dept., University of California, San Diego: Growth of hybridomas and other lymphoid cells in defined medium.

G. Fischer, Institute of Neurobiology, University of Heidelberg, Federal Republic of Germany: Cultivation of mouse cerebella cells in defined media.

H. Fong,[1] W. L. Chick,[2] and G. H. Sato,[1] [1]Dept. of Biology, University of California, San Diego; [2]Dept. of Biochemistry, Massachusetts Medical School, Worcester: Hormones and factors that stimulate growth of rat islet tumor cell line in serum-free medium.

G. R. Grotendorst,[1] H. E. J. Seppa,[1] G. R. Martin,[1] H. K. Kleinman,[1] C. D. Stiles,[2] and C. D. Scher,[2] [1]NIDR, National Institutes of Health, Bethesda, Maryland; [2]Sidney Farber Cancer Institute, Boston, Massachusetts: The platelet-derived growth factor is a chemoattractant for fibroblastic cells.

M. Iio[1] and D. A. Sirbasku,[2] [1]Dept. of Life Sciences, Kumamoto Women's University, Japan; [2]Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Partial purification of mammary tumor cell estromedins from pregnant sheep uterus.

M. J. Jackson and S. Shin, Albert Einstein College of Medicine, Bronx, New York: The role of inositol in mammalian cell growth in culture.

F. E. Leland, D. Danielpour, and D. A. Sirbasku, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Studies of the endocrine, paracrine, and autocrine control of mammary tumor cell growth.

J. G. Liehr, B. B. DaGue, A. M. Ballatore, and D. A. Sirbasku, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: The multiple roles of estrogen in estrogen-dependent renal clear-cell carcinoma of Syrian hamster.

R. W. Lim and S. D. Hauschka, Dept. of Biochemistry, University of Washington, Seattle: Differential epidermal growth factor (EGF) responsiveness and EGF receptor modulation in a clonal line of mouse myoblasts and a differentiation-defective variant.

H. Masui and T. White, University of California, San Diego: Correlation between in vivo tumorigenicity and growth characteristics of tumor cell subpopulations in human astrocytoma.

H. K. Meiss[1] and M. Marcus,[2] [1]Dept. of Cell Biology, New York University Medical School, New York; [2]Dept. of Genetics, Hebrew University, Jerusalem, Israel: Cell-cycle arrest points in nontumorigenic and tumorigenic cell lines, dependency on growth factors.

K. Miyazaki, H. Masui, and G. Sato, Dept. of Biology, University of California, San Diego: Control factors for keratinization of human bronchogenic epidermoid carcinoma cells.

J. B. Moo and D. A. Sirbasku, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Role of estrogens and growth factors in pituitary tumor cell growth.

J. H. Nielsen, Hagedorn Research Laboratory, Copenhagen, Denmark: Growth hormone as a growth factor for normal pancreatic islet cells in primary culture.

A. Richmond,[1] D. H. Lawson,[1,2] and D. W. Nixon,[1,2] [1]Clinical Research Facility and [2]Division of Hematology-Oncology, Dept. of Medicine, Emory University School of Medicine, Atlanta, Georgia: Release of an autostimulatory growth activity by human malignant melanoma cells.

H. Rochefort,[1] D. Chalbos,[1] E. Coezy,[1] M. Garcia,[1] P. Vic,[2] F. Vignon,[1] and B. Westley,[1] [1]INSERM;

[2]Laboratoire d' Histologie, Faculté de Médecine, Montpellier, France: Effect of estrogen on cell proliferation, ultrastructure, and secretory proteins in human breast cancer cell lines.

M. Salas-Prato, Dept. of Biology, University of California, San Diego: Growth of fetal mouse liver primary cells in serum-free medium.

D.S. Salomon,[1] L.A. Liotta,[1] J.-M. Foidart,[2] and M. Yaar,[2] [1]NCI and [2]NIDR, National Institutes of Health, Bethesda, Maryland: Synthesis and turnover of basement membrane components by mouse embryonal carcinoma cells in serum-free hormone-supplemented medium.

S.S. Seaver,[1] S.M. Baird,[2] J. van der Bosch,[3] and G. Sato,[3] [1]Dept. of Molecular Biology, Vanderbilt University, Nashville, Tennessee; [2]Dept. of Pathology, VA Medical Center, San Diego, California; [3]Dept. of Biology, University of California, San Diego: The effects of hormones on the growth and gene expression of a primary chick oviduct tissue culture system.

N. Shimizu, W.K. Miskimins, S. Gamou, and Y. Shimizu, Dept. of Cellular and Developmental Biology, University of Arizona, Tucson: Genetics of insulin receptors and differentiation of 3T3-L1 preadipocytes.

C.E. Smart, R.S. Slaughter, and J.E. Lever, Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Lysosomotropic compounds stimulate dome formation (a differentiated characteristic of MDCK kidney epithelial cell cultures).

R. Weinstein,[1] G.A. Hoover,[1] M.B. Stemerman,[1] and T. Maciag,[2] Depts. of [1]Medicine and [2]Pathology, Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts: Fibronectin dependence for attachment in vitro—Fibroblast vs smooth muscle cell.

W. Wharton, E. Leof, E. O'Keefe, and W.J. Pledger, Cancer Research Center, University of North Carolina, Chapel Hill: Reevaluation of the role of cyclic AMP in the control of cell-cycle traverse.

L. Wilkins,[1] G. Szabo,[1] L. Connell,[1] R. Nemore,[2] and T. Maciag,[2] [1]Harvard School of Dental Medicine; [2]Beth Israel Hospital, Boston, Massachusetts: Growth of enriched human melanocyte cultures.

E.L. Wilson, C. Dutlow, and E.B. Dowdle, Dept. of Clinical Science and Immunology, Medical School, Cape Town, South Africa: Characterization of a new hormonally responsive human breast carcinoma cell line.

R.A. Wolfe[1] and G.H. Sato,[2] [1]Dept. of Molecular Genetics, Hoffmann-La Roche Inc., Nutley, New Jersey; [2]Dept. of Biology, University of California, San Diego: Continuous serum-free culture of the N18TG-2 neuroblastoma, the C6BU-1 glioma, and NG108-15 neuroblastoma X glioma hybrid cell lines.

I. Yamane, M. Kan, Y. Minamoto, and Y. Amatsuji, Institute for TB and Cancer, Tohoku University, Sendai, Japan: α-Cyclodextrin—A partial substitute for bovine albumin in serum-free culture of mammalian cells.

Session 11: *Normal vs. Cancer Cells in Defined Media*

Chairperson: R. Sager, Harvard University Medical School, Boston, Massachusetts

A.B. Pardee and P.V. Cherington, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: On the basis for loss of epidermal growth factor requirement by transformed cells in defined medium.

R. Sager, F. Bennett, and B.L. Smith, Sidney Farber Cancer Institute, Boston, Massachusetts: Serum-free growth of CHEF/18 and mutant cell lines.

W.J. Pledger, B. Chou, E. Leof, N. Olashaw, E. O'Keefe, J.J. Van Wyk, and W.R. Wharton, University of North Carolina School of Medicine, Chapel Hill: Platelet-derived growth factor—Cellular responses and possible mode of action.

S. Powers, D. Alcorta, N. Nicholson, S. Chen, and R. Pollack, Dept. of Biological Sciences, Columbia University, New York, New York: Transformation to serum independence specifically leads to reduced hormone requirements for colonial cell growth.

A.B. Roberts, M.A. Anzano, C.A. Frolik, and M.B. Sporn, NCI, National Institutes of Health, Bethesda, Maryland: Transforming growth factors—Characterization of two classes of factors from both neoplastic and nonneoplastic tissues.

Session 12: *Mammary Cell Growth in Defined Media*

Chairperson: T. Kano-Sueoka, University of Colorado, Boulder

T. Kano-Sueoka and J.E. Errick, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: Role of phosphoethanolamine, ethanolamine, and prolactin on mammary cell growth.

D.A. Sirbasku,[1] J.B. Officer,[1] and F.E. Leland,[1] and M. Iio,[2] [1]Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, Houston; [2]Dept. of Life Sciences, Kumamoto

Women's University, Japan: Evidence for a new role of pituitary-derived hormones and growth factors in mammary tumor cell growth in vivo and in vitro.

S. Nandi, W. Imagawa, Y. Tomooka, R. Shiurba, and J. Yang, Cancer Research Laboratory, University of California, Berkeley: Growth requirements for mammary epithelial cells in primary culture in the presence and absence of serum—Effects of in vivo mammogenic hormones.

W. R Kidwell, L. A. Liotta, D. Salomon, and J. Zwiebel, Laboratory of Pathophysiology, NCI, National Institutes of Health, Bethesda, Maryland: Growth-factor effects on mammary epithelial cell proliferation and basement membrane synthesis.

M. R. Banerjee, R. Ganguly, N. M. Mehta, N. Ganguly, and P. Majumder, Tumor Biology Laboratory, University of Nebraska, Lincoln: Regulation of selective gene expression during differentiation of an isolated whole mammary organ in a hormonally defined medium.

M. R. Stampfer,[1] A. J. Hackett,[1] J. Bartley,[1] J. P. Leung,[2] T. S. Edgington,[2] and H. S. Smith,[1] [1]Lawrence Berkeley Laboratory, Peralta Cancer Research Institute, Berkeley; [2]Scripps Clinic and Research Center, La Jolla, California: Expression of tumor-specific properties by human mammary epithelium in culture.

M. Lippman,[1] H. Nawata,[1] D. Bronzert,[1] F. Vignon,[2] and H. Rochefort,[2] [1]NCI, National Institutes of Health, Bethesda, Maryland; [2]Endocrinology Unit, INSERM, Montpellier, France: Anti-estrogen-resistant variants of hormone responsive MCF-7 human breast cancer cells.

Session 13: *Neuronal Tissue in Defined Media*

Chairperson: J. E. Bottenstein, University of California, Los Angeles

A. Michler-Stuke and J. E. Bottenstein, Dept. of Pediatrics, University of California School of Medicine, Los Angeles: Growth of human and rat glioma cell lines in defined medium.

R. S. Morrison and J. de Vellis, Dept. of Anatomy, University of California, Los Angeles: Regulation of proliferation and differentiation of astrocytes in a defined medium.

H. A. Armelin, A. G. Gambarini, and M. C. S. Armelin, Instituto de Quimica, Universidade de São Paulo, Brazil: Pituitary FGF's and the growth of rat C6 glial cell variants.

H. G. Coon and C. N. Sinback, NCI, National Institutes of Health, Bethesda, Maryland: Cultures of rat neuroblasts that divide and differentiate in vitro.

M. Darmon[1,2] and G. Sato,[2] [1]Institut Pasteur, Paris, France; [2]Dept. of Biology, University of California, San Diego: Control of cell differentiation by the extracellular environment in an embryonal carcinoma cell line.

R. Bunge, M. Bunge, D. Carey, D. Higgins, L. Iacovitti, D. Kleinschmidt, and F. Moya, Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri: Functional expression in neural tissue cultured in defined medium.

A. Tixier-Vidal, A. Faivre-Bauman, C. Loudes, and J. Puymirat, Collège de France, Paris: Expression of neuronal functions by mouse fetal hypothalamic cells cultured in hormonally defined medium.

R. Goodman, Dept. of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia: Pheochromocytoma cell lines in hormonally defined medium.

Concluding Remarks: A. Pardee, Sidney Farber Cancer Institute, Harvard University, Boston, Massachusetts

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Molecular and Cellular Control of Muscle Development

September 8–September 13

Arranged by **Henry F. Epstein,** *Baylor College of Medicine,* **James I. Garrels,** *Cold Spring Harbor Laboratory,* **Stephen J. Kaufman,** *University of Illinois School of Basic Medical Sciences,* **Mark L. Pearson,** *Frederick Cancer Research Center*

212 participants

Session 1: *Proteins and Regulation*

Chairperson: H.F. Epstein, Baylor College of Medicine, Houston, Texas

H.F. Epstein,[1] D.M. Miller III,[1] L.A. Gosset,[2] and R.M. Hecht,[2] [1]Dept. of Neurology, Baylor College of Medicine, Houston; [2]Dept. of Biophysical Sciences, University of Houston, Texas: Myosin and paramyosin expression in normal and mutant nematode embryogenesis.

S. Lowey, P.A. Benfield, G.F. Gauthier, D.D. LeBlanc, and G. Waller, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Characterization of myosins from embryonic and developing chicken pectoralis muscle.

R.G. Whalen, S.M. Sell, C. Pinset, and L. Bugaisky, Dept. of Molecular Biology, Pasteur Institute, Paris, France: Biochemical characterization of myosin heavy chain isozymes in adult and developing muscles.

R.C. Strohman, E. Bandman, and R. Matsuda, Dept. of Zoology, University of California, Berkeley: Stability of expression of myosin isozymes, heavy chains, and light chains in long-term cultures.

R. Zak, A.W. Everett, R.A. Chizzonite, and W.A. Clark, Depts. of Medicine and of Pharmacological and Physiological Sciences, University of Chicago, Illinois: Analysis of molecular variants of myosin heavy chains in rabbit ventricles during normal growth and after treatment with thyroid hormone.

J.I. Garrels, J.J.-C. Lin, and F. Matsumura, Cold Spring Harbor Laboratory, New York: Protein synthesis and phosphorylation in myogenic cell lines studied by computer-analyzed two-dimensional gel electrophoresis.

Session 2: *Gene Structure and Expression I*

Chairperson: R.A. Firtel, University of California, San Diego

M. McKeown and R.A. Firtel, Dept. of Biology, University of California, San Diego: The actin multigene family of *Dictyostelium*.

T.L. Thomas,[1] R.H. Scheller,[2] L.B. McAllister,[1] R.J. Britten,[1] and E.H. Davidson,[1] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Institute of Cancer Research, Columbia University College of Physicians & Surgeons, New York, New York: Linkage and expression of sea urchin actin genes.

B.J. Bond,[1] N. Davidson,[1] S. Falkenthal,[1] E.A. Fyrberg,[2] W. Mattox,[1] and V. Parker,[1] [1]California Institute of Technology, Pasadena; [2]Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: Studies of muscle protein genes of *Drosophila*.

C.P. Ordahl, J. Fornwald, C. Ovitt, T. Cooper, and A. Calman, Dept. of Anatomy, Temple University Medical School, Philadelphia, Pennsylvania: Structure and developmental regulation of muscle-regulated gene sets.

P. Gunning, J. Engel, and L. Kedes, Howard Hughes Medical Institute Laboratory and Dept. of Medicine, Stanford Medical School, Veterans Administration Medical Center, Palo Alto, California: Characterization of the human actin gene family.

D. Hirsh, J. Files, S. Carr, and M. Krause, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder: The actin genes of *C. elegans*.

U. Nudel, M. Shani, R. Zakut, D. Katcoff, J. Calvo, Y. Carmon, M. Finer, and D. Yaffe, Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel: Studies on the structure of genes coding for contractile proteins.

Session 3: *Gene Structure and Expression II*

Chairperson: M.L. Pearson, Frederick Cancer Research Center, Frederick, Maryland

M.L. Pearson[1] and M.M. Crerar,[2] [1]Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland; [2]Biologicals, Toronto, Canada: RNA polymerase II mutants defective in myogenesis.

B. Nadal-Ginard, R.M. Medford, H. Nguyen, R. Gubits, and E. Bekesi, Albert Einstein College of Medicine, Bronx, New York: Regulation of myosin heavy chain gene expression during in vitro L_6E_9 cell myogenesis.

M. Caravatti,[1] B. Robert,[2] A Minty,[2] A. Weydert,[2] S. Alonso,[2] A. Cohen,[2] P. Daubas,[2] and M. Buckingham,[2] [1]Friedrich Miescher Institute, Basel, Switzerland; [2]Dépt. de Biologie Moléculaire, Pasteur Institute, Paris, France: The messengers coding for myosins and actins—Their expression during terminal differentiation of a mouse muscle cell line.

M. Shani, D. Zevin-Sonkin, O. Saxel, Y. Carmon, D. Katcoff, U. Nudel, and D. Yaffe, Dept. of Cell Biology, Weizmann Institute of Science, Rehovot, Israel: Control of biosynthesis of muscle-specific myosin heavy chain, myosin light chain 2, and α-actin during myogenesis.

R. Schwartz and W. Zimmer, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas: Amplification of the skeletal α-actin gene during myogenesis.

J. C. Perriard,[1] U. B. Rosenberg,[1] P. Gerschwiler,[1] A. Frischauf,[2] H. Lehrach,[2] and H. M. Eppenberger,[1] [1]Institute for Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland; [2]European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany: Isoprotein switches as indicators of gene regulation during the myogenic differentiation.

R. V. Storti,[1] V. Blautch,[1] A. Szwast,[1] D. Mischke,[2] and M.-L. Pardue,[2] [1]Dept. of Biological Chemistry, University of Illinois Medical Center, Chicago; [2]Dept. of Biology, Massachusetts Institute of Technology, Cambridge: Molecular cloning, characterization, and organization of *Drosophila* muscle genes.

M. Ballivet, J. Patrick, J. Lee, and S. Heinemann, Neurobiology Laboratory, Salk Institute, San Diego, California: Progress on cloning of the nicotinic acetylcholine receptor genes.

Session 4: *Gene Structure and Expression III*

Chairperson: C. P. Emerson, Jr., University of Virginia, Charlottesville

K. E. M. Hastings and C. P. Emerson, Jr., Dept. of Biology, University of Virginia, Charlottesville: cDNA clone analysis of mRNA regulation during myogenesis.

D. Feldman and S. Benoff, Dept. of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn: Differential utilization of myosin heavy chain mRNA isoforms during development in skeletal muscle cultures.

P. K. Umeda, C. Kavinsky, A. M. Sinha, H.-J. Hsu, S. Jakovcic, and M. Rabinowitz, Depts. of Medicine and of Biochemistry, University of Chicago, Illinois: Molecular cloning of myosin heavy chain cDNAs from chick embryo skeletal muscle.

J. Karn and A. D. McLachlan, Laboratory of Molecular Biology, Medical Research Council, Cambridge, England: Nucleotide sequence of a myosin heavy chain gene—Genetical and protein structural implications.

D. G. Moerman, S. Bolten, and R. H. Waterston, Dept. of Genetics, Washington University, St. Louis, Missouri: Studies on mutationally altered myosins in *C. elegans*.

A. J. Minty,[1] S. Alonso,[1] M. Caravatti,[2] A. Cohen,[1] P. Daubas,[1] and M. Buckingham,[1] [1]Dept. of Molecular Biology, Pasteur Institute, Paris, France; [2]Freidrich Miescher Institute, Basel, Switzerland: The number and structure of mouse actin genes.

A. S. Havaranis, P. Bragg, and S. M. Heywood, Genetics and Cell Biology Section, University of Connecticut, Storrs: Cytoplasmic utilization of liposome encapsulated myosin heavy chain mRNP during muscle cell differentiation.

Session 5: *Poster Session*

R. E. Allen,[1] K. C. Masak,[2] P. K. McAllister,[2] and G. Robinson,[2] [1]Depts. of Nutrition and Food Science and Animal Sciences; [2]Dept. of Food Science and Human Nutrition, Michigan State University,

East Lansing: Age-related changes in α-actin accumlation in satellite-cell-derived myotubes.

A.S. Baldwin, Jr., E.L.W. Kittler, and C.P. Emerson, Jr., Dept. of Biology, University of Virginia, Charlottesville: A quail fast skeletal muscle troponin I gene is linked to other fast-type genes.

E. Bandman, R. Matsuda, and R.C. Strohman, Dept. of Zoology, University of California, Berkeley: Myosin isozymes during chicken fast muscle development in vivo and in culture.

S.I. Bernstein,[1] A.W. Glenn,[1] C.P. Emerson, Jr.,[1] and J.J. Donady,[2] [1]Dept. of Biology, University of Virginia, Charlottesville; [2]Dept. of Biology, Wesleyan University, Middletown, Connecticut: Isolation, identification, and chromosomal mapping of a *Drosophila* myosin heavy chain gene.

G.S. Butler-Browne,[1] S. Cuénoud,[1] L. Bugaisky,[1] K. Schwartz,[2] and R.G. Whalen,[1] [1]Dept. of Molecular Biology, Pasteur Institute, Paris, France; [2]INSERM, Unit 127, Paris, France: Immunocytochemical studies of transitions of myosin isozymes in development.

W.R. Crain and A.D. Cooper, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Fine structure analysis of two sea urchin actin genes.

M.Y. Fiszman, D. Montarras, and F. Gros, Dept. of Molecular Biology, Pasteur Institute, Paris, France: Characterization of tropomyosin in various types of chick embryo muscles and study of their developmental changes.

G.A. Freyer, T.C. Gilliam, D. Chisolm, and J. Robbins, Dept. of Biochemistry, University of Missouri, Columbia: Isolation of normal chicken myosin heavy chain (MHC) plasmids, dystrophic chicken genomic MHC recombinants, and detection of MHC mRNA during development.

L.I. Garfinkel and B. Nadal-Ginard, Albert Einstein College of Medicine, Bronx, New York: Cloning and characterization of muscle mRNAs.

C.S. Giometti,[1] M.J. Danon,[2] and N.G. Anderson,[1] [1]Division of Biological and Medical Research, Argonne National Laboratory, Illinois; [2]Dept. of Neurology, University of Illinois Medical Center, Chicago: Analysis of human muscle biopsies by two-dimensional electrophoresis.

R.M. Gubits and B. Nadal-Ginard, Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Changes in the chromatin structure of a myosin heavy chain gene during LEG9 myoblast differentiation.

D. Hornig and B. Nadal-Ginard, Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Characterization of adult skeletal muscle myosin heavy chain (MHC) genes of rat.

S.B. Jakowlew,[1] P. Khandekar,[1] K. Datta,[1] A. Ahmed,[1] A.M. Zarraga,[1] H.-H. Arnold,[2] and M.A.Q. Siddiqui,[1] [1]Roche Institute of Molecular Biology, Nutley, New Jersey; [2]University of Hamburg, Federal Republic of Germany: Regulation of expression of cardiac muscle specific genes in early chick embryo.

V. Mahdavi, M. Periasamy, and B. Nadal-Ginard, Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Analysis of the cardiac myosin heavy chain genes in the adult rat.

R. Matsuda, E. Bandman, and R.C. Strohman, Dept. of Zoology, University of California, Berkeley: Myosin isozymes during chicken slow muscle development in vivo and in culture.

P.A. Merrifield and I.R. Konigsberg, Dept. of Biology, University of Virginia, Charlottesville: Myosin synthesis and the cell cycle during myogenesis in vitro.

D. Montarras, M.Y. Fiszman, and F. Gros, Dept. of Molecular Biology, Pasteur Institute, Paris, France: Synthesis of myosin light chains and tropomyosin subunits by quail embryo myoblasts differentiated in vitro.

B.M. Paterson, J.D. Eldridge, Z. Zehner, and M.C. O'Neill, NCI, National Institutes of Health, Bethesda, Maryland: Isolation and preliminary characterization of two different α-actin genes and one β-actin gene in the chicken.

C.E. Rozek and N. Davidson, Dept. of Chemistry, California Institute of Technology, Pasadena: Isolation and preliminary characterization of a myosin heavy chain gene from *Drosophila melanogaster*.

S. Sarkar, A.K. Mukherjee, S.R. Chandrika, and R.K. Roy, Dept. of Muscle Research, Boston Biomedical Research Institute, and Dept. of Neurology, Harvard Medical School, Boston, Massachusetts: Studies on regulatory processes of chick muscle development—A translation inhibitory 10S RNP; abundant class muscle-specific mRNA sequences; and isozymic changes of myosin light chains and tropomyosin subunits.

G. Silver and J.D. Etlinger, Dept. of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn: Calcium-dependent control of myosin light chain synthesis and accumulation in skeletal muscle cultures.

D.W. Winkelmann and S. Lowey, Rosenstiel Center, Brandeis University, Waltham, Massachusetts: Immunoquantitation of an adult myosin during chicken pectoralis muscle development.

R. Wydro, H.T. Nguyen, R. Gubits, and B. Nadal-Ginard, Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Myosin heavy chain is coded by a multigene family with highly conserved domains.

Z.E. Zehner, M.C. O'Neill, and B.M. Paterson, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of the chick cytoskeleton gene, vimentin.

Session 6: *Membrane Events*

Chairperson: S. J. Kaufman, University of Illinois, Urbana

J. Prives, L. Hoffman, T. Ross, and N. Serafin, Dept. of Anatomical Sciences, State University of New York, Stony Brook: Interaction of cell surface proteins with the cytoskeletal framework of embryonic muscle cells in culture.

S. J. Kaufman and D. M. Ehrbar, Dept. of Microbiology and School of Basic Medical Sciences, University of Illinois, Urbana: Association of myoblast cell surface determinants with submembranous components.

D. M. Fambrough,[1] A. G. Engel,[2] and T. L. Rosenberry,[3] [1]Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland; [2]Dept. of Neurology, Mayo Clinic, Rochester, Minnesota; [3]Dept. of Pharmacology, Case Western Reserve University, Cleveland, Ohio: Neuromuscular acetylcholinesterase explored with monoclonal antibodies.

J. M. Gardner and D. Fambrough, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: The biosynthesis and expression of fibronectin during myogenesis in vitro.

A. F. Horwitz, N. Neff, A. Sessions, C. Lowrey, C. Smalley, and A. Tovar, Dept. of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia: Membrane events in myoblast fusion.

W. J. Strittmatter,[1,2,3] C. E. Bright,[3] and S. B. Elias,[1,3] Dept. of [1]Neurology, [2]Biochemistry and [3]Program in Neuroscience, Baylor College of Medicine, Houston, Texas: Rat myoblast fusion requires catalytically specific proteases.

S. M. Fernandez and B. A. Herman, Dept. of Physiology, University of Connecticut Health Center, Farmington: Membrane dynamics and lectin cell surface topography during myoblast fusion.

R. J. Przybylski[1] and R. G. MacBride,[1,2] [1]Developmental Biology Center, Case-Western Reserve University, Cleveland, Ohio; [2]Dept. of Anatomy, Oral Roberts University, Tulsa, Oklahoma: The effect of lactose-lectin treatment of prefusion skeletal muscle cultures on cell behavior and myotube formation.

Session 7: *Committment and Embryogenesis*

Chairperson: F. E. Stockdale, Stanford University, California

F. E. Stockdale, M. Crow, P. Olson, G. Schwartz, and T. Hunt, Depts. of Medicine and Biology, Stanford University, California: The developmental lineage of fast and slow muscles.

P. H. Bonner and T. H. Morgan, School of Biological Sciences, University of Kentucky, Lexington: Neural induction of chick skeletal myoblast differentiation.

I. R. Konigsberg, Dept. of Biology, University of Virginia, Charlottesville: Differentiating quail myocytes are in a true G_0 state.

S. Hauschka, R. Rutz, and C. Haney, Dept. of Biochemistry, University of Washington, Seattle: Regional distribution and cell lineage states of myogenic cells during early stages of vertebrate limb development.

T. Linkhart, C. Clegg, R. Lim, G. Merrill, J. Chamberlain, and S. Hauschka, Dept. of Biochemistry, University of Washington, Seattle: Control of mouse myoblast commitment to terminal differentiation by mitogens.

H. Holtzer, P. Antin, S. Forrey-Schaudies, T. Friedman, and S. Tapscott, Dept. of Anatomy, University of Pennsylvania School of Medicine, Philadelphia: Taxol-treated postmitotic myoblasts form interdigitating microtubule-myosin arrays lacking actin filaments.

Session 8: *Poster Session*

E. K. Bayne and D. M. Fambrough, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: An extracellular matrix-plasma membrane interaction in skeletal muscle.

W. V. Bleisch and V. N. Luine, Rockefeller University, New York, New York: Effect of testosterone on growth, cholinergic enzymes, and receptors in an androgen sensitive muscle.

B. W. Cherney and A. I. Caplan, Developmental Biology Center, Case Western Reserve University, Cleveland, Ohio: Measurement of poly(ADP-ribose) synthetase activity during muscle development.

G. A. Cates and P. C. Holland, Dept. of Biochemistry, University of Saskatchewan, Saskatoon, and Montreal Neurological Institute, Canada: Immunochemical characterization of myoblast cell surface proteins.

W. C. Claycomb, Dept. of Biochemistry, Louisiana State University School of Medicine, New Orleans: The terminally differentiated adult cardiac muscle cell in culture.

C.H. Clegg and S.D. Hauschka, Dept. of Biochemistry, University of Washington, Seattle: Heterokaryon analysis of myoblast commitment to terminal differentiation.

R. Ehrismann,[1,2] D.E. Roth,[2] H.M. Eppenberger,[1] and D.C. Turner,[1,2] [1]Institute of Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland; [2]Dept. of Biochemistry, Upstate Medical Center, State University of New York, Syracuse: Fibronectin structure and myoblast attachment.

B.M. Gilfix and B.D. Sanwal, Dept. of Biochemistry, University of Western Ontario, London, Canada: Lectin-resistant myoblasts.

J.S. Gordon,[1] E. Winter,[2] D. Levy,[2] and C. Tuminello,[1] [1]Dept. of Anatomical Sciences and [2]Cellular and Developmental Biology Program, State University of New York, Stony Brook: Chromosomal protein synthesis and the regulation of myogenesis.

S.J. Kaufman and D.M. Ehrbar, Dept. of Microbiology and School of Basic Medical Sciences, University of Illinois, Urbana: Association of myoblast cell surface determinants with submembranous components.

S.F. Konieczny,[1] J. McKay,[2] and J.R. Coleman,[2] [1]Biomedical Division and [2]Division of Biology and Medicine, Brown University, Providence, Rhode Island: Isolation and characterization of terminally differentiated chicken and rat skeletal muscle myoblasts.

J.B. Lawrence,[1] S.F. Konieczny,[2] and J.R. Coleman,[1] [1]Division of Biology and Medicine and [2]Biomedical Division, Brown University, Providence, Rhode Island: Extinction of myogenic properties in somatic cell heterokaryons.

D.C. Linden[1] and M.S. Letinsky,[2] [1]Dept. of Physiology, University of California School of Medicine; [2]Jerry Lewis Neuromuscular Research Center, Los Angeles, California: Development of the cutaneous pectoris muscle in *Rana catesbeiana*—A light and electron microscopic study.

G.E. Morris, School of Biological Sciences, University of Sussex, Brighton, England: Myoblast cell culture studies of possible calcium and cyclic nucleotide involvement in physiological modulation of muscle gene expression.

H.T. Nguyen and B. Nadal-Ginard, Dept. of Cell Biology, Albert Einstein College of Medicine, Bronx, New York: Isolation and characterization of a mutant myoblast cell line temperature sensitive for commitment.

C.A. Quinn and F.S. Walsh, Institute of Neurology, London, England: Characteristics of a myogenic rat x human muscle cell hybrid.

L.S. Quinn and M. Nameroff, Dept. of Biological Structure, University of Washington, Seattle: Quantitative analysis of clonal myogenesis in the chick embryo.

L.S. Quinn and M. Nameroff, Dept. of Biological Structure, University of Washington, Seattle: Bromodeoxyuridine uncouples myosin synthesis from creatine phosphokinase synthesis at the terminal division in the myogenic lineage.

P.G. Pauw[1] and J.D. David,[2] [1]Dept. of Genetics, Iowa State University, Ames; [2]Division of Biological Sciences, University of Missouri, Columbia: Accelerated degradation of low molecular weight surface proteins during fusion in L_6.

L.L. Richer and R.J. Schmaltz, United States Meat Animal Research Center, USDA, Clay Center, Nebraska: Serum-borne factors affecting cell proliferation in serum-free, primary cultures of fetal pig myoblasts.

S. Scarpa,[1] B.W. Uhlendorf,[2] J. Chen,[2] and G. Cantoni,[2] [1]Istituto di Biochimica Applicata, Universita di Roma, Istituto Superiore di Sanità, Rome, Italy; [2]NIMH, National Institutes of Health, Bethesda, Maryland: The effect of concanavalin A and other lectins on myoblast line L5 differentiation.

E. Siegel, B. Mroczkowski, T. McCarthy, and S. Heywood, Dept. of Genetics and Cell Biology, University of Connecticut, Storrs: Characterization of translational control RNA isolated from muscle mRNP particles.

F.E. Stockdale, B.K. Grove, G. Schwartz, and M. Yaross, Depts. of Medicine and Biology, Stanford University, California: Quantitative characterization of cell surface determinants on myogenic cells during differentiation.

D.C. Turner,[1,2] M. Chiquet,[1] J. Lawton,[2] P. Dollenmeier,[1] and R. Ehrismann,[1,2] [1]Institute of Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland; [2]Dept. of Biochemistry, Upstate Medical Center, State University of New York, Syracuse: Contact guidance of myogenic cells by fibrils of fibronectin.

H. Vandenburgh, Dept. of Pathology and Laboratory of Medicine, Miriam Hospital and Brown University, Providence, Rhode Island: Orientation of developing myotubes by a moving substratum.

M.J.O. Wakelam and D. Pette, Faculty of Biology, University of Konstanz, Federal Republic of Germany: Changes in the metabolism of inositol-phospholipids during myoblast fusion.

W.E. Wright, Dept. of Cell Biology, University of Texas Health Science Center, Dallas: Evidence for a DNA-based mechanism for the inhibition of myogenesis by 5′-bromodeoxyuridine.

R.J. Zalin, Dept. of Anatomy, University College, London, England: Factors affecting the response of chick myoblasts to their developmental signal (prostaglandin E_1).

B. Zani, G. Cossu, S. Adamo, M. Pacifici, and M. Molinaro, Institute of Histology and General

Embryology, University of Rome, Italy: Requirement of protein synthesis for the inhibition of muscle-specific phenotype by tumor promoters in culture.

Session 9: Morphogenesis of the Cytoskeleton and the Contractile Apparatus

Chairperson: D. Fischman, Down State Medical Center, State University of New York, Brooklyn

D. A. Fischman, Dept. of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn: Myofibrillar assembly—Current paradoxes to be resolved.

T. Masaki, D. Bader, T. Obinata, and D. A. Fischman, Dept. of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn: Immunological analysis of myosin isoforms during chick myogenesis.

H. F. Epstein, J. M. MacKenzie, Jr., S. A. Berman, and D. M. Miller III, Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Myosin synthesis and assembly in normal and mutant nematode body-wall muscle.

E. Lazarides,[1] B. L. Granger,[1] D. L. Gard,[1] J. Breckler,[1] S. I. Danto,[2] and D. A. Fischman,[2] [1]Division of Biology, California Institute of Technology, Pasadena; [2]Dept. of Anatomy and Cell Biology, Downstate Medical Center, State University of New York, Brooklyn: Steps in the assembly of the Z-disc in muscle cells.

K. Wang, Clayton Foundation Biochemical Institute, University of Texas, Austin: Myofibrillar connections—The role of titin, nebulin, and intermediate filaments.

H. M. Eppenberger, T. Doetschman, J. C. Perriard, E. Strehler, D. Studer, and T. Wallimann, Institute of Cell Biology, Swiss Federal Institute of Technology, Zurich, Switzerland: Myomesin in cross-striated muscle cells.

Session 10: Neuromuscular Junction Formation

Chairperson: Z. W. Hall, University of California Medical School, San Francisco

L. Silberstein and Z. W. Hall, Dept. of Physiology, University of California Medical School, San Francisco: Association of acetylcholine receptor clusters with extracellular matrix in myotube cultures.

M. W. Cohen, F. Moody-Corbett, and P. R. Weldon, Dept. of Physiology, McGill University, Montreal, Canada: Influence of nerve on the formation and survival of sites of acetylcholine receptor and cholinesterase localization on muscle cells in culture.

M. M. Salpeter and T. R. Podleski, Section of Neurobiology and Behavior, Cornell University, Ithaca, New York: ACh receptor on primary rat muscle cells redistribute to reach junctional site densities after exposure to soluble CNS extracts.

U. J. McMahan, Dept. of Neurobiology, Stanford University School of Medicine, California: The role of extracellular matrix in regeneration of the neuromuscular junction.

L. Rubin, Rockefeller University, New York: Basement lamina components affecting acetylcholine receptor localization in cultured chick myotubes.

Session 11: Poster Session

D. S. Dhillon and A. L. Harvey, Dept. of Physiology and Pharmacology, University of Strathclyde Glasgow, Scotland: Comparison of the development of myotubes cultured from thymus and skeletal muscle tissue of neonatal rats.

G. K. Dhoot[1] and S. V. Perry,[2] Dept. of [1]Immunology and [2]Biochemistry, University of Birmingham, England: Role of nerve activity on the development of muscle cell phenotypes.

M.S. Ecob, Muscular Dystrophy Research Laboratories, University of Newcastle-upon-Tyne, England: Nerve-muscle interaction studied in an organotypic culture system.

H.F. Elson, Dept. of Pathology, University of California, San Diego: An extracellular matrix defect in dystrophic human skeletal muscle cell cultures.

M.R. Emmerling and R.L. Rotundo, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Maryland: Synthesis and cellular distribution of acetylcholinesterase molecular forms in quail muscle cell cultures.

Y. Kidokoro, P. Brehm, and R. Gruener, Salk Institute, San Diego, California: Developmental changes in acetylocholine receptor density and channel properties in *Xenopus* nerve-muscle culture.

J.B. Parent,[1] J. Baumgold,[2] and I. Spector,[3] [1]Howard University Cancer Center, Washington, DC; [2]NIMH; [3]Laboratory of Biochemical Genetics, National Institutes of Health, Bethesda, Maryland: Development of the sodium channel during muscle differentiation in tissue culture.

R. Sebbane and J.P. Merlie, Dept. of Biological Sciences, University of Pittsburgh, Pennsylvania: Studies of the biogenesis of the skeletal muscle ACh receptor.

G.R. Slater, Muscular Dystrophy Research Laboratories, University of Newcastle-upon-Tyne, England: Post-natal maturation of acetylcholine receptor distribution at mouse nerve-muscle junctions.

M.A. Smith[1] and C.R. Slater,[2] [1]Dept. of Biology, University of California, San Diego; [2]Muscular Dystrophy Research Laboratories, Newcastle General Hospital, Newcastle-upon-Tyne, England: Acetylcholine receptors at developing chick neuromuscular junctions.

B.A. Wolitzky, H.L. Segal, and M.S. Hudecki, Division of Cell and Molecular Biology, State University of New York, Buffalo: Rates of protein synthesis and degradation in normal and dystrophic primary muscle cultures.

R. Yasin, F.S. Walsh, D.N. Landon, and E.J. Thompson, Institute of Neurology, University of London, England: Cellular, biochemical, and immunological characterization of two human myogenic cell clones.

L. Ziskind-Conhaim, Dept. of Physiology, University of California Medical School, San Francisco: Effects of denervation and electrical inactivity on the formation of acetylcholine receptor clusters in embryonic rat muscles.

Session 12: *Muscle Development and Human Disease*

Chairperson: S.H. Appel, Baylor College of Medicine, Houston, Texas

S.H. Appel, R.G. Smith, K. Vaca, and K. Ojika, Dept. of Neurology, Baylor College of Medicine, Houston, Texas: Motor neuron survival, neurite extension, and acetylcholine (ACH) synthesis are enhanced by muscle-derived proteins.

H.M. Blau and C. Webster, Dept. of Pharmacology, Stanford University School of Medicine, California: Isolation and characterization of pure populations of human normal and dystrophic muscle cells.

S. Fuchs, Dept. of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel: An autoimmune response to acetylcholine receptor in myasthenia gravis.

A.F. Miranda, S. Shanske, and S. DiMauro, H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related Disorders, Columbia University, New York, New York: Isoenzyme transitions in human muscle development.

F.S. Walsh and S. Dhut, Institute of Neurology, London, England: Surface antigens phenotypes of human muscle cells.

This meeting was supported in part by funds from the National Science Foundation, the Frederick Cancer Research Center, and the Muscular Dystrophy Association.

BANBURY CENTER 1981 Activities

Banbury Center's fourth year of small conferences on environmental health risks, with the addition of small meetings focused on emerging areas of molecular biology and on the effects of molecular biological advances on society at large, took place as the national government rapidly changed its policies toward environmental regulation in particular and the support of science in general. The new tax-cutting Reagan administration began at once to ease restrictions on industry that had multiplied over a decade of mounting concern about the impact of advanced technology, particularly in the areas of mining and chemicals, on the health of workers and the general population. The changed political climate created problems for Banbury Center in achieving its goal of a reasonable balance of views in its conferences. Previously, the need was to assure adequate representation of scientific skepticism about the size of the environmental health risk problem. Now, the problem was assuring that what might be called an alarmist view would be heard, while continuing to concentrate on facts rather than opinions or ethical issues.

Such pressures merely forced us to redouble efforts to assure the objectivity of the spring conference on "Quantification of Occupational Cancer," whose proceedings came out in December as Banbury Report 9, and of our fall conference on "Environmental Effects on Maturation." The proceedings of the latter meeting, which considered possible special vulnerabilities of children to environmental chemicals, will be published in the spring of 1982 as Banbury Report 11.

The success of these meetings, and the expansion of the Banbury program to include such conferences on molecular biology as those on "Gene Amplification and Aberrant Chromosomal Structures" (October) and "Construction and Use of Mammalian Viral Vectors" (December), and on broader impacts of fundamental biology ("Patenting of Life Forms," October), reinforced confidence in the original view of the beautiful Robertson estate as an ideal place for meetings on urgent technical issues of biological science and public policy.

The establishment of the center, after the late Charles S. Robertson's magnificent gifts to the Laboratory in 1973 and 1976, was dictated by an increasing awareness of the social importance and usefulness of molecular biology. Recombinant DNA techniques had been applied in an important way to short-term testing of possible dangers from chemicals in the environment. The techniques themselves were the subject of years of intense, and now largely resolved, debate about the possibility of health risks. Meanwhile, scientists became increasingly conscious of the power of recombinant DNA techniques for understanding mechanisms of cancer induction as well as normal cellular development and of the rapidly expanding opportunities for using the new techniques in industrial microbiology. All of these subjects are of intense interest to the scientists working at Cold Spring Harbor Laboratory.

Sammis Hall

The sense of fulfillment of earlier hopes sharpened feelings of loss at the death in Florida, on May 2, 1981, of Charles Robertson, who had maintained a lively interest in the new uses of what had been his family home for 40 years. A few days after his death, members of the Banbury Center and Laboratory staff considered it an honor to attend a beautiful service in memory of Mr. Robertson at St. John's Episcopal Church in Cold Spring Harbor.

Not long after, on July 19, a muggy, overcast Sunday, a landmark in the development of Banbury Center was passed with the dedication of our 16-bedroom guest house, Sammis Hall. The Palladian-style structure, designed in 1978-1979 by the architectural firm of Moore, Grover, and Harper, was named for Mr. Robertson's mother's family. Accompanied by occasional showers, the ceremonies were held under a yellow-and-white-striped tent on the lawn next to the deck of the Banbury Meeting House. Among these attending were Mr. Robertson's sister, Mrs. Donald Rose, who has her summer home here, and such other members of the family as Mr. and Mrs. William Robertson and Dr. and Mrs. Walter Meier, who also are neighbors.

Built with the aid of grants of $150,000 from the Kresge Foundation and $225,000 from the Max Fleischmann Foundation, Sammis Hall pleasingly supplements the family comfort of Robertson House. Now, a total of 37 overnight guests can be accommodated on the Banbury estate, with our extra guests (already a usual feature of Banbury conferences) housed in Blackford Hall at the main Laboratory grounds across Cold Spring Harbor.

Excavation for Sammis Hall began in the fall of 1979, and the structure began rising in March 1980. A year later its first guests participated in the conference on "Quantification of Occupational Cancer." By the end of 1981, no less than 20 groups of conference and course participants had stayed in Sammis Hall for periods of from two days to three weeks, enjoying its charming views of surrounding trees and a unique central hall, stretching more than two stories up to a dozen skylights.

Dissemination of Findings

Publication of conference proceedings has been an integral part of the Banbury program from the beginning. The small size of the Banbury conference room necessarily limits attendance to between 40 and 50 people, most of them active researchers. There is little room for observers, such as members of the press, at our technical conferences. Thus, maximizing the usefulness of bringing together such groups of researchers, who do not meet together often, demands swift publication. Hence, Banbury Center strives to publish a Banbury Report six or seven months after each conference.

Our first Banbury Report was published in April 1979. Another followed by the end of that year and four more were published in 1980. In 1981 we published another three. These were Banbury Report 7 on *Endogenous Factors in Gastrointestinal Cancer,* Banbury Report 8 on *Hormones and Breast Cancer,* and Banbury Report 9 on *Quantification of Occupational Cancer.* As always, we were grateful for the splendid cooperation of the scientists, organizers, and participants, who observed our tight deadlines. Preparations began for publication of Banbury Reports 10 and 11, covering our meetings on patenting and environmental risks for children.

With the aid of a $25,000 purchase of copies of Banbury Reports 7 and 8 by the National Cancer Institute for distribution to grantees, total sales of Banbury Reports exceeded $140,000 for the year and $350,000 since the start of the publication program. The sales met most of the costs of book production and distribution.

Our second major informational program involves seminars specifically designed for groups, such as journalists and legislative aides, who have a major role in setting policy. Supported by a $100,000 grant from the Alfred P. Sloan Foundation (which, along with the Esther A. and Joseph Klingenstein Fund financed the initial operations of Banbury Center), we began holding seminars for journalists in 1981.

The first of these, for the editorial staff of *Newsday,* the Long Island newspaper, covered such environmental health issues as ground water pollution, diet and cancer, and chemical waste disposal. The second, for the editorial staffs of the magazines of Time, Inc., covered the scientific background of current advances in genetic engineering and reviewed the initial steps in the creation of a genetic engineering industry. The aim of both seminars was to bring scientists together with editorial staffs at all levels to hear

Sammis Hall, rear view

informal presentations and ask many questions, so as to open the door to future contacts on specific news stories and allow managers of news-gathering efforts to confront sources usually seen only by reporters. We were gratified that the publisher and the president of *Newsday*, Mr. David Laventhol and Mr. Donald Wright, and Mr. Henry Anatole Grunwald, Editor-in-Chief of Time, inc., were able to attend.

Conferences and Courses

The variety of meetings held at Banbury increased during 1981. Besides two informational seminars, two environmental health risk conferences, two conferences on emerging areas of molecular biology, and a conference on the social impact of molecular biology, Banbury also was the site of two conferences on neurobiology (as in 1980), two courses on neurobiology, and a workshop on tumor and transplantation antigens and provided housing for some of the participants in nine major conferences held at Cold Spring Harbor Laboratory.

The conference on "Neurobiology of the Leech," from June 29 to July 2, was organized by John Nicholls of Stanford University, Kenneth Muller of the Carnegie Institution of Washington, and Gunther Stent of the University of California at Berkeley (who also spoke at the dedication of Sammis Hall). The conference on "Methods for Measuring Global Neural Activity," from August 2 to 8, was organized by David Zipser of Cold Spring Harbor Laboratory and Peter Hand of the University of Pennsylvania School of Veterinary Medicine under a Sloan Foundation grant.

The workshop on "Tumor and Transplantation Antigens," from July 2 to 8, was organized (for the second year in a row) by Arnold Levine of the State University of New York at Stony Brook, who completed a term as an Institutional Trustee of the Laboratory.

The conference on "Gene Amplification and Aberrant Chromosomal Structures," supported by the National Institutes of Health and by a special fund established by Bankers Life of Chicago, was organized by Robert T. Schimke of Stanford University. It was held from October 4 to 7.

Organizing our second annual conference on "Construction and Use of Mammalian Viral Vectors," held from December 4 to 6, was Yakov Gluzman of the scientific staff of Cold Spring Harbor Laboratory.

Representing divergent views on the problem of quantification of occupational cancer, the organizers of the conference, held from March 29 to April 2, were Richard Peto of the University of Oxford, who doubts that cancer mortality due to environmental chemicals is rising among middle-aged persons where trends can be most accurately measured, and Marvin Schneiderman of Clement Associates, who has analyzed studies indicating a rising trend of cancer incidence.

Although a regulatory problem underlay the interest of the United States Environmental Protection Agency and the U.S. Department of Labor in a Banbury conference on special environmental health risks for children, the conference focused on scientific work related to immature humans and animals in general. The conference, entitled "Environmental Effects on Maturation," was held from November 1 to 4 and was organized by Vilma Hunt of Pennsylvania State University, Kate Smith of EPA's Health Effects Research Laboratory in Cincinnati, and Dorothy Worth of Tufts University School of Medicine. The regulatory problem in the background is estimating the degree of risk for farm children entering fields that have been sprayed with pesticides.

Banbury staffers Lynda Moran (left) *and Beatrice Toliver* (right) *with Banbury Center Director, Victor McElheny*

The conference on "Patenting of Life Forms," held from October 18 to 21, grew out of the considerable confusion among both patent lawyers and scientists about the implications of the Supreme Court's 1980 decision that life forms themselves are patentable along with processes for creating them or using them. The organizers were Norton Zinder of Rockefeller University, an Institutional Trustee of Cold Spring Harbor Laboratory, Niels Reimers, Director of Technology Licensing of Stanford University, and David W. Plant, partner in the New York City patent law firm of Fish & Neave.

Courses and conferences at Banbury Center brought some 400 scientists, lawyers, and journalists to Banbury and Cold Spring Harbor Laboratory. A further 300 scientists were guests at Banbury during Laboratory conferences. Our guests came from the United States and 18 foreign countries.

Support

A wholly new enterprise like Banbury Center has to struggle for financing. During 1981 financial support strengthened notably.

The Environmental Protection Agency and Department of Labor joined in a cooperative agreement to support the conference on "Environmental Effects on Maturation." The National Institutes of Health and Bankers Life, following up their support of the International Symposium on Aging and Cancer in Washington, D.C., in September, 1980, sponsored the October conference on "Gene Amplification and Aberrant Chromosomal Structures."

Very gratifying was notification of the award by the National Cancer Institute of funds to hold a conference on the "Possible Role of Nitrosamines in Human Cancer," April 4 to 7, 1982. The Kaiser Family Foundation of Menlo Park, California, made a very generous grant to support the holding of the Banbury conference on "Prospects for Gene Therapy: Fact and Fiction," February 5 to 7, 1982, and publication of its findings. The Burroughs Wellcome Fund granted $15,000 for a conference on hereditary factors in cancer, which will focus on probing for specific genes, to be held in October 1982.

Support from corporations, which had reached $85,000 in 1980 (our first year of seeking such assistance), virtually doubled to $169,500 in 1981. Of great help were letters of appeal from Alexander C. Tomlinson of our Board of Trustees which accompanied the Banbury proposal. Sustaining support in 1981 came from ten corporate sources: Bristol-Myers Fund, Dow Chemical, E.I. duPont deNemours, Exxon Education Foundation, Getty Oil, International Business Machines, Eli Lilly, New York Life, Phillips Chemical, and Texaco Philanthropic Foundation. Just after the turn of the year, Conoco joined the ranks with a contribution to the overall program.

A total of 15 companies, including Dow, duPont, and Lilly, contributed a total of $43,000 toward the October conference on "Patenting of Life Forms." The other contributors were: Baxter Travenol Laboratories, Chevron Research Company, Exxon Research and Engineering Company, Hoffman LaRoche, Inc., Johnson and Johnson, Merck Sharp and Dohme Research Laboratories, Monsanto Company, National Distillers and Chemical Corporation, Pfizer, Inc., Schering-Plough Corporation, Smith Kline and French Laboratories, and The Upjohn Company.

For the December conference on "Construction and Use of Mammalian Viral Vectors," two companies, Applied Molecular Genetics, Inc., and Bethesda Research Laboratories, joined 10 others, including duPont, Lilly, and Monsanto, which had contributed to the first mammalian vectors meeting in 1980. The other repeating contributors were Abbott Laboratories, Biogen N.V., Cetus Corporation, Collaborative Research, Inc., Genentech, Inc., Molecular Genetics, Inc., and New England BioLabs, Inc.

The roster of corporations contributing at least once to Banbury Center in the last two years has reached 32. Many of these companies have given generously to Cold Spring Harbor Laboratory over the last decade. It is a pleasure to record our gratitude to new and old contributors.

Journalists Workshop for Newsday: Environmental Health Risks

January 16–18

Session 1 *Water Resources and Human Health*

C.C. Johnson, C.C. Johnson and Associates, Silver Spring, Maryland: The numerous unknowns in making policy to assure water quality.

J. Cotruvo, Environmental Protection Agency, Washington, DC: The Environmental Protection Agency's priorities in assuring water quality.

M. Kavanaugh, Montgomery Engineers, Washington, DC: Engineering water treatment with a view to reuse.

J. Tripp, Environmental Defense Fund, New York, New York: Legal struggles over watershed protection in the Northeast.

Session 2 *The Health Effects of Chemical Waste Disposal*

J. Highland, Environmental Defense Fund, Washington, DC: Dimensions of the problem.

R. Albert, New York University Medical Center, New York: Difficulties of measurement.

Session 3 *Diet and Cancer*

C. Mettlin, Roswell Park Memorial Institute, Buffalo, New York

P. Newberne, Massachusetts Institute of Technology, Cambridge

Quantification of Occupational Cancer, March 29–April 2

Varying viewpoints concerning the extent of the contribution of occupation to overall cancer incidence were presented against a background of epidemiological issues, study methods, statistical approaches, and cancer and chemical production trends. Precise evaluations were made about the role of asbestos exposure in lung cancer and mesothelioma incidence as well as radiation and lung cancer incidence, and the confounding factors of cigarette smoking and the healthy worker effect were considered. Industry-sponsored surveillance programs were discussed, as were classification systems, record linkage, exposure-based case-control studies, population-based large cohorts, and the use of standardized mortality ratios vs. proportional mortality ratios. A concluding roundtable explored the prospect of identifying high-risk groups and reducing cancer in industrial countries.

Session 1 *Asbestos and Other Mineral Fibers*

Chairperson: E.D. Acheson, University of Southampton, England

I.J. Selikoff, Mount Sinai School of Medicine, New York, New York: Constraints in estimating occupational contributions to current cancer mortality in the United States.

P.E. Enterline, University of Pittsburgh Graduate School of Public Health, Pennsylvania: Proportion of cancer due to exposure to asbestos.

W.J. Blot, National Cancer Institute, NIH, Bethesda, Maryland: Cancer among shipyard workers.

J. Peto, Imperial Cancer Research Fund, Oxford, England: Trends in mesothelioma incidence in the United States and the forecast epidemic due to asbestos exposure during World War II.

J.C. McDonald, London School of Hygiene and Tropical Medicine, England: Mesothelioma as an index of asbestos impact.

W.J. Nicholson, Mount Sinai School of Medicine, New York, New York: Cancer from occupational asbestos exposure—Projections 1980–2000.

Session 2 *Radiation Risks—Animal Experiments*

Chairperson: M. Schneiderman, Clement Associates, Inc., Washington, DC

S. Darby, National Radiological Protection Board, Harwell, England: Exposure to ionizing radiation and cancer mortality among workers.

A.M. Stewart and George W. Kneale, University of Birmingham, England: Analysis of Hanford data—Delayed effects of small doses of radiation delivered at slow-dose rates.

E.P. Radford, University of Pittsburgh Graduate School of Public Health, Pennsylvania: Radon daughters in the induction of lung cancer in underground miners.

R. Saracci, International Agency for Research on Cancer, Lyon, France: The IARC monograph program on the evaluation of the carcinogenic risk of occupational carcinogens.

G.M. Paddle, ICI Central Medical Group, Wilmslow, Cheshire, England: A strategy for the identification of carcinogens in a large, complex chemical company.

Session 3 *Industry-wide Cancer Experience—Methodological Problems*

Chairperson: M.A. Silverstein, International Union, United Auto Workers, Detroit, Michigan

M.S. Gottlieb, Tulane University School of Medicine, New Orleans, Louisiana: Mortality studies on lung, pancreas, esophageal, and other cancers in Louisiana.

R.R. Monson, Harvard University School of Public Health, Boston, Massachusetts: An estimate of the percentage of occupational cancer among a group of rubber workers.

M. Karstadt, Mount Sinai School of Medicine, New York, New York: A survey of availability of epidemiologic data on humans exposed to animal carcinogens.

M.E. Warshauer, Memorial Sloan-Kettering Cancer Center, New York, New York: A prospective study of morbidity and mortality in petroleum industry employees in the United States—A preliminary report.

Session 4 *Cancer in the US—Recent Trends and Proportion Due to Occupation*

Chairperson: M.H. Sloan, National Cancer Institute, NIH, Silver Spring, Maryland

R. Peto, University of Oxford, England: Trends in US cancer onset rates.

D.L. Davis, Environmental Law Institute, Washington, DC: Estimating cancer causes—Problems in methodology, production, and trends.

H.M. Rosenberg, National Center for Health Statistics, Hyattsville, Maryland: NCHS data resources for studying occupational cancer mortality.

Session 5 *Special Problems of Methodology*

Chairperson: B.W. Karrh, E.I. duPont deNemours & Company, Wilmington, Delaware

M.S. Legator, University of Texas Medical Branch, Houston: A holistic approach to monitoring high-risk populations by short-term procedures.

S.G. Austin, Union Carbide Corporation, New York, New York: An industry-sponsored mortality surveillance program.

O. Wong, Biometric Research Institute, Inc., Washington, DC: An epidemiologic study of workers potentially exposed to brominated chemicals—With a discussion of multifactor adjustment.

R.J. Waxweiler, National Institute for Occupational Safety and Health, Cincinnati, Ohio: Quantification of differences between proportionate mortality ratios and standardized mortality ratios.

J.J. Beaumont, National Institute for Occupational Safety and Health, Cincinnati, Ohio: Occupational data sets appropriate for proportionate mortality ratio analysis.

C.P. Wen, Gulf Oil Corporation, Houston, Texas: A population-based cohort study of brain tumor mortality among oil refinery workers with a discussion of methodological issues of SMR and PMR.

Session 6 *Methodology*

Chairperson: E. Bingham, University of Cincinnati Medical Center, Ohio

F.D. Hoerger, Dow Chemical Company, Midland, Michigan: Indicators of exposure trends.

S.K. Hoar, National Cancer Institute, NIH, Bethesda, Maryland: Epidemiology and occupational classification systems.

J. Siemiatycki, Institut Armand-Frappier, Laval-des-Rapides, Quebec, Canada: Exposure-based case control approach to discovering occupational carcinogens—Preliminary findings.

O. Axelson, Linkoping University, Sweden: Guiding experiences on the etiology of acute myeloid leukemia.

S.W. Samuels, Industrial Union Department, AFL–CIO, Washington, DC: The international context of carcinogen regulation—Benzidine.

S. Milham, Jr., Washington State Department of Social and Health Services, Olympia: Proportion of cancer due to occupation in Washington State.

H. Tulinius, Icelandic Cancer Registry, Reykjavik: Cancer incidence and occupations in an area of low air pollution.

H. Falk, Center for Disease Control, Atlanta, Georgia: Hepatic angiosarcoma registries—Implications for rare-tumor studies.

Session 7 *Broad Approaches to Occupational Cancer Quantification*

Chairperson: J. Cairns, Harvard School of Public Health, Boston, Massachusetts

J.B. Swartz and S. Epstein, University of Illinois Medical Center, Chicago: Problems in assessing risk from occupational and environmental exposure to carcinogens.

A.J. McMichael, Commonwealth Scientific and Industrial Research Organization, Adelaide, SA, Australia: Differentiating work from life-style in cancer causation.

E.D. Acheson, University of Southampton, England: Towards a strategy for the identification of occupational carcinogens in England and Wales—A preliminary report.

M.R. Alderson, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surrey, England: Occupational studies—The use of national and industrial comparisons or an internal analysis.

H.B. Demopoulos, New York University Medical Center, New York: The value of contemporary demographic controls in evaluating cancer incidence and mortality rates in heavily industrialized urban areas in the United States.

T. Hirayama, National Cancer Research Institute, Tokyo, Japan: Proportion of cancer attributable to occupation obtained from a census, population-based, large cohort study in Japan.

Session 8 *Future Needs*

Chairperson: G.W. Beebe, National Cancer Institute, NIH, Bethesda, Maryland

G.W. Beebe, National Cancer Institute, NIH, Bethesda, Maryland: Record linkage and needed improvements in existing data resources.

M.E. Smith, Health Division, Statistics Canada, Ottawa: Long-term medical follow-up in Canada.

Journalists Workshop for Time, Inc.: DNA

May 3-4

Session 1 *Scientific Successes with Recombinant DNA*

J.D. Watson, Cold Spring Harbor Laboratory, New York: Comments on the political and technical history of recombinant DNA.
R. Roberts, Cold Spring Harbor Laboratory, New York: General introduction to the scientific techniques of working with DNA.
P. Leder, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland: Jumping genes.
M. Ptashne, Harvard University, Cambridge, Massachusetts: Bacteria as factories.

Session 2 *Consequences of Damage to DNA*

B.N. Ames, University of California, Berkeley: Mutagenesis.
M.S. Fox, Massachusetts Institute of Technology, Cambridge: DNA repair.
J.L. German III, New York Blood Center, New York: Chromsome damage.
W.S. Hayward, Rockefeller University, New York, New York: Viruses.

Session 3 *Long Shots in Applications of DNA Technology*

J. Schell, University of Cologne, Germany: Plants.
R. Jaenisch, Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany: The introduction of DNA into the germ lines of mice.
M. Wigler, Cold Spring Harbor Laboratory, New York: Cancer genes.

Session 4 *The Business of DNA*
Chairperson: J. Tooze, European Molecular Biology Organization, Heidelberg, Federal Republic of Germany

Discussants:
N. Zinder, Rockefeller University, New York, New York
R. Fildes, Biogen, Inc., Cambridge, Massachusetts
E.G. Jaworski, Monsanto Company, St. Louis, Missouri
R.E. Cape, Cetus Corporation, Berkeley, California
F. Pass, Molecular Genetics, Inc., Edina, Minnesota

Neurobiology of the Leech, June 29-July 2

Crucial support for Cold Spring Harbor Laboratory's program of conferences, courses, and year-round research in the neurosciences is provided by the Marie H. Robertson Fund for Neurobiology. This fund, established in 1976 through the Banbury Foundation by the family of Mr. Charles S. Robertson, honors the memory of Mr. Robertson's wife, who died in 1972. At first the fund, which provides $75,000 annually was used largely to support the summer teaching program in neurobiology, which includes laboratory courses given on the main Laboratory grounds in the village of Laurel Hollow and lecture courses given at Banbury Center in the village of Lloyd Harbor. But in 1979, after receipt of a substantial training grant from the National Institutes of Health, and added support from the National Science Foundation, it was decided to use some of the Marie H. Robertson funds to support summer workshops and to make possible one or two specialized meetings each year at Banbury Center.

Opening Discussion: When is a neuronal circuit complete?
Moderator: I. Parnas, Hebrew University of Jerusalem, Israel

Session 1
Chairperson: K.J. Muller, Carnegie Institution of Washington, Baltimore, Maryland

S. Blackshaw, University of Glasgow, Scotland: Sensory cells and motoneurones.
W.O. Friesen, University of Virginia, Charlottesville: Physiology and anatomy of sensillar movement receptors.
J.Y. Kuwada, University of California, San Diego: Development of identified neurons in the leech CNS.
A.P. Kramer, University of California, Berkeley: Development of morphological variation of mechanosensory cells in *Haementerial ghilianii.*
I. Parnas, Hebrew University of Jerusalem, Israel: Expansion of the receptive fields of leech nociceptive cells following deletion of single neurons.

Session 2
Chairperson: J.G. Nicholls, Stanford University School of Medicine, California

D.A. Weisblat, University of California, Berkeley: Cell lineage in glossiphoniid neurogenesis.
S.S. Blair, University of California, Berkeley: Alteration of cell patterning through single cell ablation in the early embryo of the leech.
G.S. Stent, University of California, Berkeley: Somite formation in the leech embryo.
J. Fernandez, University of Chile, Santiago: Organization of the germinal plate and formation of 32 body segments in embryos of *Hirudo medicinalis.*
J. Hernandez, University of Chile, Santiago: Formation of ganglionic primordia in embryos of *Hirudo medicinalis.*
Evening Discussion: What use is the leech, if any?
Moderator: G.S. Stent, University of California, Berkeley

Session 3
Chairperson: K.J. Muller, Carnegie Institution of Washington, Baltimore, Maryland

K.J. Muller, Carnegie Institution of Washington, Baltimore, Maryland: Leech synapses.
E. Macagno, Columbia University, New York, New York: The close association of the intraganglionic dendritic fields of T cells.
R. Stewart, Columbia University, New York, New York: Anatomy of leech segmental ganglia, with some comments about segmental and species differences.
A. Mason, Carnegie Institution of Washington, Baltimore, Maryland: Modulatory effects of the retzius cells on longitudinal muscle of the leech *Hirudo medicinalis*.
R.T. Sawyer, Penclawdd, West Glamorgan, South Wales: Leech biology and behavior.

Session 4
Chairperson: A.E. Stuart, University of North Carolina, Chapel Hill

G.S. Stent, University of California, Berkeley: Neuronal circuits.
R.L. Calabrese, Harvard University, Cambridge, Massachusetts: Metastable coordination of the heartbeat in the leech *Hirudo medicinalis.*
E.L. Peterson, Harvard University, Cambridge, Massachusetts: Coordination and phase control in the heartbeat timing oscillator of *Hirudo*.
M. Pelligrino, University of Pisa, Italy: Effects of destruction of single HE cells in the CNS of the leech.
C. Lent, Brown University, Providence, Rhode Island: Physiology and anatomy of the Leydig cells within the leech nervous system.
B. Payton, Memorial University of Newfoundland, St. Johns, Canada: The giant leech of Newfoundland.
Evening Discussion: Development and Regeneration
Moderator: J. Jansen, University of Oslo, Norway

Session 5
Chairperson: G.S. Stent, University of California, Berkeley

B. Wallace, Stanford University, California: Neurochemistry.
D. Stuart, University of California, Berkeley: Fluorescent staining of monoamine neurons in embryonic and adult leech.

A.L. Kleinhaus, Yale University School of Medicine, New Haven, Connecticut: Variation of membrane properties and pharmacological sensitivities among identified leech neurons.
W. Kristan, University of California, San Diego: Diversity of leech behavioral responses mediated by identified mechanosensory and motor neurons.
J.C. Weeks, University of Washington, Seattle: Identified neurons mediating swim initiation and pattern generation in the leech.
B.M. Salzberg, University of Pennsylvania, Philadelphia: Multiple-site optical recording of membrane potential—Prospects for optical recording from a reconstructed leech "nervous system."

Session 6
Chairperson: W. Kristan, University of California, San Diego

B. Zipser, Cold Spring Harbor Laboratory, New York: Specific neuron labeling.
J.G. Nicholls, Stanford University School of Medicine, California: Regeneration and plasticity.
L.P. Henderson, Stanford University School of Medicine, California: Serotonergic transmission between isolated leech neurones in culture.
E.J. Elliott, Carnegie Institution of Washington, Baltimore, Maryland: Axon and synapse regeneration in the absence of glia.

Workshop on Tumor and Transplantation Antigens, July 2-8

The development of DNA cloning and monoclonal antibody technologies has provided new research directions in the area of tumor and transplantation antigens. Both formal research presentations and extensive discussions were held with a group of scientists representing several disciplines: tumor virology, immunology, cell biology, genetics, and biochemistry. This workshop provided the group with an opportunity to compare and contrast observations from different experimental systems and to synthesize emerging general principles. The workshop was held at a time when new directions and ideas are emerging from an expanded research effort in this area.

Session 1

B. Bloom, Johns Hopkins University, Baltimore, Maryland: Mechanisms of tumor recognition and rejection.

Session 2

R. Herberman, National Institutes of Health, Bethesda, Maryland: NK cells and tumor rejection.
R. North, Trudeau Institute, Inc., Saranac Lake, New York: T-cell mediated suppression of tumor immunity.
F. Lilly, Albert Einstein College, Bronx, New York: Genetic loci of the mouse involved in tumor rejection.
S.-I. Shin, Albert Einstein College, Bronx, New York: Tumor growth and rejection in nude mice.
W. Dove, University of Wisconsin, Madison: Teratocarcinoma transplantation rejection.
T. Boon, Ludwig Institute for Cancer Research, Brussels, Belgium: New transplantation antigens of teratocarcinomas.

Session 3

E. Blankenhorn, University of Pennsylvania, Philadelphia: MHL—Genetics and monoclonal antibodies.
S. Weissman, Yale University, New Haven, Connecticut: H-2, HLA, DNA clones.
M. Steinmetz, California Institute of Technology, Pasadena: H-2, HLA, DNA clones.
L. Silver, Cold Spring Harbor Laboratory, New York: T-locus genetics and biochemistry.

Session 4

R. Weinberg, Massachusetts Institute of Technology, Cambridge: Transformation antigens.
N. Hopkins, Massachusetts Institute of Technology, Cambridge: DNA-mediated transfer of chemically transformed cell phenotype.
D. Herlyn and M. Herlyn, Wistar Institute, Philadelphia, Pennsylvania: Melanoma antigens.
O. Witte and N. Rosenberg, University of California, Los Angeles, and Tufts University Medical School, Boston, Massachusetts: Abelson virus transformation.
M. Hayman, Imperial Cancer Research Fund Laboratories, London, England: Acute leukemia viruses.
W. S. Hayward, Rockefeller University, New York, New York: Slow developing leukemias.

Session 5

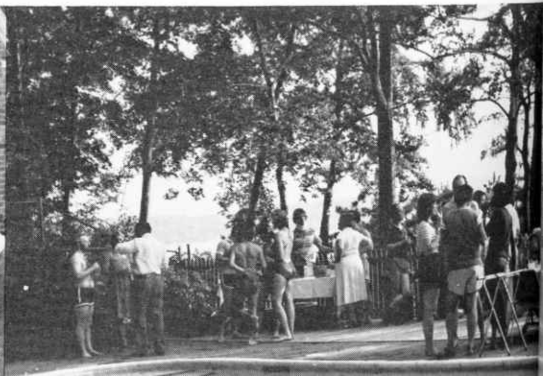
J. Brugge, State University of New York, Stony Brook: RSV *src*.
E. Racker, Cornell University, Ithaca, New York: Cellular *sarc*.
B. Sefton, Salk Institute, San Diego, California: Cellular substrates of *src*.
E. M. Scolnick, National Cancer Institute, Bethesda, Maryland: Rodent *src*.
T.V. Rajan, Albert Einstein College, Bronx, New York: Antibody immunoselection of cell surface antigens.
A. Linnenbach, Wistar Institute, Philadelphia, Pennsylvania: Human monoclonals.

Session 6

D. Lane[1] and E. Tucker Gurney[2], [1]Imperial College of Science and Technology, London England; [2]University of Utah, Salk Lake City: Monoclonal antibodies to SV40 tumor antigens.
S. Tevethia, Pennsylvania State University, Hershey: SV40-T-transplantation antigens.
K. Choudhury and Y. Ito, National Institutes of Health, Bethesda, Maryland: Polyoma T-antigens.
A.J. Levine, State University of New York, Stony Brook: p50-54 cellular tumor antigen.

Session 7 *Roundtable Discussion: New and Old Concepts of Tumor and Transplantation Antigens*
Discussants:

I. Weissman, Yale University, New Haven, Connecticut
B. Sefton, Salk Institute, San Diego, California
T. August, Johns Hopkins University, Baltimore, Maryland
R. Weinberg, Massachusetts Institute of Technology, Cambridge
T.V. Rajan, Albert Einstein College, Bronx, New York



Methods for Measuring Global Neural Activity Workshop

August 2-6

The last of three workshops in the Sloan Foundation-supported series devoted to the interface between neuro- and cognitive sciences was held this summer at Banbury Center. The workshop topic was motivated by the growing realization that in order to better understand the functioning of the brain, information must be obtained about the coherence of neural activity in many brain structures. Extraordinary technical difficulties confront studies of this kind and the workshop was devoted to a variety of new approaches which have promise for overcoming these barriers. Among the topics discussed extensively were the use of the deoxyglucose method for labeling active neurons; the use of arrays of multiple electrodes for recording from many places simultaneously; the measurement of the microblood flow in the brain which has been found to be indicative of the level of neural activity; and use of specialized dyes and optical techniques to follow the functioning of large arrays of neurons. The general tenor of the workshop was that a real beginning had been made on the problem of measuring global neural activity, but that a large amount of technical innovation and developmental work still remains to be done.

Session 1

D. Zipser[1] and P. Hand[2], [1]Cold Spring Harbor Laboratory, New York; [2]University of Pennsylvania School of Veterinary Medicine, Philadelphia: Welcoming and introductory remarks.

S. Kety, McLean Hospital, Belmont, Massachusetts: Estimation of circulation and metabolism of the brain by means of inert diffusible tracers.

Session 2

G. Gerstein, University of Pennsylvania, Philadelphia: Simultaneous recording from many neurons—A cost/benefit analysis.

L. Sokoloff, National Institutes of Health, Bethesda, Maryland: Metabolic mapping of local functional activity in the central nervous system with radioactive deoxyglucose.

M. P. Stryker, University of California, San Francisco, School of Medicine: Measurements of the relationship between glucose utilization and neural activity in the cat's visual cortex.

M. Mishkin, National Institutes of Health, Bethesda, Maryland: The visual system visualized with the 2-DG technique.

C. Kennedy, National Institutes of Health, Bethesda, Maryland: Local metabolic responses during motor activity.

D. Flood, University of Rochester Medical Center, New York: Methods of examining the response of neural population applied to the modification of orientation preferences of cats reared in striped cylinders.

P. Hand, University of Pennsylvania, Philadelphia: Functional plasticity produced by sensory disuse or enrichment.

C.R. Gallistel, University of Pennsylvania, Philadelphia: Objective quantitative data reduction with computer-assisted image analysis.

Session 3

M. Des Rosiers, University of Montreal, Quebec, Canada: An overview of the various difficulties (technical and biological) encountered in attempting to apply the DG method at the cellular level while respecting the physiological prerequisites of the method.

D.B. Kelley, Princeton University, New Jersey: Measuring activity in single neurons with [^{3}H] 2-deoxy-D-glucose.

C. Smith, National Institutes of Health, Bethesda, Maryland: A method for the determination of local rates of protein synthesis in the nervous system.

B. Agranoff, University of Michigan, Ann Arbor: Regional brain protein synthesis in rat brain after hypoglossal axotomy.

L. B. Cohen, Yale University, New Haven, Connecticut: Optimal methods for measuring spike activity.

J. Haselgrove, University of Pennsylvania, Philadelphia: Spectroscopic analysis of the metabolic intermediates of living and freeze-trapped brains.

J. Greenberg, University of Pennsylvania, Philadelphia: Flow-metabolism couple in the brain.

R.R. Miselis, University of Pennsylvania, Philadelphia: Application of [^{14}C] iodoantipyrine cerebral blood flow method to a behavioral problem — The vasomotor hypothesis for drinking to angiotensin II.

Session 4

D. Ingvar, University Hospital, Lund, Sweden: Normal and abnormal distribution of activity in the human cerebral cortex.

P. Roland, Bispebjerg Hospital, Copenhagen, Denmark: Macrophysiological dissection of the human brain.

M. Reivich, University of Pennsylvania, Philadelphia: FDG technique—Physiologic and clinical studies.

M. Phelps, University of California, Los Angeles, School of Medicine: The measurement of local cerebral glucose metabolism in man with positron computed tomography—Factors which effect the accuracy of local estimates and applications in visual and auditory stimulations.

W. Cobbs, University of Pennsylvania, Philadelphia: Abnormalities in cerebral glucose metabolism in patients with visual field defects.

R. Bajcsy, University of Pennsylvania, Philadelphia: Perception and recognition of 3-D shapes—A computer science point of view.

J.A. Feldman, University of Rochester, New York: Global activity questions suggested by connectionist theory.

C.J. Vierck, Jr., University of Florida College of Medicine, Gainesville: The spinal somatosensory pathways as model systems for neural coding by convergent interactions.

Session 5

J.H. Kaas, Vanderbilt University, Nashville, Tennessee: Microelectrode mapping methods for subdividing the somatosensory system.

E.L. Schwartz, New York University Medical Center, New York: Computational anatomy in striate and extrastriate primate visual cortex—Spatial mapping as a structural basis for perceptual coding.

M. Gazzaniga, New York Hospital-Cornell Medical Center, New York: Cognitive testing of the separate hemispheres—Split brain approaches to metabolic studies.

Gene Amplification and Aberrant Chromosomal Structures

October 4–7

During the past 5 years there has developed a body of literature and a group of investigators concerned with chromosomal abnormalities in cultured animal cells and in human tumors. These abnormalities include the presence of extrachromosomal elements called double minute chromosomes as well as expanded regions of chromosomes called homogeneously staining regions. Other investigators studying mechanisms of resistance to various agents that kill cells, including cancer chemotherapeutic agents, have found that one common mechanism of resistance involves selective amplification of specific genes. The amplified genes can be present in homogeneously staining regions of chromosomes, or can occur on double minute chromosomes. Thus, these two different areas of investigation—chromosomal abnormalities and drug resistance resulting from gene amplification—appear to be converging.

In October 1981, approximately 50 investigators, including workers in such diverse fields as bacterial genetics, DNA repair-replication, drug resistance, chromosome structure, and developmental biology met at Banbury Center to share current results and concepts.

Session 1 *Examples of Gene Amplification*

Chairperson: G. R. Stark, Stanford University Medical Center, California

P. C. Brown, Stanford University, California: Gene amplification and methotrexate resistance in cultured animal cells.

C. Bostock, University of Edinburgh, Scotland: Amplification of *dhfr* genes in mouse cells.

J.R. Bertino, Yale University School of Medicine, New Haven, Connecticut: Gene amplification in a human leukemia line K-562.

M.C. Weiss, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France: *dhfr* amplification in rat hepatoma variants.

J.L. Biedler and P.W. Melera, Sloan-Kettering Institute for Cancer Research, Rye, New York: Antifolate and vincristine resistance in animal cells.

M. Tien Kuo, University of Texas System Cancer Center, Houston: Vincristine-resistant Chinese hamster ovary cells.

C.T. Caskey, Baylor College of Medicine, Houston, Texas: *hprt* variants and reversion resulting from gene amplification.

K.E. Mayo, University of Washingotn, Seattle: Regulation of amplified and transfected mouse metallothionein genes.

I. Andrulis, The Hospital for Sick Children, Toronto, Ontario, Canada: Amplification of asparagine synthetase genes in CHO cells.

Session 2 *Gene Amplification*

Chairperson: J.L. Biedler, Sloan-Kettering Institute for Cancer Research, Rye, New York

G. Levan, University of Gothenburg, Sweden: Double minute chromosomes, C-minus chromosomes in mouse cell lines.

J.M. Trent, University of Arizona Health Sciences Center, Tucson: Metothrexate treatment and chromosomal aberrations in human tumors.

J.D. Minna, National Naval Medical Center, Bethesda, Maryland: Chromosomal aberrations in human oat cell tumor cell lines.

S.A. Endow, Duke University Medical Center, Durham, North Carolina: Replication of ribosomal genes in *Drosophila*.

A.C. Spradling, Carnegie Institution of Washington, Baltimore, Maryland: Gene amplification during development in *Drosophila*.

R.J. Schwartz, Baylor College of Medicine, Houston, Texas: Actin gene amplification in muscle development.

V. Walbot, Stanford University, California: Nuclear gene amplification in maize and B chromosomes.

R.E. Kellems, Baylor College of Medicine, Houston, Texas: Control of dihydrofolate reductase DNA replication and mRNA production.

Session 3 *Molecular Structures of Amplified DNA Sequences*

Chairperson: C. Bostock, University of Edinburgh, Scotland

R.T. Schimke, Stanford University, California: *dhfr* gene structure and structure of amplified DNA sequences in various mouse cell lines.

J. Hamlin, University of Virginia, Charlottesville: Amplified DNA sequences in MTX-resistant CHO cells.
L.A. Chasin, Columbia University, New York, New York: Dihydrofolate reductase gene structure in amplified and *dhfr*-deficient CHO cells.
G.R. Stark, Stanford University Medical Center, California: On the structure of amplified DNA sequences in PALA-resistant cells.
G.M. Wahl, Salk Institute for Biological Studies, San Diego, California: Analysis of CAD gene amplification using molecular cloning, gene transfer, and cytogenetics.
F. Gilbert, University of Pennsylvania School of Medicine, Philadelphia: Chromosomal aberrations in human neuroblastomas and retinoblastomas.
P.E. Barker, Yale University School of Medicine, New Haven, Connecticut: Structure of aberrant chromosome structures in human tumor cell lines.
H. Hubbell, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania: Molecular structure of DMs from a human colon carcinoid cell line.
D.L. George, Johns Hopkins University School of Medicine, Baltimore, Maryland: Amplified DNA sequences in mouse tumor cells—Association with DMs and HSRs.

Session 4 *Mechanisms of Gene Amplification*

Chairperson: R.T. Schimke, Stanford University, California

S. Lavi, Weizmann Institute of Science, Rehovot, Israel: Carcinogen-mediated amplification of DNA sequences in CHO cells.
T.D. Tlsty, Stanford University, California: Enhanced generation of MTX resistance in cultured animal cells.
A. Varshavsky, Massachusetts Institute of Technology, Cambridge: Phorbol esters and gene amplification.
M. Botchan, University of California, Berkeley: Chromosome excision and/or amplification promoted by viral origins of replication.
P.C. Hanawalt, Stanford University, California: DNA repair pathways in mammalian cells.
W.A. Haseltine, Sidney Farber Cancer Institute, Boston, Massachusetts: Studies on DNA repair in bacteria.
R.H. Rownd, Northwestern University Medical School, Chicago, Illinois: Use and lack of use of insertion sequences in amplification events in bacteria—Possible mechanisms of amplification in bacteria.

Session 5 *Chromosomal Alterations*

Chairperson: S.A. Latt, The Children's Hospital Medical Center, Boston, Massachusetts

S.A. Latt, The Children's Hospital Medical Center, Boston, Massachusetts: Use of flow sorting to study amplified DNA sequences.
R. Kaufman, Massachusetts Institute of Technology, Cambridge: Transfection and amplification of *dhfr* genes in animal cells.
J. Roberts, Columbia University College of Physicians and Surgeons, New York, New York: Amplification and correction of transformed genes.
J.D. Rowley, University of Chicago, Illinois: Nonrandom chromosome changes in human leukemia.
J.L. German III, New York Blood Center, New York: Human chromosomes and the generation of somatic cell diversity.
J. Yunis, University of Minnesota, Minneapolis: High-resolution chromosome banding in the study of human neoplasia and birth defects.
G. Klein, Karolinska Institute, Stockholm, Sweden: Gene dosage, gene expression, and tumorigenesis.

Patenting of Life Forms, October 18–21

The Supreme Court's 1980 decision that artificial strains of microorganisms themselves could be patented, as well as processes for making or using these strains, symbolized for many people the growing commercialization of techniques of molecular biology which hitherto had been regarded as tools for investigating the foundations of genetics. The decision brought two cultures, patent law and fundamental biology, into sudden contact. Each knew little of the other. Both biologists and lawyers began asking to what extent patenting of inventions, so central to the pharmaceutical industry but with a much more subtle impact on the electronics industry, would govern the commercialization of the new recombinant DNA techniques. To remove some of the uncertainty, leading patent lawyers and scientists heard reviews by scientists of some of the immense diversity inherent in manipulations of microorganisms and by lawyers of the application of patent law to such organisms. The conference concluded with a review of recent changes in American and European patent laws and practices and the possibility of more change soon.

Session 1 *Scientific Issues*

J. Hicks, Cold Spring Harbor Laboratory, New York: The life cycle of the common microbial hosts, with emphasis on yeast—the most complex.

M. Scharff, Albert Einstein College of Medicine, Bronx, New York: Monoclonal antibodies. What defines a permanent cell line, differentiating what is novel or unique; what parameters distinguish one hybridoma from another?

C.M. Croce, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania: Monoclonal antibody systems with potential for diagnosis of pancreatic and other forms of cancer.

H. Heyneker, Genentech, Inc., South San Francisco, California: Vector systems and expressions in *E. coli.*

A. Ullrich, Genentech, Inc., South San Francisco, California: Gene transfer—Moving a human gene to a mouse genome.

S. Brenner, Cambridge University Medical School, England: Recombinants that are the same but different.

Session 2 *Open Forum on Scientific Issues*

Chairperson: N.D. Zinder, Rockefeller University, New York, New York

Discussants:

N.H. Carey, Celltech, Slough, Berkshire, England
P.A. Sharp, Massachusetts Institute of Technology, Cambridge
J. Davies, Biogen, SA, Geneva, Switzerland
J. Sambrook, Cold Spring Harbor Laboratory, New York

Session 3 *Legal Issues*

D.W. Plant, Fish & Neave, New York, New York: Primer on law on patents and other intellectual property.

B.I. Rowland, Townsend and Townsend, Palo Alto, California: Should the fruits of genetic engineering be patentable?

V. Vossius, Vossius, Vossius, Tauchner, Heunemann, Rauh, Munich, Germany: Discussion of items I and II as they relate to European and German patent law.
T.D. Kiley, Genentech, Inc., South San Francisco, California: Assuming patentability, what property rights should accrue to workers in this field?
J.W. Schlicher, Genentech, Inc., South San Francisco, California: The extent to which antitrust and patent misuse law limits the manner in which patents are acquired, enforced, and licensed.
N.J. Reimers,[1] R.G. Ditzel,[2] and W.P. O'Neill,[3] [1]Stanford University, California; [2]University of California, Berkeley; [3]DNAX Research Institute, Palo Alto, California: Problems in enforcing patents and in commercializing patented subject matter in the genetic engineering field.
A.H. MacPherson, Skjerven, Morrill, Jensen, MacPherson, & Drucker, Santa Clara, California: Surviving in an industry that largely eschews patents—electronics.

Session 4 *Open Forum on Legal Issues*
Chairperson: Hon. G. Rich, United States Court of Customs and Patent Appeals, Washington, DC

Discussants:
G.M. Gould, Hoffmann-La Roche, Inc., Nutley, New Jersey
C. H. Herr, E.I. duPont deNemours & Company, Wilmington, Delaware
A.E. Tanenholtz, US Department of Commerce, Patent & Trademark Office, Washington, DC

Session 5 *Revision in Patent Law and the Patent Court System Affecting the Patenting of Life Forms*

Discussants:
N.E. Noonan, House Committee on Science and Technology, Washington, DC
E.L. Bernard, Bernard, Rothwell & Brown, Washington, DC
G.M. Karny, Office of Technology Assessment, US Congress, Washington, DC

Environmental Effects on Maturation, November 1-4

To what extent are such environmental factors as nutrition and chemicals, artificial or natural, having such effects on children as stunting their physical growth or mental development, predisposing them to such long-term effects as cancer, or increasing the chance that these children, when they become adults, will have children of their own with birth defects? Experts on human, primate, and small animal development, including biochemists, pediatricians, psychologists, and pharmacologists, considered what is presently known about environmental risks for children, including risks to the developing brain, liver, and reproductive organs. The issues were considered in order to help plan research for better understanding of the risks faced by farm workers' children in an environment where pesticides are used heavily. Among many issues, participants considered the paradox presented by young animals actually showing increased resistance to some chemicals, even though, as rapidly growing organisms, the animals may have been expected to show increased vulnerability.

Session 1 *Routes of Exposure: Skin and Lung*
Chairperson: V. Hunt, Pennsylvania State University, University Park

H.I. Maibach, University of California School of Medicine, San Francisco: Percutaneous absorption—Neonate compared to the adult.
L. Frank, University of Miami School of Medicine, Florida: Maturational aspects of exidant-induced lung injury.
R.C. Spear, University of California, Berkeley: Farmworker exposure to pesticide residues—Reflections on differential risk.

Session 2 *Routes of Exposure: Gastrointestinal Tract*
Chairperson: D. Barltrop, Westminster Children's Hospital, London, England

D. Barltrop, Westminster Children's Hospital, London, England: Lead/nutrition.
C.M. Schiller, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Effects of toxins on gastrointestinal function—Developing systems.
S.J. Henning, University of Houston, Texas: Development of feeding behavior and digestive function.
E.P. Savage, Colorado State University, Fort Collins: Pesticides in human breast milk.

Session 3 *Metabolism, Liver and Gastrointestinal*

Chairperson: G.L. Lucier, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

H.P. Hoensch, University of Tubingen, Federal Republic of Germany: Absorption and metabolism of xenobiotics in the intestine.

E.S. Vesell, Pennsylvania State University, Hershey: Dynamically interacting factors that affect the response of developing individuals to toxicants.

S.D. Murphy, University of Texas Health Science Center, Houston: Toxicity and metabolism of organophosphorus insecticides in developing rats.

G.L. Lucier, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Imprinting of hepatic metabolism by neonatal hormone.

Session 4 *Kidney and Immune Systems*

Chairperson: L.I. Kleinman, University of Cincinnati College of Medicine, Ohio

L.I. Kleinman, University of Cincinnati College of Medicine, Ohio: The effect of lead on the maturing kidney.

R.J. Kavlock, US Environmental Protection Agency, Research Triangle Park, North Carolina: The ontogeny of the hydropenia response in neonatal rats and its application in developmental toxicology studies.

J.N. Udall, Massachusetts General Hospital, Boston: Macromolecular transport across the developing intestine.

M.I. Luster, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Altered immune functions in rodents perinatally treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, phorbol-12-myristate-13-acetate and benzo(a)pyrene.

T. Kalland, University of Bergen, Norway: Long-term effects on the immune system of an early life exposure to diethylstilbestrol.

Session 5 *Central Nervous System*

Chairperson: R.J. Bull, US Environmental Protection Agency, Cincinnati, Ohio

K. Suzuki, Albert Einstein College of Medicine, Bronx, New York: Development of myelin.

R.B. Kearsley, New England Medical Center Hospital, Boston, Massachusetts: Cognitive assessment of developing infants.

L.W. Reiter, US Environmental Protection Agency, Research Triangle Park, North Carolina: Organo-tin exposure in developing rodents.

R.E. Bowman, University of Wisconsin Primate Laboratory, Madison: Some behavioral sequelae of toxicant exposure during development.

R.C. Vannucci, Cornell University Medical College, New York, New York: Vulnerability of the immature brain to hypoxia-ischemia.

Session 6 *Maturation of the Reproductive System, Including Neuroendocrine Aspects*

Chairperson: J.H. Clark, Baylor College of Medicine, Houston, Texas

J.H. Clark, Baylor College of Medicine, Houston, Texas: Sex steroids and maturation in the female.

B.S. McEwen, Rockefeller University, New York, New York: Estrogens—Influences on brain development and neuroendocrine function.

C.W. Bardin, The Population Council, New York, New York: The differentiation of the male reproductive tract.

R.W. Goy, Wisconsin Regional Primate Research Center, Madison: The developmental actions or effects of androgens in Rhesus monkeys.

D. Kupfer, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Xenobiotics.

D.R. Mattison, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland: Prepubertal ovarian toxicity.

P. Walker, Le Centre Hospitalier de l'Universite Laval, Quebec, Canada: Long-term susceptibility resulting from brief perinatal manipulation of thyroid function.

Session 7 *Epidemiological Perspectives*

Chairperson: H.W. Berendes, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland

J.E. Norman, Jr., National Research Council, Washington, DC: Breast cancer in women irradiated in early life.

R. Illsley, Institute of Medical Sociology, Aberdeen, Scotland: Impact of early-life factors on pregnancy outcomes.

D. Snowdon, Loma Linda University School of Health, California: Age at baptism into the Seventh-Day Adventist Church and risk of death due to ischemic heart disease—A preliminary report.

G.G. Nahas, College of Physicians & Surgeons of Columbia University, New York, New York: The Ledermann model applied to the frequency of marihuana use among U.S. high school seniors.

Session 8 *Lessons from Pharmacokinetics and Human Responses*

Chairperson: C.M. Berlin, Jr., Pennsylvania State University, Hershey

L.K. Garrettson, Virginia Commonwealth University, Richmond: Age-related development of theophylline—Pharmacokinetic responses of children.

B.R. Sonawane, University of Pennsylvania, Philadelphia: Developmental aspects of acetaminophen hepatotoxicity—Influence of age and acute starvation.

J.S. Partin, State University of New York, Stony Brook: Reye's syndrome and its association with salicylates.

A.H. Neims, University of Florida College of Medicine, Gainesville: Some drug host aspects of drug therapy in pediatrics.

J.M. Tanner, University of London, England: Conference summary.

Construction and Use of Mammalian Viral Vectors, December 3-6

At this conference 49 DNA and RNA tumor virologists presented and discussed the most recent advances in the use of tumor viruses as cloning vectors. The progress in this field can be judged by comparing this conference with the 1980 Banbury meeting on Construction and Use of Mammalian Viral Vectors. One year ago, the only viral vectors constructed and used were SV40 and adenovirus; papilloma and RNA tumor viruses were merely discussed as potential vectors. In contrast, during the 1981 meeting, work with a great variety of vectors, including both DNA tumor viruses (SV40, polyoma, papilloma, adenoviruses, herpesvirus) and RNA tumor viruses (MoMLV, MoMSV, HaMSV, ASV, SNV, MMTV) was presented.

Session 1 *SV40*

Chairperson: T. Shenk, State University of New York, Stony Brook

G.C. Fareed, University of California, Los Angeles: Expression of influenza virus hemagglutinin using SV40 vectors.

M.J. Gething, Imperial Cancer Research Fund Laboratories, London, England: Cell-surface expression and secretion of influenza hemagglutinin from simian cells infected with SV40-HA vectors.

P. Gruss, NCI, National Institutes of Health, Bethesda, Maryland: The expression of viral and cellular p21 *ras* genes using SV40 as a vector.

D.H. Hamer, NCI, National Institutes of Health, Bethesda, Maryland: Regulation of a metallothionein gene cloned in animal virus vectors.

M. Horowitz, Massachusetts Institute of Technology, Cambridge: SV40 as a vector for cloning eukaryotic sequences and controlling elements.

R. Treisman, Harvard University, Cambridge, Massachusetts: Expression of human β-globin genes in Cos-7 and HeLa cells.

A. Levinson, Genentech, Inc., South San Francisco, California: Expression of hepatitis B surface antigen using SV40-based vectors.

Session 2 *RNA Tumor Viruses*

Chairperson: G. Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland

E.M. Scolnick, NCI, National Institutes of Health, Bethesda, Maryland: Properties of transmissible retroviruses containing the thymidine kinase gene of HSV.

S. Watanabe, University of Wisconsin, Madison: Encapsidation sequence required for retrovirus vectors.

C. Tabin, Massachusetts Institute of Technology, Cambridge: The utilization of a retrovirus as a eukaryotic vector for transmitting cloned DNA sequences.

J. Sorge, Cold Spring Harbor Laboratory, New York: Retrovirus vector independent of selectable markers.

R. Mulligan, Massachusetts Institute of Technology, Cambridge: Construction of transmissible retrovirus cloning vehicles carrying dominant-acting genetic markers.

Session 3 *Papilloma, Polyoma, Simian Virus*

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

P.M. Howley, NCI, National Institutes of Health, Bethesda, Maryland: Expression of selective traits in mouse cells transformed by a BPV69T-SV2*gpt* hybrid DNA.

R. Breathnach, Faculte de Medicine de Strasbourg, France: Bovine papilloma virus I-pBR322 and polyoma-pBR322 recombinants as eukaryotic vectors.

D. DiMaio, Harvard University, Cambridge, Massachusetts: Intact bovine papilloma virus-human DNA recombinant plasmids that propagate as episomes in mouse and bacterial cells.

M. Botchan, University of California, Berkeley: Enhanced transformation mediated by bovine papilloma virus.

M. Fried, Imperial Cancer Research Fund Laboratories, London, England: Use of a polyoma virus vector.

R. Contreras, Laboratorium voor Moleculaire Biologie, Gent, Belgium: Expression of human fibroblast interferon β_1 gene by transfection of monkey cells with an SV40 vector.

P.J. Southern, Scripps Clinic and Research Foundation, La Jolla, California: Mammalian cell transformation with SV40 hybrid plasmid vectors.

Session 4 *RNA Tumor Viruses*

Chairperson: E.M. Scolnick, NCI, National Institutes of Health, Bethesda, Maryland

S. Broome, Harvard University, Cambridge, Massachusetts: A Rous sarcoma virus *gag* gene product modulates on RNA levels in transfected cells.

E. Gilboa, Princeton University, New Jersey: Transduction and expression of nonselectable genes using retrovirus-derived vectors.

G. Vande Woude, NCI, National Institutes of Health, Bethesda, Maryland: Use of retroviral sequences in cotransfection, activation, and rescue of *onc* genes.

I.M. Verma, Salk Institute, San Diego, California: Expression and regulation of rat growth hormone gene in mouse cells.

G.L. Hager, NCI, National Institutes of Health, Bethesda, Maryland: Analysis of glucocorticoid regulation by linkage of the mouse mammary tumor virus promoter to a viral oncogene.

M. Kriegler, University of California, Berkeley: A retroviral LTR contains a new type of eukaryotic regulatory element.

Session 5 *Adenovirus, Herpesvirus*

Chairperson: T. Grodzicker, Cold Spring Harbor Laboratory, New York

C.S. Thummel, University of California, Berkeley: Precise positioning of SV40 DNA in adenovirus expression vectors by a combination of in vitro and in vivo recombination.

Y. Gluzman, Cold Spring Harbor Laboratory, New York: Helper-independent adenovirus vector.

K.L. Berkner, Massachusetts Institute of Technology, Cambridge: Adaptation of adenovirus as a cloning vehicle.

N. Frenkel, University of Chicago, Illinois: A novel eukaryotic-virus vector—The herpes simplex virus amplicon.

N.D. Stow, University of Glasgow, Scotland: Propagation of foreign DNA sequences using a novel herpes simplex virus vector.

B. Howard, National Institutes of Health;, Bethesda, Maryland: Vectors which may be propagated by integration into the *E. coli* chromosome.

Postgraduate Training Program

SUMMER 1981

The Postgraduate Training Program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects which are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Neurobiology of Behavior, June 5-June 18

INSTRUCTORS

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

This course was designed to introduce students to cellular approaches to the study of behavior and learning. The lectures provided an intensive coverage of four main areas: 1) general principles of behavior and cellular neurobiology; 2) simple forms of behavior, learning, and motivation; 3) initiation and maintenance of complex locomotor sequences, including voluntary movement and motor learning; 4) communication. To illustrate general principles, suitable systems for study were selected from both invertebrate and vertebrate behavior. To put the cellular work into perspective, selected examples were also taken from human behavior and its abnormalities.

PARTICIPANTS

Belardetti, Francesco, M.D., University of Pisa, Italy
Ford, Paul M., B.S., National Jewish Hospital, Denver, Colorado
Garg, Ajay P., B.S., Schenectady, New York
Hofmann, Michael J., B.S., University of Connecticut, Storrs
Lane, Steven R., B.A., University of California, San Francisco
Miyashita, Yasushi, M.S., University of Tokyo, Japan
Mortin, Lawrence I., B.S., Washington University, St. Louis, Missouri
Nelson, Lorene M., B.S., University of Colorado, Denver
Norris, Brian J., Texas Technological University, Lubbock
Nye, Jeffrey Scott, B.A., Harvard Medical School, Boston, Massachusetts
Parpal, Leslie A., M.A., Temple University, Philadelphia, Pennsylvania
Rapaport, Ray, B.A., Wayne State University, Detroit, Michigan
Reichert, Heinrich, Ph.D., Stanford University, California
Richerson, George B., B.S., University of Iowa, Iowa City
Rosenheimer, Julie L., B.A., University of Wisconsin, Madison
Symonds, Laura L., B.S., University of Pennsylvania, Philadelphia
Zabala, Nelson A., M.D., IVIC, Caracas, Venezuela

SEMINARS

Kandel, E.R., Columbia University. *Introduction to the cellular study of behavior.*
Koester, J., Columbia University. *Introduction to the biophysics of behavior.*
Kandel, E.R., Columbia University. *Morphology and physiology of synaptic transmission.*
Koester, J., Columbia University. *Repetitive firing properties and the control of behavior.*
Kandel, E.R., Columbia University. *Learning I. Habituation.*
———. *Learning II. Sensitization.*
Wine, J. Stanford University. *Nerve circuitry for simple behavioral acts and their control.*
Baudry, M., University of California. *Long-term plasticity in the vertebrate CNS.*
Levine, R., University of Washington. *Hormones and behavior.*
Kupfermann, I., Columbia University. *Motivation.*
Wurtz, R., National Institutes of Health. *Attention.*
Pearson, K., University of Alberta. *Introduction to motor sequences.*
———. *Central and reflex control of movements.*
———. *Walking in the cat.*
Getting, P., University of Iowa. *Rhythm generation in invertebrate motor systems.*
Friesen, O., University of Virginia. *Neuronal control of leech swimming.*
Ghez, C., Columbia University. *Voluntary movements in mammals.*
Evarts, E., National Institute of Mental Health. *Cerebral control of voluntary movement.*
Thach, W.T., Jr., Washington University. *Does the cerebellum learn motor programs?*
Fuchs, A., University of Washington. *Adaptive regulation in the oculomotor system.*
Nottebohm, F., Rockefeller University. *Introduction to communication.*
———. *Brain pathways for vocal learning.*
Suga, N., Washington University. *Brain pathways for sound processing in bats.*
Zaidel, E., University of California. *Language processing by the right and left hemisphere.*
Ojemann, G., University of Washington. *A surgeon's view of language processing pathways.*
Geschwind, N., Harvard Medical School. *Apraxia.*
Zigmond, R., Harvard Medical School. *Use and disuse: Effects on neurotransmitter synthesis.*
———. *The role of neurotransmitters in behavioral abnormalities.*

Nervous System of the Leech, June 6–June 26

INSTRUCTORS

Nicholls, John, M.D., Ph.D., Stanford University Medical School, California
Parnas, Itzhak, Ph.D., Hebrew University, Jerusalem, Israel
Muller, Kenneth, Ph.D., Carnegie Institution of Washington, Baltimore, Maryland
Zipser, Birgit, Ph.D., Cold Spring Harbor Laboratory, New York

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

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

The final phase of the course consisted of devising and performing original experiments, some of which proved to be of sufficient interest to be pursued in greater detail. For example, individual nerve cells in the leech were stained with specific monoclonal antibodies. Techniques for dissecting and maintaining individual indentified cells and keeping them alive in culture, as well as the use of Pronase injection for killing single cells were listed by the students.

PARTICIPANTS

Dhanjal, Sukhvinder S., B.S., Institute of Neurology, London, England
French, Kathleen A., Ph.D., University of North Carolina, Chapel Hill
Hockfield, Susan, Ph.D., Cold Spring Harbor Laboratory, New York
Laiwand, Roni, M.S., Hebrew University, Jerusalem, Israel
Macdonald, Beth L., B.A., University of Connecticut, Farmington
McKay, Ronald, Ph.D., Cold Spring Harbor Laboratory, New York
Schwarz, Jurgen R., Ph.D., University of Hamburg, Federal Republic of Germany
Shankland, Martin S., Ph.D., University of California, Berkeley
Tolbert, Leslie P., Ph.D., Harvard Medical School, Boston, Massachusetts
Zhang, Ren-Ji, State University of New York, Plattsburgh

SEMINARS

Blackshaw, S., Stanford Medical School. *Touch cells.*
———. *Nociceptive cells.*
Friesen, O., University of Nigeria. *Receptors to waves in the fluid.*
Kristan, W., University of California, San Diego. *Swimming.*
———. *Development of reflexes.*
Muller, K., Carnegie Institution of Washington. *Electronics for intracellular recording.*
———. *Motor cells.*
———. *Abnormal sensory and motor cells.*
———. *The S cell: An interneuron.*
———. *Regeneration.*
———. *Structure of synapses.*
Nicholls, J., Stanford Medical School. *Introduction: Why the leech?*
———. *Sensory cells.*
———. *After potentials and conduction block.*
———. *Synaptic transmission.*
———. *Cultured cells: Chemical and electrical connections.*
———. *Presynaptic inhibition and modulation of transmitter release.*
———. *Accumulation and glia.*
Parnas, I., Hebrew University. *Conduction block.*
———. *Spread of receptive fields after killing single cells with Pronase.*
Stent, G., University of California, Berkeley. *Swimming and heartbeat.*
———. *Development of receptive fields.*
Weisblat, D., University of California, Berkeley. *Development of the leech.*
Zipser, B., Cold Spring Harbor Laboratory. *Neural control of sexual function in the leech.*
———. *Monoclonal antibodies to specific cells.*

The Mammalian Central Nervous System, June 6–June 29

INSTRUCTORS

Burke, Robert, M.D., National Institutes of Health, Bethesda, Maryland
Hubel, David, M.D., Harvard Medical School, Boston, Massachusetts
Malpeli, Joseph, Ph.D., University of Illinois, Chicago
Rymer, William Z., M.D., Northwestern Medical School, Chicago, Illinois
Sherk, Helen, Ph.D., Harvard Medical School, Boston, Massachusetts

This workshop offered laboratories and lectures on the mammalian central nervous system. It began with four days of lectures by Robert Burke and David Hubel on the spinal cord, motor control, and the visual system. These were followed by a day of demonstrations of recording from cat spinal motorneurons and muscle afferents and lateral geniculate neurons. The remaining 18 days were

devoted to four series of laboratory experiments on the cat CNS, with the eight participants working in pairs, spending four to five days on each series. In all labs, participants learned the appropriate surgical techniques, the preparation of microelectrodes, and histological procedures for reconstructing microelectrode tracks. In one lab participants studied the reflex activity of motorneurons in the spinal cord using both intracellular and extracellular recording techniques. Functional properties of the associated motor units were identified and synaptic connections from various cutaneous and muscle afferent systems were examined. In another lab, the participants recorded extracellularly from auditory cortex and used retrograde transport of horseradish peroxidase to look for evidence of a columnar structure to interhemispheric auditory projections. The other two labs focused on extracellular recording from visual cortex (areas 17, 18, 19, and the Clare-Bishop area) and subcortical visual structures (the lateral geniculate nucleus and the superior colliculus). The single-cell responses to visual stimuli, retinotopic organization, and functional cytoarchitecture of these structures were examined. A digital computer was used to generate poststimulus histograms. In the subcortical vision labs the participants studied orthodromic and antidromic shock-evoked driving, making use of the impulse collision technique. Retrograde transport of horseradish peroxidase was also used in the vision experiments to study the connections between cortical and subcortical structures.

PARTICIPANTS

Atkeson, Christopher G., B.A., Harvard University, Cambridge, Massachusetts
Carman, George J., B.A., University of Texas, Austin
Demeter, Steven, M.D., University of Iowa, Iowa City
Illing, Robert-Ben, M.D., Max-Planck-Gesellschaft, Munich, Federal Republic of Germany
Matsubara, Joanne A., B.A., University of California, San Diego
McDonagh, Martin J., Ph.D., The Medical School, Nottingham, England
Orbach, Harry S., Ph.D., Yale University, New Haven, Connecticut
Schumer, Robert A., Ph.D., New York University, New York

SEMINARS

Gilbert, C., Harvard University. *Functional morphology of visual cortex.*
Fetz, E., University of Washington. *New approaches to the evaluation of motor cortex function.*
Robinson, D., Johns Hopkins Medical School. *Application of control theory to the oculomotor system.*
Karten, H., State University of New York, Stony Brook. *Histochemistry of vertebrate return.*
Llinas, R., New York University. *Cellular biology of olivo-cerebellar circuits.*

Molecular Biology of Plants, June 6–June 26

INSTRUCTORS

Ausubel, Frederick M., Ph.D., Harvard University, Cambridge, Massachusetts
Bedbrook, John, R., Ph.D., CSIRO, Canberra, Australia
Smith, Stephen, Ph.D., CSIRO, Canberra, Australia
Riedel, Gerard E., Ph.D., CSIRO, Canberra, Australia

ASSISTANTS

Brown, Susan E., Harvard University, Cambridge, Massachusetts
Dunn, Barbara, B.S., Harvard University, Cambridge, Massachusetts

The three week Plant Molecular Biology Course consists of a series of lectures by the staff and by distinguished invited speakers and a series of laboratory exercises designed to acquaint the students with the fundamentals of current plant molecular biology research. The course assumes that most students come with a good background in molecular genetic techniques but with a poor understanding of general botany, plant physiology, plant sexual reproduction, and plant development. Therefore, the course begins with lectures to acquaint the students with some of the fundamental differences between plants and animals. The introductory topics are tied together by considering plants from a developmental point of view. Laboratory experiments involving whole plant anatomy, embryo and meristem culture, and somatic cell tissue culture complement the introductory lectures.

The introductory section is followed by a series of detailed lectures covering topics which are relatively unique to plants including photosynthesis, seed formation and germination, and regeneration of intact plants from somatic cultures. Among the topics covered are classical transmission genetics including transposable genetic elements, cytogenetics, plant viruses, plant-bacterial interactions, and the molecular biology of the plant organelle and nuclear genomes. The laboratory projects parallel the lectures and include experiments on (1) the mechanism of α-amylase induction in germinating seeds; (2) the manipulation of plant tissue cultures; (3) the generation of plant mutants; (4) the isolation, characterization and manipulation of plant DNAs and RNAs; and (5) cytogenetics.

GUEST INSTRUCTORS

Smith, Jane H., ARCO Plant Cell Research Institute, Dublin, California
Dellaporta, Stephen, Worcester Polytechnic Institute, Massachusetts
Sussex, Ian, Yale University, New Haven, Connecticut
Walbot, Virginia, Stanford University, California
Hanson, Maureen, University of Virginia, Charlottesville
Jones, Jonathan, Harvard University, Cambridge, Massachusetts
Varner, Joseph, Washington, University, St. Louis, Missouri
Miles, Donald, University of Missouri, Columbia

PARTICIPANTS

Boccara, Martine, Ph.D., Sloan-Kettering Institute, New York
Carbonero, Pilar, M.D., Universidad Politecnica de Madrid, Spain
Dion, Patrice, Ph.D., Université Laval, Quebec, Canada
Dowling, Elizabeth L., B.S., University of California, Berkeley
Goodman, Howard M., Ph.D., University of California, San Francisco
Hofman, Jason D., Ph.D., Dalhousie University, Halifax, Nova Scotia
Klessig, Daniel F., Ph.D., University of Utah, Salt Lake City
Lica, Lorraine M., Ph.D., University of California, Irvine
Magnusson, Ronald P., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Novak, Ulrike, B.S., Imperial Cancer Research Fund, London, England
Sanchez, Federico E., Ph.D., University of California, San Francisco
Scolnik, Pablo A., Ph.D., University of Chicago, Illinois
Selzer, Gerald B., Ph.D., National Institutes of Health, Bethesda, Maryland
Simpson, Robert B., Ph.D., University of Washington, Seattle
Woznyj, Manfred J., M.S., Universität Bielefeld, Federal Republic of Germany
Zimmer, Elizabeth A., Ph.D., Washington University, Seattle

SEMINARS

Sussex, I., Yale University. *Plant anatomy and development.*
Bedbrook, J., CSIRO. *Molecular cloning of plant nuclear genes.*

Ausubel, F., Harvard University. *Molecular biology of nitrogen fixation.*
Riedel, G., CSIRO. *Development of vectors for transforming plant cells.*
Walbot, V., Stanford University. *Plant development.*
van Montagu, M., Rijksuniversitiet Gent. *Molecular biology of crown gall tumorigenesis.*
Flavell, R., Plant Breeding Institute. *Organization of chromosomal DNA sequences in plants.*
Hanson, M., University of Virginia. *Plant tissue culture.*
Jones, J., Harvard University. *In situ hybridization in plant cytogenetics.*
Varner, J., Washington University. *Molecular biology of plant hormone action.*
Smith, S., CSIRO. *Uptake of nuclear coded proteins in chloroplasts.*
Mets, L., Carnegie Institute. *Organelle genetics.*
McClintock, B., Cold Spring Harbor Laboratory. *Controlling elements in maize.*
Burr, B., Brookhaven Laboratory. *Controlling elements in maize.*
Miles, D., University of Missouri. *Isolation and characterization of photosynthetic mutants.*
Verma, D.P., McGill University. *Molecular biology of legume root nodules.*
Bogorad, L., Harvard University. *Molecular biology of chloroplast gene expression.*
Shepard, J., Kansas State University. *Use of plant tissue culture techniques in crop improvement.*
Dellaporta, S., Worcester Polytechnic Institute. *Transformation of plant protoplasts.*
Hallick, R., University of Colorado. *Molecular biology of chloroplasts.*
Malmberg, R., Cold Spring Harbor Laboratory. *Plant tissue culture techniques.*

Animal Cell Culture, June 13-June 26

INSTRUCTORS

Sato, Gordon H., Ph.D., University of California, San Diego, LaJolla
Mather, Jennie P., Ph.D., The Population Council, Rockefeller University, New York, New York

ASSISTANT

Byer, Alicia, B.A., The Population Council, Rockefeller University, New York, New York

Recent investigations into the role of serum in cell culture medium have shown that the serum requirement for many established cell lines can be replaced by specific mixtures of hormones and purified serum factors. This approach has been used successfully to prolong viability and function of primary cultures, specifically select for one cell type in primary cultures, and establish cell lines of cell types not previously maintained in culture. Participants brought their own cell lines and/or selected one of the established lines provided and designed and carried out experiments to define a serum-free medium for these cells. Participants also prepared primary cultures of several cell types in serum-free media. Laboratory exercises were supplemented by lectures and discussions with invited speakers expert in various aspects of cell culture and hormonal control of cell proliferation and differentiation. Special thanks go to Dr. Coon, Dr. Taub, and Br. Barnes for arranging laboratory exercises in specialized aspects of the use of serum-fee hormone-supplemented media.

PARTICIPANTS

Aprison, Barry S., M.S., Brandeis University, Waltham, Massachusetts
Billeter, Rudolf, Ph.D., National Institutes of Health, Bethesda, Maryland
Brock, Tommy, Ph.D., University of Alabama, Birmingham
Bullock, Phyllis, M.S., Rush University, Chicago, Illinois
Burczak, John, M.S., Michigan State University, East Lansing
Eschenbruch, Margaret, Ph.D., Friedrich Miescher Institute, Basel, Switzerland
Fisher, Gordon, Ph.D., University of Sherbrooke, Quebec, Canada
Friderici, Karen, M.S., Michigan State University, East Lansing
Furth, Mark E., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Hill, Julian B., M.D., NCI, National Institutes of Health, Bethesda, Maryland
Jemma, Cristina, Ph.D., Superiore Istituto di Sanita, Rome, Italy
Ron, Dina, M.S., Ben Gurion University, Beer Sheva, Israel
Schairer, Hans, Ph.D., Gesellschaft fur Biotechnologische, Braunschweig, Federal Republic of Germany
Smith, Carole, Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Valentich, John, Ph.D., University of Texas, Houston
Van Eldik, Linda, Ph.D., Rockefeller University, New York, New York
Vignon, Francoise, Ph.D., INSERM, Montpellier, France

SEMINARS

Coon, H., National Institutes of Health. *Serum-free culture of thyroid.*
Taub, M., State University of New York, Buffalo. *Growth of kidney cells in serum-free medium.*
McKeehan, W., W. Alton Jones Cell Science Center. *Optimizing media for serum-free culture hormone nutrient interactions.*
Barnes, D., University of Pittsburgh. *Role of attachment factors in serum-free culture.*
Phillips, D., Rockefeller University. *Use of scanning and electron microscopy to study hormone action in cell culture.*
Lane, D., Johns Hopkins University. *Role of insulin in differentiation of adipocytes.*
Pawelek, J., Yale University. *Regulation of growth and differentiation in melanoma cells in culture.*
Yamada, K., National Institutes of Health. *Structure and function of fibronectin.*
Glass, J., Beth Israel Hospital. *Transferrin and iron uptake.*
Haour, F., INSERM, *Hormonal control of gonadatropin receptors and response.*
Sherman, M., Roche Institute. *Embryonic development in culture.*

The Synapse and Sensory Transductor, July 9–July 29

INSTRUCTORS

Rahamimoff, Rami, M.D., Hebrew Unversity Medical School, Jerusalem, Israel
McMahan, U.Jack, Ph.D., Stanford University, California
Stevens, Charles F., M.D., Ph.D., Yale University, New Haven, Connecticut
Yoshikami, Doju, Ph.D., University of Utah, Salt Lake City

This course was designed for graduate students and research workers interested in the structure and function of synapses and sensory receptors. It consisted of lectures; readings of papers; group discussions; presentations of selected topics by participants; seminars by instructors, faculty, and participants; and demonstrations. After the course ended, some of the participants stayed on for a workshop.

PARTICIPANTS

Antelman, Bruce M., B.A., Harvard Medical School, Boston, Massachusetts
Baker, Anthony J., B.A., University of Bristol, England
Behrens, Maria Isabel, M.D., Catholic University, Santiago, Chile
Bodmer, Rolf A., B.S., Friedrich Miescher Institute, Basel, Switzerland
Drotar, AnnaMarie, M.S., University of Colorado, Boulder
Dye, John C., B.A., University of California, San Diego
Fallon, Justin, R., Ph.D., University College London, England
Frair, Patricia M., Ph.D., McGill University, Montreal, Canada
Greene, Glenn J., M.S., California Institute of Technology, Pasadena
Krause, James E., Ph.D., University of Pittsburgh, Pennsylvania
Lechleiter, James D., B.S., University of Arizona, Tucson
Nunez, Ramon H., M.D., University of Connecticut, Storrs
Nussinovitch, Itzhak, Hadassah Medical School, Jerusalem, Israel
Oland, Lynne A., M.S., University of North Carolina, Chapel Hill
Paggi, Paola, B.S., University of Rome, Italy
Pfeiffer, Friedhelm, B.S., Max-Planck-Institut für Psychiatrie, Munich, Federal Republic of Germany
Porter, Seth, B.A., Dartmouth Medical School, Hanover, New Hampshire
Sawyer, Carol E., University of Pennsylvania, Philadelphia
Sherman-Gold, Rivka, M.S., Weizmann Institute, Rehovot, Israel
Silberberg, Shai D., B.S., Hadassah Medical School, Jerusalem, Israel
Tempel, Bruce L., M.A., Princeton University, New Jersey
Vergara, Cecilia, B.S., Harvard University, Boston, Massachusetts

SEMINARS

McMahan, J., Stanford University. *Structure of peripheral synapses.*
———. *Structure of central synapses.*
———. *Localization of acetylcholine receptors at the neuromuscular junction.*
———. *Localization of acetylcholinesterase at the neuromuscular junction.*
———. *The vesicle hypothesis.*
———. *Degeneration of the neuromuscular junction.*
———. *Regeneration of the neuromuscular junction.*
Rahamimoff, R., Hebrew University. *Principles of signaling in the nervous system.*

———. *The ionic basis of membrane potentials: The forces.*
———. *Ionic fluxes through excitable membranes.*
———. *The resting membrane potential.*
———. *The action potential: Phenomenology.*
———. *Cable properties and conductance of the action potential.*
———. *The ionic basis of the action potential currents.*
———. *Ionic conductances and models for action potentials.*
———. *Quantal release of transmitter.*
———. *The role of calcium ions in transmitter release.*
———. *Sodium ions as secondary regulators of quantal liberation of acetylcholine.*
———. *Frequency modulation of transmitter liberation.*
Yoshikami, D., University of Utah. *The excitatory postsynaptic potential.*
———. *The ionic basis of the excitatory postsynaptic potential.*
———. *The inhibitory postsynaptic potential.*
———. *Electrical synapses.*
———. *Microphysiology of the neuromuscular junction.*
———. *Slow synaptic potentials.*
———. *Synaptic activity visualized by autoradiography.*
Stevens, C., Yale University. *The voltage dependence of the acetylcholine channel.*
———. *Fluctuation analysis of postsynaptic membrane channels.*
———. *Relaxation techniques in the study of the acetylcholine channel.*
———. *Application of noise analysis and single-channel recording in the study of local anesthetic action and desensitization: Evidence, predictions, and intuition.*
Goodenough, D., Harvard Medical School. *Gap junctions: Structure as revealed by x-ray diffraction and electron microscopy.*
———. *Cell biology of gap junctions.*
Sigworth, F., Max-Planck Institute. *Properties of sodium channels from fluctuation analysis and single-channel recording.*
Corey, D., Yale University. *Techniques of single-channel recording.*
Schwartz, E., University of Chicago. *First events in vision: Generation of response in vertebrate rods.*
Horn, J., Harvard Medical School. *Slow muscarinic inhibition in sympathetic ganglia.*
Purves, D., Washington University School of Medicine. *Specificity in neural development.*
———. *Synaptic rearrangements during development.*
Patterson, P., Harvard Medical School. *The neural crest.*
———. *The role of environment in the development of neurons in tissue culture.*
Jackson, M., University of California, Los Angeles. *Applications of single-channel recording techniques in tissue-cultured muscle and nerve.*
Nicholls, J., Stanford University School of Medicine. *Regeneration in the leech nervous system.*
Jan, L., University of California, San Fransicso. *Peptides in the nervous system.*
———. *Peptidergic transmission in sympathetic ganglia.*
Herbert, E., University of Oregon. *Processing of polyprotein precursors of endocrine ACTH and enkephalens.*
———. *Regulation of synthesis; processing and release of constituent hormones of pro-opiomelanocortin and use of recombinant DNA techniques to study gene expression.*
Bowers, C., Harvard Medical School. *Neural regulation of pineal biochemistry.*
Frank, E., Harvard Medical School. *Monosynaptic reflexes in the spinal cord.*
———. *Synaptic transmission in the spinal cord.*
Shapiro, E. and R. Kretz, Columbia University. *Post-tetanic poteniation of an identified synapse in Aplysia is correlated with a Ca^{2+}dependent K^+ current in the presynaptic neuron: Direct evidence for Ca^2 accumulation.*

Kado, R., CNRS. *Sodium channels in oocytes.*
Lee Rubin, L., Rockefeller University. *Neuromuscular junction development. I and II.*
———. *Neurotoxins.*
Albrecht-Buehler, G., Cold Spring Harbor Laboratory. *Control of tissue cell movement.*
Fambrough, D., Carnegie Institution. *Acetylcholinesterase forms and distribution.*
———. *Acetylcholine receptor structure.*
———. *Acetylcholine receptor turnover.*
———. *Myasthenia gravis.*
———. *Acetylcholine receptor biosynthesis.*
———. *Regulation of acetylcholine receptors.*
Black, I., Cornell College of Medicine. *The catecholamine synapse.*
———. *Postnatal and prenatal development of the catecholamine system.*
———. *Development of the peptidergic system.*
Rahamimoff, H., Hebrew University. *Properties, purification, and reconstitution of calcium transport systems from isolated nerve terminals.*
Raviola, E., Harvard Medical School. *Cell biology of the retina.*
———. *Connectivity in the retina.*
Baylor, D., Stanford University School of Medicine. *Overview of signaling in the retina.*
———. *Visual transduction in rods and cones.*
———. *Electrical analysis of transduction in rods.*
———. *Cell interactions in the vertebrate retina.*
Hudspeth, J., California Institute of Technology. *Mechanical transduction in the acoustico-lateralis systems.*
———. *Physiology of hair cells.*
Chiarandini, D., New York University. *Ca^{2+} electrical activity in mammalian skeletal muscle.*
Stefani, E., Instituto Politecnico Nacional. *Ca^{2+} currents in frog skeletal muscles.*
Zipser, B., Cold Spring Harbor Laboratory. *Studies with monoclonal antibodies to the leech nervous system.*
Ascher, P., Ecole Normale Superieure. *Potassium responses to neurotransmitters in* Aplysia *neurons.*

Molecular Cloning of Eukaryotic Genes, June 30–July 30

INSTRUCTORS

Maniatis, Thomas, Ph.D., Harvard University, Cambridge, Massachusetts
Fritsch, Edward F., Ph.D., Michigan State University, East Lansing
Engel, Doug, Ph.D., Northwestern University, Chicago, Illinois

ASSISTANTS

Weiss, Michael A., B.S., Harvard University, Cambridge, Massachusetts
Van de Woude, Susan, Harvard University, Cambridge, Massachusetts
Bates, Paul F., B.S., Michigan State University, East Lansing

The course is designed to give "hands on" experience in the isolation and analysis of eukaryotic genes. Experiments performed during the course include double-strand cDNA synthesis; poly(dG):poly(dC) tailing; DNA fragment isolation from gels; ^{32}P labeling of nucleic acids in vitro; preparation and isolation of λ arms and preparation of eukaryotic DNA inserts for λ cloning; preparation of in vitro packaging extracts; formation of eukaryotic λ libraries; screening of the eukaryotic DNA libraries in λ phage by plaque hybridization screening and by recombinational screening; formation of libraries in cosmid vectors; specific methods for subcloning fragments of eukaryotic DNA in plasmid vectors; screening of plasmids for insert fragments by size and by filter colony hybridization screening; and Northern and chromosomal Southern blotting analyses. Laboratory demonstrations, prepared by outside experts, detail the identification of recombinants by RNA filter selection and subsequent in vitro translation. Topics covered include the transient replication and expression of genes in SV40 subclones of chromosomal genes transfected into simian cells and the expression of isolated genes introduced into cultured cells by microinjection. Through the seminar series, students are also introduced to a wide variety of topics in which recombinant DNA studies have proven to be indispensable in the analysis of the organization and expression of genes in a wide variety of organisms and eukaryotic viruses.

PARTICIPANTS

Allen, Deborah, B.S., Imperial Cancer Research Fund, London, England
Arai, Ken-ichi, Ph.D., University of Tokyo, Japan

Batter, David K., B.S., Cornell University, New York, New York
Deikman, Jill, B.A., University of California, Berkeley
Diesseroth, Albert B., Ph.D., National Institutes of Health, Bethesda, Maryland
Gefter, Malcolm L., Ph.D., Massachusetts Institute of Technology, Cambridge
Goodbourn, Stephen, B.A., Medical Research Council, Oxford, England
Lusis, Aldons J., Ph.D., University of California, Los Angeles
Magun, Bruce E., Ph.D., University of Arizona, Tucson
Oliver, Noelynn, B.A., Stanford University, California
Petko, Lawrence J., B.A., University of Chicago, Illinois
Riley, Sylvia C., B.S., University of Virginia, Charlottesville
Schopf, Thomas J.M., Ph.D., University of Chicago, Illinois
Sly, William S., M.D., Washington University, St. Louis, Missouri
Van Ommen, Gert-Jan, Ph.D., University of Amsterdam, The Netherlands
Vezza, Anne C., Ph.D., Rockefeller University, New York, New York

SEMINARS

Bishop, M., University of California, San Francisco. *Molecular biology of RNA tumor viruses.*
Davis, R., Stanford University Medical School. *Electron microscopy of DNA.*
——. *Molecular biology of yeast.*
McKnight, S., Carnegie Institution. *Structure and function of eukaryotic promoters.*
Roberts, R., Cold Spring Harbor Laboratory. *Restriction enzymes, DNA sequencing and computer analysis of sequence information.*
Bender, W., Harvard Medical School. *Molecular biology of* Drosophila melanogaster.
Wigler, M., Cold Spring Harbor Laboratory. *DNA mediated gene transfer.*
Seed, B., Harvard University. *Gene isolation by recombination in* E. coli.
Sambrook, J., Cold Spring Harbor Laboratory. *DNA tumor viruses.*
DiMaio, D., Harvard University. *Directed mutagenesis.*
Rubin, G., Harvard Medical School. *Transposable elements in* Drosophila.
Mulligan, R., Massachusetts Institute of Technology. *Eukaryotic cloning and expression vectors.*
Roberts, B., Harvard Medical School. *Identification of specific cDNA clones by hybrid-selection/translation procedures.*
Grodzicker, T., Cold Spring Harbor Laboratory. *Molecular biology of adenoviruses.*
Mellon, P., Harvard University. *Transient expression of cloned genes in cells in culture.*
Efstratiadis, A., Harvard Medical School. *Eukaryotic gene structure and evolution.*
Engel, D., Northwestern University. *Chicken globin genes.*
Maniatis, T., Harvard University. *Molecular genetics of human globin genes.*
Ptashne, M., Harvard University. *Expression of eukaryotic genes in bacteria.*
Ausubel, F., Harvard University. *Molecular biology of plants.*
Hood, L., California Institute of Technology. *Molecular basis of antibody diversity.*
Capecchi, M., University of Utah. *Microinjection of cloned DNA into cells in culture.*

Advanced Bacterial Genetics, June 30-July 20

INSTRUCTORS

Berman, Michael, Ph.D., Frederick Cancer Research Center, Frederick, Maryland
Enquist, Lynn W., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Silhavy, Thomas, Ph.D., Frederick Cancer Research Center, Frederick, Maryland

ASSISTANTS

Bear, Susan, Ph.D., National Institutes of Health, Bethesda, Maryland
Emr, Scott, Ph.D., Frederick Cancer Research Center, Frederick, Maryland
Jackson, Dolores, B.S., Frederick Cancer Research Center, Frederick, Maryland

Students performed a variety of genetic manipulations in the bacterium *E. coli* using the insertion element Tn*10*. Insertions which prevent utilization of maltose, ribose, and galactose were isolated and characterized genetically. In addition, insertions in a positive regulatory gene required for expression of the major outer membrane porin proteins were isolated. Insertions near and in the structural genes for

these porin proteins were isolated as well. Certain *Tn10* insertions were used as a selective marker to perform localized mutagenesis of particular regions of the chromosome. From such experiments, a number of deletion and point mutations were obtained.

The genetic analysis of porin regulation relied on the use of fusions between these genes and the *lac* operon. The students isolated additional gene fusions in vivo and in vitro. Using a defective Mu phage, fusions between *lacZ* and various target genes were constructed in vivo. The students isolated specialized transducing phages carrying different operon fusions and then used in vitro recombination to clone these fusions on a plasmid vector. In other experiments random DNA restriction enzyme fragments were cloned into a plasmid vector allowing the isolation of gene fusion encoding hybrid β-galactosidase proteins. The students analyzed the plasmid clones for the extent of inserted DNA and the relative levels of β-galactosidase production.

Phage λ vectors carrying DNA fragments encoding either the porin regulatory gene, *ompR* or proline biosynthetic genes were used in mutagenesis experiments. Students isolated deletion and point mutations in these genes and characterized them by genetic crosses and restriction enzyme analysis. These phages were used to map genetically various chromosomal mutations obtained by the students.

Gene libraries of *E. coli* and several other gram-negative bacteria were constructed using phage λ. Pools of these hybrid phages were screened for phages carrying porin regulatory genes and β-galactosidase genes. Students identified and characterized such phages using complementation selection, plaque hybridization, and restriction analysis.

PARTICIPANTS

Baird, David G., Ph.D., Agricultural Research Council, Newbury, England
Belas, M. Robert, B.A., University of Maryland, College Park
Downs, Karen M., M.S., University of Illinois, Chicago
Fahnestock, Stephen R., Ph.D., University of Wisconsin, Madison
Goff, Stephen A., B.A., Harvard Medical School, Cambridge, Massachusetts
Hoiseth, Susan K., B.A., Stanford University, California
Hollstein, Monica C., Ph.D., University of California, Berkeley
Hove-Jensen, Bjarne, Institute of Biological Chemistry, Copenhagen, Denmark
Jones, Robin M., M.S., University of Western Ontario, London, Canada
Lohman, Timothy M., Ph.D., University of Oregon, Eugene
Maloy, Stanley R., Ph.D., University of California, Irvine
Masker, Warren E., Ph.D., Oak Ridge National Laboratory, Tennessee
Reha-Krantz, Linda, Ph.D., University of Alberta, Edmonton, Canada
Schweizer, Herbert P., B.A., University of Konstanz, Federal Republic of Germany
Shurvinton, Claire E., B.S., University of Nottingham, England
Vaisanen, Eino, B.S., University of Helsinki, Finland

SEMINARS

Stahl, F., University of Oregon. *Update on Chi, and* E. coli *recombinator.*
Heffron, F., Cold Spring Harbor Laboratory. *Transposable elements.*
Gottesman, M., NCI, National Institutes of Health. *The bacteriophage lambda: An overview.*

———. *Problems of gene regulation in bacteriophage lambda.*

Davis, R., Stanford University School of Medicine. *Expression of heterologous genes in* E. coli.

Weinstock, G., Frederick Cancer Research Center. *Application of new techniques to new problems in bacterial genetics.*

Botstein, D., Massachusetts Institute of Technology. *Applying new technology to old problems in bacterial genetics.*

Roth, J., University of Utah. *Why the chromosome is organized the way it is.*

Ptashne, M., Harvard University. *How the λ repressor and Cro work.*

Sternberg, N., Frederick Cancer Research Center. *Dissection and analysis of components that regulate the replication and partitioning of the stringent plasmid replicon P1.*

Messing, J., University of Minnesota. *DNA template selection by cloning.*

Basic Neuroanatomical Methods, July 5–July 25

INSTRUCTORS

Jones, Edward G., M.D., Ph.D., Washington University Medical School, St. Louis, Missouri
Hand, Peter, D.V.M., Ph.D., University of Pennsylvania, Philadelphia
Pickel, Virginia M., Ph.D., Cornell University Medical College, New York, New York

ASSISTANTS

Wise, Steven P., Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Hendry, Stewart H.C., B.A., Washington University, St. Louis, Missouri
McClure, Bertha, Washington University, St. Louis, Missouri
Hand, Carol, B.A., University of Pennsylvania, Philadelphia

This was primarily a laboratory course designed for research workers interested in learning basic and advanced neuroanatomical techniques. Participants carried out an extensive set of laboratory exercises involving most of the commonly used methods and there were lectures and discussions by the instructors and visiting speakers.

Classical methods covered included: perfusion fixation; embedding in different media; frozen, paraffin, celloidin, and plastic sectioning; conventional cell staining methods; lesion making; silver impregnation of normal and degenerating nerve fibers; brightfield, darkfield, and fluorescence microscopy, photomicrography, and electron microscopy.

Newly developed methods included histochemical, radiochemical, and immunocytochemical methods for tracing neuronal pathways in terms of axoplasmically transported material, neurotransmitter-related compounds, or metabolic activity.

PARTICIPANTS

Commins, Deborah L., B.A., University of California, Irvine
Denaro, Frank J., M.A., State University of New York, Stony Brook
Dodd, Jane, Ph.D., Harvard Medical School, Boston, Massachusetts
Eckmiller, Marion, University of Dusseldorf, Federal Republic of Germany
Fujita, Shinobu C., Ph.D., California Institute of Technology, Pasadena
Manning, Donald C., B.S., Johns Hopkins University, Baltimore, Maryland
McKay, Ronald, Ph.D., Cold Spring Harbor Laboratory, New York
Millington, William R., Ph.D., Johns Hopkins University, Baltimore, Maryland
Sclar, Gary, M.S., University of California, Berkeley
Wirsig, Celest, B.A., University of Florida, Gainesville

SEMINARS

Hendrickson, A., University of Washington. *Autoradiographic methods.*
Swanson, L., Salk Institute. *Flourescent dye tracers.*
Cuello, C., University of Oxford. *Monoclonal antibodies.*
Peters, A., Boston University. *The golgi-EM method.*
Kelly, D., Princeton University. *The initiated 2 D-G method.*
LaVail, J., University of California, San Francisco. *Retrograde axonal transport.*
Raviola, A., Harvard University. *Freeze fracture-etch methods.*

Electrophysiological Methods, July 21–August 10

INSTRUCTORS

Kehoe, JacSue, Ph.D., Ecole Normale Superieure, Paris, France
Chiarandini, Dante, M.D., New York University, New York
Stefani, Enrique, M.D., Instituto Politecnico Nacional, Mexico City, Mexico

GUEST LECTURER

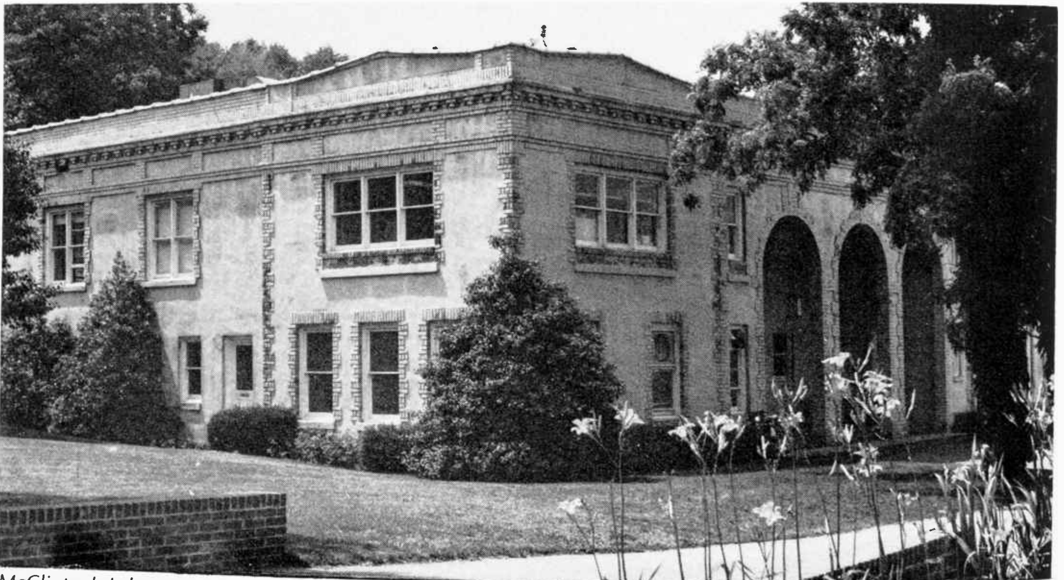
Kado, Ray, Ph.D., Centre National de la Recherche Scientifique, Gif-sur-Yvette, France

In this neurobiology course the neuromuscular junction of the frog and the central ganglia of the mollusk *Aplysia* were used as experimental preparations for training students in basic electrophysiological methods for cellular neurobiology. Examination of certain characteristics of the resting, action and synaptic potentials of these two preparations served as a basis for introducing the following techniques: microdissection, fabrication of single and multibarreled capillary microelectrodes; intracellular recording of membrane voltage changes (in so-called current clamp) and membrane currents (using a slow voltage clamp); intracellular and extracellular application of ions and drugs (by ionophoresis and pressure injections); and intracellular staining of *Aplysia* neurons.

The first 3 days of the course were devoted to lectures and exercises on electronics for cellular neurobiologists given by Ray Kado. The last 2 days of the course were devoted to individual experimental projects chosen by the students. These projects permitted them to try techniques not taught in the course and to use biological preparations that they intended to study when they returned to their own laboratories.

PARTICIPANTS

Chapron, Yves, D.Sc., CENG, Grenoble, France
Chesler, Mitchell, M.A., New York University, New York
Corman, Bruno J., Ph.D., Centre d'Etudes Nucleaires, Saclay, France
Davis, Robin L., M.A., Stanford University, California
Evans, Michael G., B.S., University of Bristol, England
Gurney, Alison M., B.S., University College London, England
Ignatius, Michael J., B.S., Stanford University, California
Penit, Jacqueline, College de France, Paris
Rubin, Leona J., University of Colorado, Boulder
Tublitz, Nathan, B.A., University of Washington, Seattle



McClintock Laboratory

Introduction of Macromolecules into Mammalian Cells

July 22–August 10

INSTRUCTORS

Capecchi, Mario, Ph.D., University of Utah, Salt Lake City
Cooper, Geoffrey, Ph.D., Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

ASSISTANTS

Lane, Mary Ann, Ph.D., Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts
Fraser, Laurie, B.S., University of Utah, Salt Lake City

This laboratory course focused on methods of introducing biologically active macromolecules into living cells. Emphasis was placed on transferring DNA into cultured cells by transfection and by nuclear injection using glass micropipettes. Laboratory exercises included (1) transfection of DNA containing viral and cellular transforming genes; (2) microinjection and transfection of DNAs containing selectable biochemical markers; and (3) introduction of proteins into cells by fusion with loaded red cell ghosts and liposomes.

These exercises were supplemented with group discussions and lectures by invited speakers who considered the application of these methods to the isolation and functional characterization of structural genes and their regulatory sequences, evaluation of in vitro mutagenesis, and analysis of the biological activity of proteins and messenger RNAs.

PARTICIPANTS

Bond, Beverely J., B.A., California Institute of Technology, Pasadena
Caboche, Michel C., Ph.D., INRA, Versailles, France
Carbone, David P., B.A., Johns Hopkins University, Baltimore, Maryland
Chaudry, Furzana, B.S., Imperial Cancer Research Fund, London, England
Chin, Daniel J., Ph.D., University of Texas, Dallas
Crews, Stephen T., B.A., California Institute of Technology, Pasadena
Dyson, Julian, B.S., Imperial Cancer Research Fund, London, England
Glanville, Niall F., Ph.D., Fred Hutchinson Cancer Center, Seattle, Washington
Heinrich, Guenther F., Ph.D., Institute for Immunology, Basel, Switzerland
Jackson, Marian J., M.S., Albert Einstein College, Bronx, New York
James, Robert J., B.S., Washington University, Seattle
Katinka, Michael D., Ph.D., Pasteur Institute, Paris, France
Maresca, Antonella, Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Nowak, Barbara, M.S., University of Erlangen, Federal Republic of Germany
Santangelo, George M., B.A., Yale University, New Haven, Connecticut
Wang, Yuan, Max-Planck-Institut für Biochemie, Munich, Federal Republic of Germany

SEMINARS

Cooper, G., Sidney Farber Cancer Institute. *Analysis of cellular transforming genes by transfection.*
Capecchi, M., University of Utah. *DNA transfer by microinjection.*
Kurtz, D., Cold Spring Harbor Laboratory. *Hormonal control of gene expression.*
Scolnick, E.M., NCI, National Institutes of Health. *Biogical activities of SFFV DNA.*
Van de Woude, G., NCI, National Institutes of Health. *Cellular mechanisms associated with the expression of the transforming phenotype of transfected DNA.*
Weinberg, R., Massachusetts Institute of Technology. *Transforming sequences and proteins of nonvirus-induced tumors.*
Wigler, M., Cold Spring Harbor Laboratory. *DNA mediated gene transfer as a genetic tool.*
Ruddle, F., Yale University. *Gene transfer by chromosomes, DNA, and microinjection.*
McBride, W.W., National Institutes of Health. *Chromosome-mediated gene transfer.*
Stacey, D., Roche Institute. *Biological activities of injected retroviral DNA molecules.*
Grodzicker, T., Cold Spring Harbor Laboratory. *Introduction of foreign genes into mammalian cells using adenovirus vectors.*
Feramisco, J., Cold Spring Harbor Laboratory. *Microinjection of antibodies in living cells.*
Hager, G., National Cancer Institute. *Hormone-responsive regulatory sequences of mouse mammary tumor virus DNA.*
Livingston, D., Sidney Farber Cancer Institute. *Biological activities of SV40 T antigens.*
Mulligan, R., Massachusetts Institute of Technology. *Animal cell cloning vehicles.*
Fraley, R., Monsanto Company. *Liposome-mediated DNA transfer.*

Yeast Genetics, July 22-August 10

INSTRUCTORS

Fink, Gerald, Ph.D., Cornell University, Ithaca, New York
Sherman, Fred, Ph.D., University of Rochester, New York
Hicks, James, Ph.D., Cold Spring Harbor Laboratory, New York

ASSISTANT

Kosiba, Bradley, University of Rochester, New York

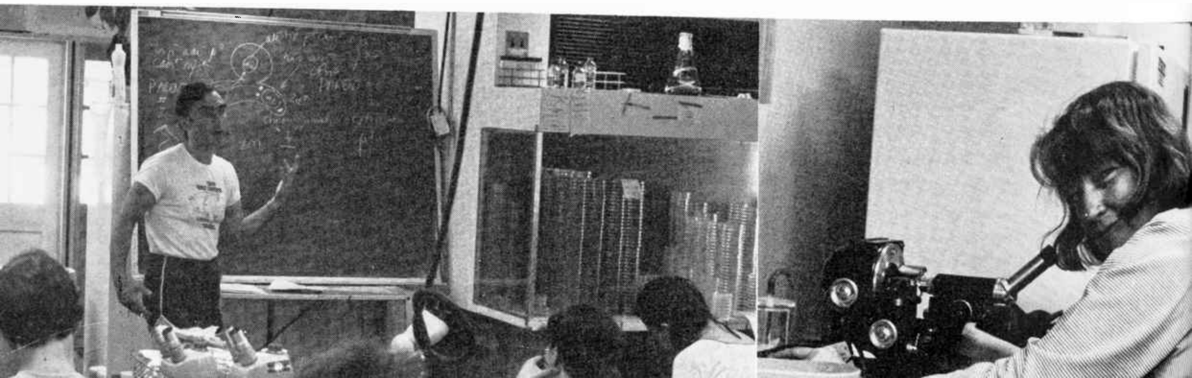
This program emphasized the major techniques used in molecular biology and genetic analysis of yeast, including the following: the isolation and characterization of chromosomal and mitochondrial mutants; tetrad analysis; chromosomal assignment of unknown numbers; mitotic recombination; allelic and complementation tests; and analysis of heterothallic and homothallic characters. Recombinant DNA techniques including yeast transformation, filter hybridization, and gel electrophoresis were applied to cloning and genetic analysis of yeast DNA.

PARTICIPANTS

Arai, Naoko, Ph.D., University of Tokyo, Japan
Ballario, Paola, B.S., University of Rome, Italy
Engler, Michael J., Ph.D., Harvard Medical School, Boston, Massachusetts
Gonzalez, Alicia M., M.S., Universidad Nacional, Mexico City, Mexico
Gupta, Naba K., Ph.D., University of Nebraska, Lincoln
Hieter, Philip A., B.A., National Institutes of Health, Bethesda, Maryland
Hsu, Yun-Pung, Ph.D., Purdue University, West Lafayette, Indiana
Lindquist, Susan L., Ph.D., University of Chicago, Illinois
Locht, Camille, M.S., Catholic University of Louvain, Belgium
Proffitt, John H., B.S., Oregon State University, Cornvallis
Pulitzer, John F., Ph.D., International Institute of Genetics, Naples, Italy
Sadowski, Paul D., Ph.D., University of Toronto, Ontario, Canada
Schmeissner, Ursula M., Ph.D., NCI, National Institutes of Health, Bethesda, Maryland
Stahl, Franklin W., Ph.D., University of Oregon, Eugene
Waxman, Lloyd H., Ph.D., Harvard Medical School, Boston, Massachusetts
Yocum, Rogers, Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Dutcher, S.K., Rockefeller University, *Pleiotrophic effects of cell-division cycle mutants.*
Broach, J., State University of New York, Stony Brook. *The yeast plasmid Scp1.*
Petes, T.D., University of Chicago. *Structure and replication of yeast DNA*
———. *Genetic analysis of repeated genes.*
Warner, J., Albert Einstein College of Medicine. *Coordinate expression of ribosomal genes.*
McLaughlin, C.S., University of California, Irvine. *Protein synthesis.*
Hicks, J., Cold Spring Harbor Laboratory. *Regulation of mating types.*
Thorner, J.W., University of California, Berkeley. *Control of conjugation by pheromones.*
Mortimer, R.K., University of California, Berkeley. *Genetic mapping.*
Fink, G.R., Cornell University. *Transposable elements.*
Homison, G., Columbia University Medical School. *Nuclear genes controlling mitochondrial gene expression.*



Hereford, L.M., Brandeis University. *Regulation of histone synthesis.*

Rosbash, M., Brandeis University. *Organization, expression, and splicing of ribosomal protein genes.*

Sherman, F., University of Rochester. *Controlling regions of the cytochrome c genes.*

Esposito, R.E., University of Chicago. *Meiosis and ascospore formation.*

Szostak, J.W., Sidney Farber Cancer Institute. *Telomeres, damaged-induced genes, and mitotic recombination.*

Saunders, C., Oregon State University. *Chromatin structure in active and inactive genes.*

UNDERGRADUATE SUMMER RESEARCH PROGRAM

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 230 students have completed the course, and many have gone on to creative careers in biological sciences.

The objectives of the program are to provide (1) a greater understanding of the fundamental principals of biology, (2) an increased awareness of major problem areas under investigation, (3) better understanding of the physical and intellectual tools for modern research and the pertinence of this information to future training, and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from a large number of applicants, took part in the program, which was supported by The Camille and Henry Dreyfus Foundation, Inc. They are listed below with their laboratory sponsors and topics of research.

Kristen M. Clarke, University of Pennsylvania
Research Advisor: R. Roberts
M13 as a chimeric protein cloning system.

Lindsey Ann Criswell, University of California, Berkeley
Research Advisor: B. Stillman
Characterization of temperature-sensitive mutants of adenovirus 2.

Lisa Haas, University of California, Berkeley
Research Advisor: J. Hicks
Mapping of cloned pieces of yeast DNA which complement mutations in positve and negative regulatory elements for the unexpressed mating type loci.

Jonathan Miller, Yale University
Research Advisor: J. Stringer
Viral RNA levels in rat cells transformed by an SV40 T-antigen mutant.

Nancy Mills, Harvard University
Research Advisor: D. Zipser
The promoter region of the herpes virus thymidine kinase gene.

Roger Mosesson, Columbia College
Research Advisor: R. Harshey
Construction of mini-Mu plasmid vectors that can be used for cloning.

Craig Okada, University of Utah
Research Advisor: F. Heffron
I. *cis* inhibition of transposition.
II. Construction of a plasmid to study deletions associated with transposons.

Ron Sapolsky, University of Rochester
Research Advisor: J. Smart
Tryptic peptide mapping and sequencing analysis of proteins from adenovirus serotype 2 early regions.

Eric S. Schulze, University of California, Berkeley
Research Advisor: S. Blose
The midbody—A functional and molecular perspective.

Nick Theodorakis, Washington University
Research Advisor: S. Hughes
Sequencing the chicken β-actin gene.

Olney Fellow
Mirjana Nesin, University of Belgrade
Research Advisor: M. Wigler
Searching for human and murine transposons.

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 post-graduate staff to develop their skills in defending, organizing, and presenting their research. In addition to those listed below, seminars were given by many others involved in research at this laboratory.

1980–1981

November

Steve Dellaporta, Worcester Polytechnic Institute, Massachusetts: Direct transformation of plant protoplasts with Ti DNA.

Susumu Tonegawa, Basel Institute for Immunology, Switzerland: Isolation of the "D" region of an immunoglobin gene.

December

Hal Weintraub, Hutchinson Cancer Center, Seattle, Washington: Regulation and chromatin structure of hemoglobin genes during development.

Russell Malmberg, Michigan State University, Lansing: Biochemical, cellular, and developmental characterization of tobacco mutants that alter polyamine metabolism.

James Shapiro, University of Chicago, Illinois: The role of cointegrate resolution in the mobilization of unique DNA by transposable elements.

January

David Helfman, Emory University School of Medicine, Atlanta, Georgia: Cyclic CMP phosphodiesterase: Occurence, biological involvement, purification and characterization.

Nick Cowan, Princeton University, New Jersey: The structure of human tubulin genes.

Richard Young, Swiss Institute for Experimental Cancer Research, Lausanne: A single mouse amylase gene specifies two different tissue specific mRNAs.

February

Alice Fulton, Massachusetts Institute of Technology, Cambridge: The cytoskeletal network—Developmental reorganization and assembly in vitro and in vivo.

K. Mizuuchi, National Institutes of Health, Bethesda, Maryland: Integration in vitro.

James Broach, State University of New York, Stony Brook: Replication and recombination of the yeast 2μ plasmid.

C.J. Bostock, Medical Research Council Laboratories, University of Edinburgh, Scotland: Gene amplification in methotrexate resistant cells.

Winship Herr, Harvard University, Cambridge, Massachusetts: Probing for recombinant RNA tumor viruses in leukemic AKR mice.

March

Hugh Pelham, Carnegie Institution, Baltimore, Maryland: Control of 5S RNA synthesis.

Sara Lavi, Weizmann Institute, Rehovet, Israel: Carcinogen mediated amplification of specific DNA sequence in Chinese hamster cells.

Francis Galibert, L'Hopital Saint-Louis, Paris, France: Comparison of the nucleotide sequence of the genome of the human hepatitis B virus and the woodchuck virus.

James Haber, Brandeis University, Waltham, Massachusetts: Mechanism of yeast mating type genes transposition.

S. Rosenblatt, Weizmann Institute, Rehovet, Israel: Molecular biology of measles.

Bill Hayward, Rockefeller University, New York: ALV induced lymphoid leukosis—Activation of a cellular *onc* gene by promoter instertion.

Walter Doerfler, University of Cologne, Germany: Integration expression, and methylation of viral DNA in adenovirus type 2 and type 12 transformed and tumor cells.

Merilyn Sleigh, CSIRO, Sidney, Australia: The influenza virus haemagglutinin gene—Cloning, sequencing, and evolution.

April

Olof Sundin, Massachusetts Institute of Technology, Cambridge: Segregation leads to accumulation of catenated dimers—Dissection of the final stages of SV40 replication.

Alex Goldfarb, Max-Planck Institute, Munich, Federal Republic of Germany: Modulation of RNA polymerase activity by bacteriophage T4-induced modification of the alpha subunit.

Walter Keller, University of Heidelberg, Germany: Transcription and processing of adenoviral RNA with extracts from HeLa cells.

Werner Gobel, University of Wurzburg, Federal Republic of Germany: Hemolysin production by pathogenic *E. coli.*

May

Mark Van Montagu, Rijks University, Gent, Belgium: A little something about *Agrobacterium.*

June

Elizabeth Taparowski, Brown University, Providence, Rhode Island: Evolution of bovine satellite sequences.

Mike Krangel, Harvard Biological Laboratories, Cambridge, Massachusetts: Biosynthesis of histocompatibility antigens in normal and mutant cell lines.

July

Rolf Kemler, Pasteur Institute, Paris, France: Teratocarcinoma differentiation and the cytoskeleton.

August

David White, University of Melbourne, Australia: Antigenic determinants of influenza virus haemagglutinin recognized by T_h, T_s, T_d and B lymphocytes.

Walter Schaffner, University of Zurich, Switzerland: Expression of a genomic rabbit beta-globin gene is enhanced by a small segment of SV40 or polyoma DNA.

Karin Esami, University of Western Ontario, Canada: Aspect of the biogenesis of vaccinia virus.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and high school students the opportunity to acquire a greater understanding of their environment. Through a series of specialized field courses, students can engage in such introductory programs as Nature Detective, Seashore Life, and Pebble Pups, as well as more advanced programs such as Marine Biology and Nature Photography.

During the summer a total of 412 students participated in the Nature Study Program. Most classes were held outdoors, when weather permitted, or at the Uplands Farm Nature Preserve of the Long Island Chapter of the Nature Conservancy. The Laboratory has equipped and maintains a darkroom and classroom-laboratories at Uplands Farm for the study of field specimens collected by the students.

In addition to the four-week courses, a series of one-day marine biology workshops was offered to students, with a special workshop for adults. Studies on the marine ecology of Long Island Sound were conducted aboard the 66-foot schooner J.N. Carter chartered from Schooner, Inc. of New Haven, Connecticut. This vessel is equipped with the instrumentation necessary to study the Sound chemically, physically, and biologically. Students participated in these studies and in the actual sailing of the vessel.

PROGRAM DIRECTOR

Edward Tronolone, M.S., P.D., Science Curriculum Associate, East Williston Public Schools.

INSTRUCTORS

Rose Becker, M.S., Museum of Long Island Natural Sciences, State University of New York, Stony Brook
Kathryn Bott, M.S., Science teacher, Friends Academy
Gail Claydon, B.S., teacher, United Methodist Church of Huntington-Cold Spring Harbor
Robert Jaeger, M.S., science teacher, Mineola High School
Katherine Jindra, M.S., naturalist, Nassau County BOCES
Fred Maasch, M.Ed., biology teacher, Islip High School
James Romansky, M.S., biology teacher, Bay Shore High School
Bernadette Voras, M.S., science teacher, Shoreham-Wading River High School

COURSES

Nature Bugs
Nature Detectives
Advanced Nature Study
Pebble Pups
Rock Hounds
They Swim, Crawl, and Walk
Marine Biology
Nature Photography I & II
Fresh Water Life
Adventure Education
Marine Biology Workshop
Bird Study

LABORATORY STAFF

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J. D. Watson

ADMINISTRATIVE DIRECTOR
William R. Udry

ASSISTANT DIRECTOR FOR RESEARCH
Joseph Sambrook

DIRECTOR BANBURY CENTER
Victor McElheny

RESEARCH SCIENTISTS

Guenter Albrecht-Beuhler
Stephen Blose
Thomas Broker
Ahmad Bukhari
Louise Chow
Jeffrey Engler
James Feramisco
John Fiddes
James Garrels
Thomas Gingeras
Yakov Gluzman
Terri Grodzicker
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James Hicks
Susan Hockfield
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Amar Klar
David Kurtz
Jim Lin
Michael Mathews
Ronald McKay
Manuel Perucho
Richard Roberts
Lee Silver
Magdalene So
Bruce Stillman
Jeffrey Strathern
James Stringer
Fuyuhiko Tamanoi
William Topp
Michael Wigler
Birgit Zipser
David Zipser

VISITING SCIENTSTS

Nancy Hogg
Jorgen Johansen
Yoshiki Katoh
Pei-Mao Lin
Michele Manos
Mara Rossini

Staff as of December 1981

POSTDOCTORAL FELLOWS

Judith Abraham
Bruce Anderson
Marilyn Anderson
Frederick Asselbergs
Janet Brandsma
Joan Brooks
Stephen Dellaporta
Hans Engeser
Michele Francoeur
Greg Freyer
Richard Frisque
Mitchell Goldfarb
Marie Goradia
Richard Guilfoyle
Mary Harper
David Helfman
Margaret Hightower
John Ivy
Tohru Kamata
Thomas Kost
Deborah Kwoh
Theodore Kwoh
Chao-Hung Lee
Saundra Lemaster
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Russell Malmberg
Fumio Matsumura
Catherine Monaghan
Kim Nasmyth
Kenji Shimizu
Joseph Sorge
Olof Sundin
G. Paul Thomas
Nikos Vamvakopoulos
William Welch
Stuart Weisbrod
Masao Yamada
Clifford Yen

GRADUATE STUDENTS

Jayne Danska
Douglas Hanahan
Richard Kostricken
Daniel Levy
Zarina Manzoor
Pamela Rosman
Nora Sarvetnick
Kevin Van Doren

RESEARCH ASSISTANTS

Barbara Ahrens
Georgia Binns
William Boorstein
Ann Bushnell
Michael Cahn
Angela Calasso
Lynn Cascio
Terrance Chisum
Elizabeth Chow
Elizabeth Cilibrasi
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Concepcion Lama
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Linda Van der Wal
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Margaret Wallace
Jeanne Wiggins
Mary Wong
Jonathan Wood
Elizabeth Woodruff

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Joyce Noe
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James Sabin
Roberta Salant
Susan Schultz
Martin Spiegel
Jacqueline Terrence

GENETICS RESEARCH UNIT CARNEGIE INSTITUTION OF WASHINGTON

Alfred D. Hershey
Barbara McClintock

SUPPORTING STAFF

Patricia Barkley
Maureen Berejka
Gladys Blose
Robert Borruso
Louisa Dalessandro
Nancy D'Anna
August Dulis
Steven Eagels
Marilyn Goodwin
Gladys Kist
Eric Lockhart
Deborah Lukralle
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Michael Ockler
Herb Parsons
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Marlene Rubino
Andrea Stephenson
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Barbara Ward

PUBLICATIONS DEPARTMENT

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Judith Cuddihy
Nadine Dumser
Nancy Ford
Annette Kirk
Joan McPartlin
Douglas Owen
Elizabeth Ritcey

LIBRARY STAFF/ MARKETING

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Ellen DeWeerdt
Genemary Falvey
Susan Gensel
Amy Gibson
David Gibson
Karen Herrmann
Laura Hyman
Colleen Lanahan
Audrey Powers
Regina Schwarz

BUILDINGS AND GROUNDS DEPARTMENT

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Joseph Brodawchuk
Carol Caldarelli
Vincent Carey
Ronald Chambers
Barbara Cuff
Peter Dunn
Warren Eddy
Joseph Ellis
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Lance LaBella
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Charles Marshall
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John Meyer
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Diamond Scarduzio
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Lane Smith
Peter Stahl
Owen Stewart
Patrick Sullivan
Hans Trede
Dorothy Youngs

BANBURY CENTER

Christine Dacier
Katya Davey
Margaret McEvoy
Lynda Moran
Beatrice Toliver

FINANCIAL STATEMENT

BALANCE SHEET
year ended December 31, 1981
with comparative figures for year ended December 31, 1980

ASSETS

	1981	1980
CURRENT FUNDS		
Unrestricted		
Cash and Short-term investments	$ 1,062,039	1,343,002
Accounts Receivable	267,888	440,985
Prepaid expenses and other assets	349,506	82,112
Inventory of books	131,461	90,188
Due from Banbury Center	146,373	128,785
Total unrestricted	**1,957,267**	**2,085,072**
Restricted		
Grants and contracts receivable	2,845,237	2,540,887
Due from unrestricted fund	110,702	221,538
Total restricted	2,955,939	2,762,425
Total current funds	$ 4,913,206	4,847,497
ENDOWMENT FUNDS		
Robertson Research Fund		
Cash	395,840	(24,084)
Marketable securities (quoted market 1981—$11,111,405; 1980—$10,798,472)	10,507,785	9,016,253
Total Robertson Research Fund	10,903,625	8,992,169

LIABILITIES AND FUND BALANCES

	1981	1980
CURRENT FUNDS		
Unrestricted		
Accounts payable	$ 438,074	327,289
Mortgage payable	66,600	133,200
Due to plant fund	30,850	808,714
Due to restricted fund	110,702	221,538
Fund balance	1,311,041	594,331
Total unrestricted	1,957,267	2,085,072
Restricted		
Fund balance	2,955,939	2,762,425
Total restricted	2,955,939	2,762,425
Total current funds	$ 4,913,206	4,847,497

Olney Memorial Fund		
Cash	90	517
Marketable Securities (quoted market 1981—$20,355; 1980—$19,341)	27,538	27,538
Total Olney Memorial Fund	27,628	28,055
Total endowment funds	**$ 10,931,253**	**9,020,224**

Fund balance	$ 10,931,253	9,020,224

PLANT FUNDS

Investments	517,021	245,665
Due from unrestricted fund	30,850	808,714
Land and improvements	966,110	966,110
Buildings	6,513,210	6,006,958
Furniture, fixtures and equipment	1,408,503	1,255,429
Books and periodicals	365,630	365,630
Construction in progress	585,779	371,787
	10,387,103	10,020,293
Less allowance for depreciation and amortization	2,865,421	2,388,005
Total plant funds	7,521,682	7,632,288

PLANT FUNDS

Fund balance	$ 7,521,682	7,632,288

BANBURY CENTER

Current funds		
Unrestricted		
Cash	$ 700	600
Accounts receivable	8,101	26,293
Inventory of books	47,419	—
Due from Banbury restricted fund	42,314	—
Total unrestricted	98,354	26,893
Restricted		
Grants and contracts receivable	113,440	—
Total restricted	113,440	—
Total current funds	**211,974**	**26,893**
Endowment Funds		
Robertson Maintenance Fund		
Cash	21,660	(16,314)
Marketable securities (quoted market 1981—$1,937,629; 1980—$1,895,985)	1,882,356	1,630,397
Total endowment funds	1,904,016	1,614,083

BANBURY CENTER

Current funds		
Unrestricted		
Accounts payable	$ 85,406	57,733
Due to CSHL unrestricted fund	146,373	128,785
Fund balance	(133,245)	(159,625)
Total unrestricted	98,354	26,893
Restricted		
Due to Banbury unrestricted	42,314	—
Fund balance	71,126	—
Total restricted	113,440	—
Total current funds	211,974	26,893
Endowment funds		
Endowment funds balance	1,904,016	1,614,083

Plant funds		
Land	772,500	772,500
Buildings	792,757	412,672
Furniture, fixtures and equipment	176,285	176,285
Construction in progress	1,386	368,149
	1,742,928	1,729,606
Less allowance for depreciation	221,356	173,596
Total plant funds	1,521,572	1,556,010
Total Banbury Center	$ 3,637,562	3,196,986
Total—All funds	$ 27,003,703	24,696,995

Plant funds		
Plant funds balance	**1,521,572**	**1,556,010**
Total Banbury Center	$ 3,637,562	3,196,986
Total—All funds	$ 27,003,703	24,696,995

CURRENT REVENUES, EXPENSES AND TRANSFERS
year ended December 31, 1981
with comparative figures for year ended December 31, 1980

COLD SPRING HARBOR LABORATORY

	1981	1980
REVENUES		
Grants and contracts	$ 5,691,966	4,066,773
Indirect cost allowances on grants and contracts	2,402,569	1,910,098
Contributions		
Unrestricted	51,273	74,334
Restricted and capital	33,367	40,587
Long Island Biological Association	—	120,000
Robertson Research Fund Distribution	412,000	375,000
Summer programs	363,967	287,634
Laboratory rental	20,732	20,732
Marina rental	48,401	43,370
Investment income	266,752	181,050
Publications sales	838,687	896,245
Dining hall	387,436	327,328
Rooms and apartments	235,798	215,686
Other sources	26,211	97
Total revenues	$ 10,779,148	8,558,934
EXPENSES		
Research*	4,616,825	3,782,095
Summer programs*	747,519	631,402
Library	182,990	153,052
Operation and maintenance of plant	1,378,817	1,137,772
General and administrative	1,100,037	910,581
Depreciation	475,136	430,800
Publications sales*	655,911	656,142
Dining hall*	425,887	406,292
Total expenses	$ 9,583,122	8,108,136

BANBURY CENTER

	1981	1980
REVENUES		
Endowment income	$ 90,000	80,000
Grants & contributions	277,003	87,800
Rooms and apartments	40,196	23,027
Publications	139,455	176,575
Transfer from Cold Spring Harbor Laboratory	60,264	378,345
Total revenues	606,918	745,747
EXPENSES		
Conferences	118,715	49,182
Publications	182,079	204,210
Operation and maintenance of plant	109,199	99,564
Program administration	183,610	152,708
Depreciation	48,537	43,200
Capital plant	13,315	330,500
Total expenditures	655,455	879,364
Excess (deficit) of revenues over expenses	$ (48,537)	(133,617)

TRANSFERS		
Capital building projects	1,115,471	875,673
Banbury Center	60,264	378,345
Total transfers	1,175,735	1,254,018
Total expenses and transfers	10,758,857	9,362,154
Excess (deficit) of revenues over expenses and transfers	$ 20,291	(803,220)**

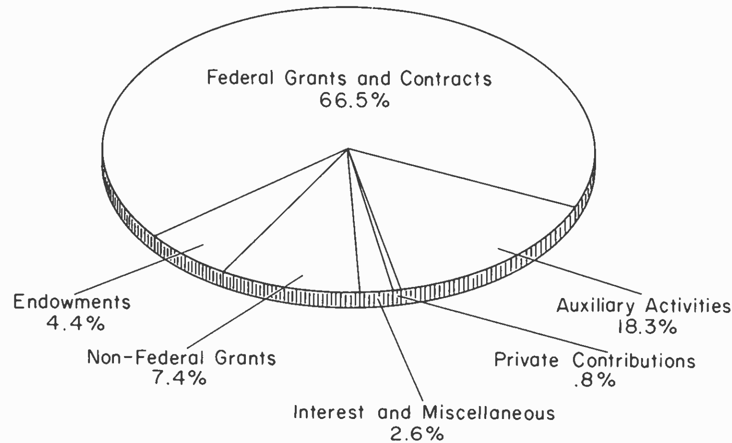
**Reported exclusive of an allocation for operation and maintenance of plant, general and administrative, library, and depreciation expenses.*

***1980 deficit primarily caused by conversion of current funds to plant funds for capital building projects.*

Note: Copies of our complete, audited financial statements, certified by our independent auditors, Peat, Marwick, Mitchell & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE

Year End December, 31, 1981



GRANTS

January 1-December 31, 1981

NEW GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	Principal Investigator and Program	Total Award	Duration of Grant
National Institutes of Health	Dr. Watson—Gene Organization Program Project	$5,626,997	4/1/81-3/31/86
	Dr. Watson—1981 Cell Proliferation Meeting	36,350	8/15/81-8/14/82
	Dr. Watson—1981 & 1983 C. *elegans* Meeting	57,750	4/1/81-3/31/84
	Dr. Watson—General Research Support	104,513	4/1/81-3/31/82
	Dr. Watson—1981 Mitrochondrial Genes Meeting	3,000	4/1/81-3/31/82
	Dr. Albrecht-Buehler—Research	525,529	5/1/81-4/30/84
	Dr. Blose—Research	326,982	12/1/81-11/30/84
	Dr. Hicks—Research	1,196,529	7/1/81-6/30/86
	Dr. Ivy—Fellowship	38,776	1/27/82-1/26/84
	Dr. Klar—Research	876,786	7/1/81-6/30/86
	Dr. Lemaster—Fellowship	34,681	6/1/81-2/15/83
	Dr. McKay—Research	288,546	7/1/81-6/30/84
	Dr. Roberts—1981 Nucleases Meeting	10,000	
	Dr. Sorge—Fellowship	63,744	6/1/81-5/31/84
	Dr. Welch—Fellowship	57,156	9/14/82-9/13/85
	Dr. B. Zipser—Research	299,219	12/1/81-11/30/84
National Science Foundation	Dr. Heffron—Research	147,000	2/15/81-1/31/83
	Dr. Hicks—Research	99,600	5/15/81-4/30/84
	Dr. Strathern—Equipment	64,845	5/15/81-4/30/82
	Dr. D. Zipser—Research	100,000	1/1/81-12/31/82
	1981 Mitrochondrial Genes Meeting —Dr. Watson	7,500	2/15/81-7/31/81
	1981 Muscle Meeting —Dr. Watson	5,000	7/1/81-12/31/81
	1981 Nucleases Meeting —Dr. Roberts	5,000	8/1/81-1/31/82
Asian Molecular Biology Biology Organization	Dr. Watson—travel funds	1,000	
American Cancer Society	Dr. Rossini—Research	42,106	9/1/80-12/31/81
	Dr. Topp—Research	15,000	2/1/81-1/31/82
	1981 Cell Meeting	3,938	9/1/81-9/30/81
Rita Allen Foundation	Dr. Hockfield—Research	30,000	7/1/81-6/30/82
Abbott Laboratories	1982 In Vitro Mutagenesis Meeting	500	

COLD SPRING HARBOR LABORATORY

Grantor	Principal Investigator and Program	Total Award	Duration of Grant
Applied Molecular Genetics, Inc.	1982 In Vitro Mutagenesis Meeting	1,000	
Bethesda Research Laboratory	1981 Nucleases Meeting	3,000	
	1981 Cell Meeting	1,000	
Boehringer Manheim Biochemicals	1981 Cell Meeting	300	
Central General Hospital	Research	2,500	
Cetus Corporation	1981 Cell Meeting	1,000	
	1981 Molecular Biology of Yeast Meeting	1,000	
	1982 In Vitro Mutagenesis Meeting	1,000	
Chevron Chemical Co.	1981 Plant Course	5,000	
Collaborative Research Corp.	1981 Cell Meeting	1,000	
The Camille and Henry Dreyfus Foundation	Dr. D. Zipser—1982 Undergraduate Research Participation	20,000	1/1/82-12/31/82
Department of Energy	1981 Symposium Support	9,000	5/1/81-4/30/82
Enzo Biochem, Inc.	1981 Nucleases Meeting	500	
Foundation for Microbiology	1982 Phycomyces Meeting	5,000	
Johnson and Johnson	1981 Cell Meeting	750	
K.C. Biological, Inc.	1981 Cell Meeting	1,000	
Esther A. and Joseph Klingenstein Fund	1981 Mammalian Brain Slice and Single Channel Recording Workshop	29,500	
	1982 Advanced Neuro-Anatomical Course and Single-Channel Recording Workshop	45,000	
Leukemia Society of America, Inc.	Dr. Engler—Fellowship	37,000	7/1/81-6/30/83
Litton Bionetics, Inc.	1981 Muscle Meeting	5,000	
Merck & Co.	1981 Muscle Meeting	1,500	
	1982 In Vitro Mutagenesis Meeting	1,000	
	Dr. So—Research	5,000	

COLD SPRING HARBOR LABORATORY

Grantor	Principal Investigator and Program	Total Award	Duration of Grant
Muscular Dystrophy Association	1981 Muscle Meeting	15,000	
	Dr. Blose—Research	16,242	1/1/81-12/31/81
	Dr. Garrels—Research	20,000	1/1/81-12/31/81
	Dr. Lin—Research	29,750	1/1/81-12/31/81
	Dr. Matsumura—Fellowship	36,000	1/1/81-12/31/82
	Dr. Welch—Fellowship	32,000	1/1/81-12/31/82
	Dr. B. Zipser—Synapse and Single-Channel Recording Workshop Support	12,000	
New England Biolabs, Inc.	1981 In Vitro Mutagenesis Meeting	2,000	
	1981 Nucleases Meeting	5,000	
New England Nuclear	1981 Nucleases Meeting	250	
	1981 In Vitro Mutagenesis Meeting	100	
Pabst Brewing Co.	1981 Nucleases Meeting	1,000	
Schleicher & Schuell, Inc.	1981 In Vitro Mutagenesis Meeting	100	
Upjohn Company	1981 Molecular Biology of Yeast Meeting	500	
Damon Runyon-Walter Winchell Cancer Fund	Dr. Abraham—Fellowship	17,000	8/1/81-7/31/82
	Dr. Brandsma—Fellowship	17,000	9/1/81-8/31/82
Yamasa Shoyo Co., Ltd.	Dr. Katoh—Research	33,000	10/1/81-9/30/83

BANBURY CENTER

Grantor	Program	Total Award	Duration of Grant
National Institutes of Health	1981 Gene Amplification Conference	$31,120	9/1/81-8/31/82
	1982 Nitrosamines Conference	32,320	9/30/81-8/31/82
Environmental Protection Agency	1981 Meeting on Response of the Developing Organism to Environmental Risks	50,000	9/1/81-8/31/82

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

Grantor	Principal Investigator and Program	Total Award	Duration of Grant
National Institutes of Health	Dr. Watson—General Research Support	$ 64,418	4/1/80-3/31/81
	Dr. Watson—Summer Workshops	470,340	4/1/77-3/31/82
	Dr. Watson—Symposium Support	187,433	4/1/79-3/31/84
	Dr. Watson—Herpes Virus Meeting	42,280	7/1/80-6/30/81
	Dr. Watson—Neurobiology Course Support	258,500	6/1/79-3/31/82
	Mr. Udry—Cancer Research Facility	1,411,011	9/15/77-indefinite
	Dr. Blose—Research	167,702	12/1/78-11/30/81
	Dr. Bukhari—Genetics Program Project	1,661,439	6/1/80-5/31/83
	Dr. Burridge—Research	131,039	4/1/80-3/31/81
	Dr. Chaconas—Fellowship	13,215	8/1/79-4/22/81
	Dr. Feramisco—Research	214,758	7/1/80-6/30/83
	Dr. Garrels—Research	323,073	4/1/79-3/31/82
	Dr. Gingeras/Roberts—Research	408,085	3/1/80-2/28/83
	Dr. Hicks—Advanced Bacterial Genetics Course	97,081	4/1/80-3/31/83
	Dr. Hicks—Research	384,703	7/1/78-6/30/81
	Dr. Klar—Research	253,000	7/1/78-6/30/81
	Dr. Kurtz—Research	179,542	4/1/80-3/31/83
	Dr. Mathews—Research	266,570	4/1/80-3/31/83
	Dr. Sambrook—Cancer Research Center	13,500,000	1/1/77-12/31/81
	Dr. So—Research	124,370	9/1/80-8/31/82
	Dr. Stringer—Fellowship	15,690	2/1/79-1/31/81
	Dr. Topp—Research	275,806	1/1/79-12/31/81
	Dr. Topp—Research	303,452	8/1/80-6/30/83
	Dr. D. Zipser—Fellowship Training	852,578	7/1/78-6/30/83
National Science Foundation	Dr. Bukhari—Research	330,000	6/15/79-5/30/82
	Dr. Garrels—Research	140,000	9/1/80-8/31/82
	Dr. Roberts—Research	345,000	8/15/80-7/31/83
	Dr. Roberts—Research	210,000	12/15/79-11/30/82
	Dr. B. Zipser—Neurobiology Course Support	60,000	7/1/79-12/31/82
	Dr. B. Zipser—Research	120,000	11/1/78-10/31/81
	Dr. D. Zipser—Research	136,000	2/15/78-1/31/81
	Dr. D. Zipser—Research	157,000	5/15/80-4/30/83
Cystic Fibrosis	Dr. Garrels—Research	87,230	7/1/80-6/30/82

COLD SPRING HARBOR LABORATORY

Grantor	Principal Investigator and Program	Total Award	Duration of Grant
Grass Foundation	Dr. B. Zipser—Neurobiology Scholarship	24,000	1980-1982
Esther A. and Joseph Klingenstein Fund	Dr. Watson—Neurobiology Course Support	60,000	5/1/79-4/30/82
New England Biolabs, Inc.	Dr. Roberts—Research	14,000	
Alfred P. Sloan Foundation	Dr. D. Zipser—Computer Workshops	31,000	6/1/79-12/31/82
Volkswagen Foundation	Dr. Watson—Neurobiology Scholarships	78,696	1980-1982
Whitehall Foundation	Dr. B. Zipser—Research	135,000	10/1/80-9/30/83
Helen Hay Whitney Foundation	Dr. Drickamer—Fellowship	24,125	9/1/79-5/31/81
	Dr. Frisque—Fellowship	29,000	9/1/80-8/31/82
Damon Runyon-Walter Winchell Cancer Fund	Dr. B. Anderson—Fellowship	35,000	10/1/80-9/30/82
	Dr. Goldfarb—Fellowship	35,000	10/1/80-9/30/82
	Dr. Lin—Fellowship	28,000	7/1/79-6/30/81
	Dr. Stillman—Fellowship	28,000	4/1/79-3/31/81

BANBURY CENTER

Grantor	Program	Total Award	Duration of Grant
Alfred P. Sloan Foundation	Public Information Workshops	$100,000	1/1/80-12/31/82

FINANCIAL SUPPORT OF THE LABORATORY

The Cold Spring Harbor Laboratory is a publicly supported educational institution chartered by the University of the State of New York and may receive contributions which are tax exempt under the provisions of the Internal Revenue Code, particularly Section 501C. In addition, the Laboratory has been formally designated a "public charity" by the Internal Revenue Service. Accordingly, it is an acceptable recipient of funds which would result from the termination of "private" foundations.

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

Methods of Contributing to Cold Spring Harbor Laboratory

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities

You can generally deduct the full amount of the gift on your income tax return, if the appreciation would have been a long-term gain, to the extent of 50% of your adjusted gross income and you need pay no capital gains tax on the stock's appreciation.

We recommend either of the following methods:

(1) Deliver the stock certificates to your broker with instructions to him to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(2) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, New York 11724. In a separate envelope send an *executed* stock power.

Bequests

Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property

Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of private foundation to "public" status on termination

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

For additional information, please contact the Administrative Director, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724, or call 516-367-8300.

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THE LONG ISLAND BIOLOGICAL ASSOCIATION

THE LONG ISLAND BIOLOGICAL ASSOCIATION (LIBA) and Its Relation to the Cold Spring Harbor Laboratory

Biological research at Cold Spring Harbor began in 1890 when the Brooklyn Institute of Arts and Sciences was looking for a place to set up a summer laboratory as the headquarters of its Department of Zoology. Representatives of the Institute were invited by Eugene S. Blackford, at that time the First Commissioner of the State of New York, to inspect a site on Bungtown Road across Northern Boulevard (25A) from the Fish Hatchery. The site was found to be ideal, and so the original Laboratory was organized with Mr. Blackford as president of the Board of Trustees. The land was leased from Mr. John D. Jones, whose family since 1819 had operated various industries including shipbuilding, coopering, and textile manufacture (later whaling, also) at the head of Cold Spring Harbor. Bungtown Road, which runs through the Lab property, got its name from the factory that specialized in making bungs—or stoppers—for barrels.

In 1892 the Laboratory's land was leased for a dollar a year from the Wawepex Society, which Mr. Jones had organized as a corporation for holding real estate and for investing funds for the propagation of fishes and for scientific research. In 1904 the Wawepex Society leased additional land to the Carnegie Institution of Washington, which wanted to locate a Department of Experimental Evolution in the Cold Spring Harbor area. Charles B. Davenport, who had been directing the Laboratory since 1896, assumed the additional duties of director of the Carnegie Institution's experimental station. It is interesting to note that Mr. Davenport lived in what came to be known later as the Carnegie Dormitory, the Victorian house on 25A built by John D. Jones before the turn of the century, and recently repainted in its original colors.

The Long Island Biological Association was established in 1924 when the Brooklyn Institute decided to discontinue its research at Cold Spring Harbor and offered its laboratory to two universities. Fortunately, a local group of interested neighbors decided to assume responsibility for the Lab, and thus LIBA came into being. For 38 years LIBA actually operated the Laboratory in conjunction with the Carnegie Institution, but in 1962 it seemed advisable for the Laboratory to be reorganized as an independent unit. Therefore, the property on which it now stands was conveyed to it by LIBA, which, however, still retains reversionary rights. Today LIBA is one of thirteen institutions participating in the support of the Laboratory, each institution being represented on the Laboratory's Board of Trustees.

What has happened, in effect, is that LIBA has become an expanding group of local "Friends of the Laboratory" who help support it through annual contributions. Also, from time to time, the Association undertakes campaigns to finance special important projects for which the Lab cannot obtain funds from the Federal Government or from other sources. For instance, in 1974, LIBA made possible building the James Laboratory Annex and the renovation of Blackford Hall; and in 1976 the re-building of Williams House.

The affairs of LIBA are handled by a board of 28 directors who are elected to office by the membership at an annual meeting. At least twice a year LIBA members are invited to bring their friends to a lecture or an open house at the Lab.

Membership in LIBA requires a minimum annual contribution (tax deductible) of $25 for a husband and wife, $15 for a single adult, $5 for a junior member (under 21). Further information can be obtained from the Long Island Biological Association, Box 100, Cold Spring Harbor, N.Y. 11724, or by telephoning the Laboratory's administrative director, Mr. Wiliam R. Udry, at (516) 367-8300.

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REPORT OF THE CHAIRMAN FOR 1981

I am pleased to report that in 1981 LIBA's membership was further expanded and that it continued to contribute generously to the Association's funds. What our next major project for the Laboratory is to be will be decided at the directors' meeting in May 1982.

As usual, members and their guests were invited to two lectures during the year. At the first, the annual Dorcas Cummings Memorial Lecture on May 10th, we had the good fortune to secure as our speaker Mr. Walter Sullivan, Science Editor of *The New York Times,* who took us into Outer Space in a fascinating talk about the search for extra-terrestrial inhabitants, based on his well known book on the subject entitled *We Are Not Alone.*

Then at the winter annual meeting we heard an exciting lecture by Dr. Thomas E. Lovejoy, vice-president of the World Wildlife Fund-U.S. and originator of a joint Brazilian-American long-term study of the ecological dynamics of forest patches in the Central Amazon. He illustrated his talk with a series of exceptionally brilliant colored slides.

At both of the directors' meetings, on April 5th and October 25th, talks were given by members of the Lab's scientific staff. The first was by Ronald McKay in which he described his research on monoclonal antibodies, which hold out great hope for the benefit of mankind in the areas of both diagnosis and therapy.

The second talk was given by Michael Wigler, who, with his teammates, has recently achieved a major breakthrough in cancer research: the isolation of a gene which transforms a healthy cell into a cancerous cell. The special purpose of these talks is to keep LIBA's directors in close touch with important developments at the Lab and to give them the opportunity to get to know the scientists involved in them.

The successful and greatly appreciated custom of giving dinner parties for the visiting scientists at the Lab's summer Symposium was continued in 1981. This year's hosts and hostesses were:

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Mr. & Mrs. Miner D. Crary, Jr.
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At the annual meeting regret was expressed that Mr. Samuel D. Parkinson's term of office as a director had expired and that Mr. Theodore Wickersham had resigned because he has moved away from Cold Spring Harbor. After giving thanks to both men for their loyal service to LIBA, Mr. Galston, as chairman of the nominating committee, placed in nomination to fill the vacant places on the Board, Mr. Charles F. Dolan and Mrs. Stephen Van R. Ulman, who were then duly elected to office.

Edward Pulling, *Chairman*
Long Island Bilogical Assn.
February 15, 1982

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