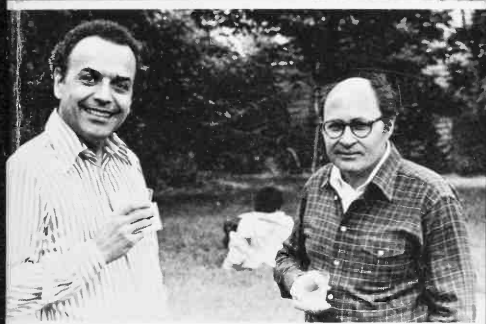




ANNUAL REPORT 1978



COLD SPRING HARBOR
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COLD SPRING HARBOR, NEW YORK

Cover: Participants at 1978 Symposium (top to bottom) G. Selzer, F. Stahl, and J. Strathern; A. Kornberg, A. Falaschi, and R. Holliday; W. Arber and D. Nathans; W. Udry, A. Bukhari, and D. Baltimore

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COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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DIRECTOR'S REPORT

The collective decisions of knowledgeable men go sour more often than we want. We should not be surprised, however, that blue ribbon solutions like those proposed several years ago for recombinant DNA research frequently go astray. High-level committees are generally called into action only when a problem arises that has no simple response, or maybe no answer at all.

This was the case when in 1973 Herbert Boyer and Stanley Cohen came upon a method for rearranging DNA molecules in the test tube to create hybrid molecules ("recombinant DNA"), which might be derived, say, partly from mouse DNA and partly from bacterial DNA. Their ability to put these recombined molecules functionally back into cells had possibly opened the way to the creation of novel life forms that might alter the course of evolution. Moreover, the procedures for making recombinant DNA were simple and cheap and if uncontrolled were likely to be universally taken up and exploited throughout the world within a year or two at most. So it was natural that concern should develop as to whether we should just plunge ahead and hope for the best or whether we should try to block the momentum of recombinant DNA research until we could be sure that we were not doing ourselves in.

But whom to turn to was not at all obvious, since recombinant DNA research was so open-ended that no one person was clearly qualified to point the way. This unsettling fact should have warned us that there might be no logical response to the existence of recombinant DNA and that we had no recourse but to move ahead. Our civilization had reached its present highly advanced state only by facing the unknown, always hoping that we could generate enough ingenuity to get us out of any jams that might arise.

Instead, the National Academy of Science made what then looked like a prudent response. It put together a high-level committee of scientists who worked with DNA with the hope that they would do more than throw dice—that somehow their past experiences would equip them for a logical response. The truth in such situations, however, is often just the opposite. But no

one likes to advertise that we may have no meaningful guide for what tomorrow will bring. Psychologically this is hard to accept, and our sanity almost demands placing more faith in experts than the facts warrant. So when we bring authorities together, we have already half-committed ourselves to following their advice. We know that if we don't, we may have to make the decision ourselves and shoulder the criticism if the wrong moves are made.

Of course, if our experts recommend action that looks as though it will block what we want to do tomorrow, we may need much persuasion before we even consider going along. Most smokers, for example, are unlikely to stop puffing in public no matter who advises the action. On the other hand, most Americans would accept a ban on supersonic airliners since we would not notice their disappearance. Only those who directly want to use the Concorde would cry "murder" and employ our legal system to the full to try and block what they would regard as capricious nonsense. They know that once a recommendation has been codified into administrative rules, the subsequent backpedaling needed to nullify them may be very hard to bring off.

We should thus be very careful not to push for regulations which we cannot logically defend. We do not know, for example, whether we are entering a warming or cooling period for the earth. So it would be a bad mistake to say we should cut down on the burning of oil, gas, and coal because if too much CO₂ is released, the atmospheric temperature will rise to unacceptable levels (the so-called greenhouse effect). In fact, we may want warmer weather to reduce our winter fuel needs. The point here is that if too many variables exist, prediction becomes impossible and we should back away from decisions that give the impression that we know more than we do.

In particular we mustn't overreact by assuming the worst possible outcome and settling into a siege response that prevents the possibility of progress if in fact nothing catastrophic happens. For example, we would destroy any chances of maintaining our national prosperity if all our governmental actions were predicated

upon the belief that the Russians were about to engage us in a nuclear war. And almost equal senseless panic would result if, say, six months of almost no rain led us to half-starve ourselves because of the fear that we were about to enter a decade of no rain.

Likewise, we shall only harm ourselves by assuming that the newly developed recombinant DNA technology poses a credible threat to our civilization. The good that can come out of its use is immense—it can revolutionize our understanding of human chromosomes, give us a practical way to make vaccines against difficult-to-grow viruses like hepatitis, and yield unlimited amounts of now scarce drugs like the antiviral agent interferon. Nonetheless, there is opposition to its use based on the conjecture that our blessings might indeed be mixed and that recombinant DNA procedures might also generate new disease agents or modify preexisting organisms so as to badly upset the earth's ecology.

Not even hints exist, however, that any of these four events would be plausible outcomes of totally unrestrained DNA research. Infectious-disease experts tell us that pathogenicity is not easy to generate, and the illegitimate interspecies DNA transfers mediated in nature by viruses have probably already tested the ecological consequence of any DNA transfer that we can now do in the laboratory. We should thus not get into a tizzy because we can't now, or even in the far distant future, have a way to disprove implausible hypotheses about how we may meet our doom. Instead we should push recombinant DNA research and development as fast as our resources permit, while keeping alert to the highly improbable event that one or more lab workers will come down with a disease that we have not seen before.

Unfortunately, we are not now acting with such bold intelligence. We are badly held back by a morass of bureaucratic regulations that are seen by most of the scientists they affect as having no intellectual validity. Alas, I was one of those who helped bring about these increasingly despised rules. Some eleven molecular biologists, of whom I was one, called in the spring of 1974 for a temporary moratorium on experiments involving either tumor virus genomes or the transfer of the genes coding for dangerous toxins from one group of bacteria to another. The moratorium was to last almost a year, until the matter could be discussed by a larger internationally based expert group that was to meet in California at the Asilomar Conference Center. In doing so, we opted for an apparently fair middle position that could satisfy both those many scientists who wanted to move swiftly and those who felt that if we were to err it should be on the side of caution.

This moratorium call, made in half-haste after only a half-day meeting at MIT, was an act I later came to badly regret. The experiments we had so casually halted were soon to strike me as much safer than many categories of

work with disease-causing agents that the microbiologists have been carrying out for decades without significant harm to themselves, much less to the public at large.

For example, experiments with tumor virus genes inserted into *E. coli* instinctively appear safer than working with the viruses themselves. Yet there are no firm regulations governing tumor virus research—the precautions to be taken being left to the individual investigator to decide. This seeming indifference to public health is not that at all. Without knowing the level of risk, if any, it is impossible to know what precautions, if any, are necessary. Any rigid rules governing tumor virus research are bound to appear capricious to many, and the only practical recommendation is a prudent respect for commonly accepted microbiological safety procedures.

Why then did we react so much more cautiously to recombinant DNA? A major reason was that the recombinant DNA procedures were not yet a necessary ingredient for our day-to-day research. Until they became so, we did not see the need to appear possibly indifferent to the public good by unilaterally ignoring those who said that science was now out of control. Moreover, Watergate was still with us and the national mood was very much to come clean with what one was up to. So we saw no harm and possibly some considerably credit in so attracting public attention. It was thought best to overestimate rather than underestimate our concerns. Later, when more experiments had been done and no one had taken ill because of recombinant DNA, we could seriously downgrade, if not forget, the whole matter. Then no one could accuse us of keeping back even our most paranoid ideas.

Unfortunately, none of us seriously questioned whether we might be alerting the public unnecessarily and by doing so give recombinant DNA doomsday scenarios a credibility they didn't deserve. For the minute the moratorium was announced, we had, in effect, asked the public to join us in the decision-making process. Why would we have actually halted our experiments if we weren't really worried? This point was not initially perceived, nor to my knowledge did anyone see the consequences of forming subcommittees to rate various types of recombinant DNA experiments for their potential risks. For if, in fact, risks could be quantitated, they would have to be real and the virtual inevitability of the public's insisting that more formal rules take over when the moratorium ended.

The trap was thus almost closed when we assembled at Asilomar in February 1975. By then there had been so much talk to the press and TV about the wisdom of our initial caution, that it was generally thought politically unacceptable to argue, as I did, that the whole conference was a mistake and we would do everyone a service

by cutting it short with a brief statement to the effect that it was logically impossible to regulate conceptual risks. Already in their opening statements the organizing committee cautioned us not to appear self-serving and instead to come up with guidelines that would have the dual virtue of having teeth yet being imaginative enough so that we would not have to stop too many scientifically valid experiments. This mood was quickly shared by most participants, and speaker after speaker rose to identify his position with that of intelligent caution.

During the next two days there were only a few minor embarrassing outcries that the logic behind the proposed rules was paper-thin if not nonexistent; virtually everyone joined together to come up with a set of internationally applicable rules to govern how everyone should subsequently work with recombinant DNA. In doing so the Asilomar congreants placed their collective reputation behind the statement that the dangers were real enough to embrace four categories of increasing presumptive risk. Some experiments were left almost unregulated while others became saddled with so many precautions that virtually all laboratories would be prevented from their execution.

At first, the Asilomar solution pleased almost every participant except those relatively few who sensed that they had been stopped without a fair hearing and that they had been stymied merely because Asilomar would have been judged a whitewash if no one had been seriously hurt. Most others left for home happy that their own experiments might be done without too much hassle if the Asilomar recommendations were soon accepted by their respective governmental authorities. The general mood was that of relief, and virtually no one faced up to what his response would have been if the majority of experiments had been proscribed as too dangerous for the ordinary lab.

Quickly, however, the uneasy truth began to emerge that Asilomar had been more of an emotional experience than a logical response to firm facts. With no experiments existing that might provide any clues to the magnitude of the theoretical bad, there was no convincing way to defend its conclusions either against those critics, like me, who saw no compelling reasons for any guidelines at all, or against the doomsday prophets who wanted all recombinant DNA work to stop. Thus, not unexpectedly, the guidelines began to unravel the moment they became open for formal reexamination.

The initial assault came when the first committee set up by NIH to receive Asilomar met in July 1975 at Woods Hole. It was chaired by Dave Hogness and composed largely of eukaryotic molecular biologists, several of whom by then had already taken up recombinant DNA in a big way. With formal rules now hanging over them, they saw Asilomar as less benign than it first perceived. Even worse, not one of them felt the

slightest apprehension about work with recombinant DNA. The only anxiety, in fact, came from the journalistic build-up of Asilomar as one of science's finest hours. Such farfetched exaggeration could only make the public far more concerned than the matter warranted. Hoping to defuse the issue, the Hogness Committee urged a general relaxation of the proposed rules. In particular they downgraded what they felt were unnecessarily harsh regulations for working with tumor viruses and mammalian DNA.

Almost immediately, however, there were counter arguments to beef up Asilomar from a group of phage workers that met here in Cold Spring Harbor a month later. They proclaimed the Woods Hole action a dangerous example of the failure of self-regulation. Making a virtue of their own immediate lack of need for recombinant DNA technologies, they viewed themselves as more objectively suited to decide which experiments should be carefully regulated. They were particularly bothered by unregulated work with tumor viruses and wanted assurances that they would not needlessly be exposed. However, being totally unqualified to discuss how tumor virus genes inserted into *E. coli* could realistically threaten human beings, they focused mostly on the putative social misuses of recombinant DNA and in particular on how it might hasten the day when we could genetically engineer human life.

NIH quickly caved in and expanded its recombinant DNA Advisory Committee, knowing that they had no logical way of choosing the Hogness Committee's recommendations over those proposed by the self-proclaimed socially conscious phage world. Moreover, with Senator Kennedy taking a populist cry that recombinant DNA was too important to be left in the scientist's hands, they wanted to be protected by a broader-based committee that would listen to all scientific viewpoints including that of the so-called concerned public. Whom from the public to choose, however, was not that obvious, and NIH's first course was to be sure that attention was given to representatives from both the new left, which for political reasons wanted to use recombinant DNA as a rallying cry for the downtrodden in order to get back at MIT, Harvard, and Berkeley, and from the Calvinistic hard core of the environmental movement, which believe that only tighter and tighter regulations could protect us from our baser motives of greed and ambition.

This ploy badly misfired as those who now found recombinant DNA regulation intellectually impossible, and thus absurd, found themselves outnumbered on the official scene. The balance tilted toward tougher regulations, and the final guidelines that emerged from NIH in June of 1976 restricted DNA work far more stringently than those proposed by Asilomar.

Like any compromise between irreconcilable posi-

tions, the new guidelines satisfied no one. For example, the environmentalists reacted with lawsuits based on NIH's presumed failure to prepare adequate environmental impact statements. And the Science for the People crowd cried that the guidelines could no be enforced over industry and that the capitalistic imperative for profits would deluge us with newly created deadly germs. Equally angry were the increasingly large number of molecular biologists who could not start their experiments until the certification of the so-called safe strains that none of them felt were at all needed. While so waiting, they could only curse themselves for acquiescing so witlessly in the Asilomar charade. Even worse, they didn't see how they could call off the whole thing without seeming erratically self-serving. Having been greatly praised by the press for their wisdom in calling for Asilomar, virtually none of them was anxious to say he thought that the whole exercise was a silly miscalculation.

Instead, most molecular biologists felt the best course was to argue in public that the Asilomar guidelines were the correct response and that if we were to follow them we would have nothing to worry about. This tactic, however, frequently created more anxiety than it dispersed, for it implied that we knew what we were protecting ourselves against. If that were so, the dangers which society faced were real and we must worry what would happen if the NIH guidelines are not strictly adhered to. Inevitably there was more and more talk about the need for some form of punitive national legislation, and drafts of proposed laws began to circulate in Congress in 1977.

Initially the virtual certainty of some form of national legislation was accepted by most molecular biologists, who at the first public go-arounds before Congress almost without exception said that some form of guidelines, if not laws, would be a good thing. This way they hoped not to antagonize those in power and so hopefully tone down the final harm. Happily, Congress got tangled up in trying to decide which form of legislation was needed, in particular whether it should forestall the passage of even tougher rules by state and local governments. This gave us hope that if we lobbied hard enough the absurdity of recombinant DNA regulation would become clear and the guidelines would gradually fade away before anyone codified them into real law.

In fact this has partly happened, though not as fast as would have occurred if we all quickly said in public what we were endlessly boring ourselves with in private. In any case, the about-face last Spring of the politically astute Senator Kennedy almost certainly signifies that recombinant DNA no longer has any political value and that public hysteria cannot be maintained indefinitely in the absence of a credible villain. To be sure, not

all senators are yet on our side, and Senator Stevenson just recently issued a lengthy report arguing for legislation. Fortunately his colleague on the Science Committee, Senator Harrison Schmidt, has issued a strong counter report and the odds now appear to be against any recombinant DNA legislation going through Congress in the foreseeable future.

Our main concern now is the speed with which NIH can rid itself of its regulatory role over DNA research. New committees must reverse the actions of old ones, and approximately one year ago there commenced a concentrated push to effect a general lowering of all guidelines and in particular to open up tumor virus genomes to recombinant DNA analysis. To do these latter experiments we then had to go to England or France where the rules were not nearly as dogmatic as ours. New ad hoc groups thus were charged by NIH's Director, Don Fredrickson, to come in with arguments for less stringent guidelines. In due course they did their job, and a by-now somewhat emboldened Recombinant Advisory Committee gave its consent last June to the desired relaxations. This process, however, was far from painless. The now absurdly lengthy procedural details which must be followed to give due process to those who disapprove any downgrading have consumed a disgracefully disproportionate amount of the NIH directorate's time. However, by late September we thought we were at last home, with only the remaining step being approval by HEW Secretary Califano. His staff, being lawyers, had no way of independently assessing the pros and cons, and we initially hoped that Secretary Califano, being a busy man, would just sign the new guidelines and move on to something to which he might make a real contribution.

Instead we found the Secretary's staff was in no hurry to sign and had homed in on the future complexion of the Recombinant Advisory Committee, which would have the power to still further downgrade the regulations. In particular they strongly objected to the proposal of NIH that the Committee be dominated by leading scientists who wanted to work with recombinant DNA. Instead they wanted an ethically balanced committee that would represent all sides of the matter.

This proposal naturally depressed many of us since it opened up the prospect of persons without any scientific qualifications deciding what science we should not do. Naturally we called on everyone we knew in the White House to alert them to this new folly within HEW. We were told, however, that we shouldn't worry, as in fact the Secretary would on December 15th OK the proposed guideline relaxations, and, moreover, that only a few of the public members would be real obstructionists and we could count on the rest to help us quietly dismantle the whole hateful artifice. Why the White House should see it necessary to put any obstruc-

tionists on board at all is beyond me since I doubt the current administration would lose more than 500 votes over the entire United States were it to announce that we can do anything we want with recombinant DNA.

Now I'm afraid we must remain very vigilant, since the new public members, by being so appointed, naturally presume they have a real job to do. It is not as if they were directors of the Postal Service and getting \$25,000 per year for twiddling their thumbs. Instead they all too clearly will be wasting their time if they routinely act as rubber stamps for a scientific community that knows it is hopeless to teach them the subtleties of molecular biology. Their self-respect may thus demand that they try to show that we scientists don't know everything; and so we must expect that they will say "no" more often than we want.

We must not fight back, however, by saying that only we scientists can judge unquantifiable conceptual risks. We are no more qualified than the man in the moon to assess such situations, and we are stewing in this mess because at Asilomar we said we were. On the contrary, if there were firm facts showing us that recombinant DNA research was leading to dangerous new bugs, then it would be totally proper for the public to help us decide what we should do next. Since, however, no data exist to let us decide rationally whether we or the public should worry at all, no Recombinant Advisory Committee should exist at all.

This point, not whether there should be public members, is the crux of the matter. We are upset by the thought of too many public members only because they may take regulation seriously—unlike almost all molecular biologists, who now painfully realize their past blunders and can now be counted on to vote themselves out of existence. But if too many public members exist, they might co-opt enough faint-hearted scientists to delay the dissolution, so that next year at this time, if not for another decade, we shall still be writing up these idiotic memoranda of understanding. We must thus call out as loudly as we can for the ability to do all forms of recombinant DNA research unfettered by any inherently mindless chains.

To act otherwise is against all the traditions that have given us modern science and we should not think ourselves at all courageous to demand the right to go ahead with new experiments as long as there is no valid reason to presume we might harm someone. Yet Secretary Califano is now proposing that we start serious experiments to show we shall not be put in danger from our newly created bugs. This is effectively an impossible demand, for some incalculable risks go along with almost all major medical or technological steps, no matter how we proceed. This fact of life cannot be avoided by the setting aside of large sums of money for "risk assessments" that necessarily have no generalized

applicability. Given the almost infinite variety of potential recombinant DNA experiments there is no way that such risk assessments could ever be done on the massive scale necessary to reassure anyone with a real brain. At best the limited highly touted examples would only waste more public monies.

Yet to my amazement Ms. Shirley Williams, the hitherto thought most competent Minister of Education and Science of the British government, has recently stated on TV that they are prepared to spend "tens of millions of pounds" (at least one-third of the entire budget of Britain's Medical Research Council) for recombinant DNA risk assessment. This extraordinary statement is a painful reflection of the gaps that exist between the two cultures, both in England and here in the United States, and we can only hope she is soon put in contact with scientists who have their feet on the ground.

How we are to educate Secretary Califano and his staff is not clear, since they seem to see the political imperative to give almost as much weight to outsiders as they do to the leaders of our profession. Why this should be so is hard to fathom, since the Secretary could not have been indifferent to quality advice in his legal days, and his public views on cigarettes show he can strongly choose sides.

We thus have no choice as scientists but to make widely known our lack of confidence in Secretary Califano's office, and the possibility that its misguided equalitarianism will deprive the American nation of the full benefits of the most powerful new research tool available for biological research.

Highlights of the Year

Homing in on the way tumor viruses make cells cancerous

Nine years ago when we started tumor virus research we chose to emphasize the papovaviruses (e.g., SV40 and polyoma) since they have unusually small chromosomes that at most can code for 5–10 genes. Hopefully only a few, if not one, would code for the key protein(s) that converts normal cells into cancerous equivalents. Now we believe only two protein products (big T and little t) have this role, and both are so-called early proteins that function in the beginning phases of the viral life cycle. T and t have the same amino terminal sequences, an inexplicable result until RNA splicing was discovered with its momentous implication that the same DNA segment could code for a multiplicity of different proteins. The larger of these two proteins, big T (m.w. ~ 95,000), was shown last year to bind specifically to tandemly repeating sequences at the origin of

DNA replication. This was a pleasing result since for several years T has been thought to function specifically in DNA initiation as opposed to elongation. Over this past year Bob Tjian has gone on to show that highly purified T has an ATPase activity, an attribute previously found for several bacterial proteins known to facilitate DNA initiation. Clearly the next step will be to develop an *in vitro* system for DNA synthesis in which T plays an essential part.

t (m.w. ~ 17,000) remains much more mysterious as there is no easy way yet known to prepare it in amounts large enough for conventional biochemical analysis. Unlike big T, which exclusively functions in the nucleus, small t resides in the cytoplasm, where work by Bill Topp suggests that it is responsible for the more disorganized cytomusculature characteristic of SV40-transformed cells. Addition of epidermal growth factor to normal cells mimics most t action, leading to the hypothesis that t functions to convert quiescent cells into cells metabolically able to commence DNA synthesis provided that specific initiation factors like T are present. Further progress is likely to depend upon the construction of genetically engineered viruses that overproduce t.

Exploitation of our splicing discovery

Last year's discovery of RNA splicing has been quickly followed up by the sequencing of the tripartite leader sequence which precedes all the late adenovirus messages. We started by making a cDNA transcript of purified fiber mRNA and inserting this through recombinant DNA procedures into an *E. coli* plasmid, which later could be highly amplified for sequencing studies. Joe Sambrook went to ICRF in London to do the recombinant DNA steps, since the guidelines then in effect here precluded work the English permitted. Subsequently Sayeeda Zain and Rich Roberts did the necessary sequencing, using the newest Sanger methodologies. Comparison of the mRNA sequence with that of the respective genomic DNA sequences showed that adeno splicing occurs at the same specific sequences where splicing occurs for ovalbumin, immunoglobulin, and hemoglobin mRNAs. The enzymes that do the splicing are probably similar, if not identical.

The exact significance of the 120-nucleotide-long leader remains unknown except for the obvious fact that it must somehow facilitate mRNA binding to eukaryotic ribosomes.

Our Symposium marks the 25th anniversary of the Double Helix

For several years we had thought that the 1978 Sym-

posium should be on DNA replication. Not only was the Double Helix found in 1953, but it was at the 18th Symposium (on Viruses) that it got its first real public presentation. Moreover, experiments on DNA replication might at last be converging on the final answer and in addition were beginning to strongly affect our thinking about crossing over. So, early in June we brought together for our 43rd annual Symposium most of the key scientists now involved with DNA replication and recombination. The formal program was our fullest yet, as was the total attendance of some 425. Our main lecture hall holds only 225 seats, so we set up closed-circuit TV in several outlying spots including a massive tent. Happily, there were not many complaints from the late arisers who never saw the live morning show, which each day started as soon as the lecture room was totally occupied. By the end of the meeting, sessions were beginning just after 8 a.m., or less than nine hours after the completion of the previous evening session.

We started out strongly with an opening talk by Arthur Kornberg, and the conclusion was delivered with the crisp elegance of Frank Stahl. Altogether there were some 153 presentations, both formal and informal, and we much look forward to the necessarily massive proceedings, which are scheduled to be ready early in May.

A very pleasant diversion from the necessarily hectic pace were the gracious dinner parties that our neighbors held for the speakers. This year we wish to thank in particular Mr. and Mrs. David Clark, Mr. and Mrs. Norris W. Darrell, Jr., Mr. and Mrs. Clarence E. Galston, Mr. and Mrs. Charles S. Gay, Mrs. Carol Lamb, Mr. and Mrs. James A. McCurdy, Mr. and Mrs. Richard Olney, Mr. and Mrs. James J. Pirtle, Mr. and Mrs. Franz Schneider, Mr. and Mrs. Stanley S. Trotman, Jr., Mrs. Ethelbert Warfield, Mr. and Mrs. Richard J. Weghorn, and Mr. and Mrs. Theodore Wickersham.

The steadily increasing size of successive Symposia naturally concerns us, since we cannot view the trend as temporary. When these meetings began, high-level biology was practiced at relatively few institutions and there was almost an *avant garde* quality to our audience. Now, however, virtually every important biological discipline has hundreds of active members and it seems almost impossible to pick an exciting topic that should have an audience of less than 300, if not 400. We have thought of building a lecture hall of, say, twice our current size; but without simultaneously expanding our dining, housing, and parking facilities, we would still be pressed to the wall. Thus common sense tells us not to plan for another lecture hall, but instead we must just accept further chaotic Symposium weeks. As long as we can pick topics in the forefront of biology, we trust the high quality and integrity of the occasions will compensate for our inadequacies as hotel keepers.

Appointment of Victor McElheny to direct our Banbury Conferences

Some 18 months ago we began searching for Foundation help to let us start up at our Banbury Conference Center a major program of small, high-powered meetings aimed at assessing the risks to humans of the ever-increasing collection of chemicals known to damage our genetic material. Many such agents initiate cancers, and how we should realistically respond to their steadily rising presence in high-technology societies is often not clear. Happily, we were able to persuade Victor McElheny, the Technology Correspondent of *The New York Times*, to consider directing our efforts, and with his leadership dangled as bait, the Klingenstein Fund and the Sloan Foundation each responded last spring with awards of \$100,000 to cover our first two years of operation.

Vic formally joined us in mid-May, just before we began our first such Banbury Meeting. Its topic was "Assessing Chemical Mutagens: The Risk to Humans." Funding came from the Environmental Protection Agency, which shared the organization of a program that brought together for three very intensive days some 25 experts in chemical mutagenesis. The proceedings were transcribed and, after extensive editing by our staff, will appear in book form in March 1979 as our first Banbury Report.

Full-scale operation of Banbury will commence in April of this coming year, and plans are now well-advanced for three risk-assessment-related meetings in the spring and three in the fall.

Planning a new residence for our Banbury guests

From the start of our planning for a meetings facility at the Robertson estate we have considered 32 participants as optimal for small, specialized conferences lasting two to three days. This number should allow everyone to meet informally, hopefully over one or more meals, yet is large enough for a diversity of outlook and experience. So we renovated the Georgian-style garage to give us a meeting room to handle this number, purchased 32 super Herman Miller chairs to go around our specially designed massive conference tables, and took comfort that the dining facility at Robertson House could, if slightly pressed, handle only 32.

The number of guests, however, that can live in the main house is a maximum of 21, and then only with double occupancy of most bedrooms. This is not always practical, and we knew initially that almost half our conference participants would have to be housed elsewhere, a far from perfect situation when a major aim is extended informal conversation.

So, with the start-up of our Risk Assessment Program assured, and Victor McElheny in command, we com-

menced plans for a small residence that would provide us with 16 single rooms. Our architects, Moore, Grover and Harper, were asked to come up with schematics for a building that would be architecturally harmonious with the Georgian-style buildings constructed for the Robertson family. Their stunning proposal, which came to us in September, is for a square residence of classical Palladian form, with a central hall around which eight rooms are located on each of the two floors. Happily, the Kresge Foundation gave its consent to use the \$150,000 in funds that it granted us last year, toward the construction costs. We have now started on the working drawings, with ground-breaking planned at the latest by late Spring. Our target completion date is March 1980, in time for that year's spring group of risk-assessment meetings.

Major changes in our scientific staff

This August, Ray Gesteland, a member of our scientific staff for 10 years, left us to join the University of Utah as the Howard Hughes Professor of Biology. Always one of our key scientists, Ray also served with great distinction as our Assistant Director for Research. We would not be where we are today were it not for his unequivocal devotion both to the Lab and to the Cold Spring Harbor community in which he, his wife Harriett, and their four children were such respected and well-liked members.



Ray Gesteland

Just before their departure more than a hundred of their friends here joined them for a nostalgic farewell evening cruise on the Great South Bay. And an inscribed clock of elegant Tiffany design was presented to them as they began their journey to Salt Lake City. We wish them all forms of success in their new lives and trust they will find new occasions to again grace our surroundings.

Also leaving our staff this fall was Jim McDougall, who has taken a senior position at the Hutchinson Cancer Center in Seattle. While here he began in-situ nucleic acid hybridization methods that finally should settle the question whether Herpes II virus is a causative agent for cervical cancer. And Bob Tjian returned to Berkeley, where he was an undergraduate, to become an Assistant Professor of Biochemistry. His superb biochemical training was an invaluable asset to James Laboratory and his departure creates a gap that will not be easily filled.

Departing at the completion of their postdoctoral training were Ray Bigelis to work at Cornell with Jerry Fink, Denise Galloway to continue work in Seattle with Jim McDougall, Walter Schaffner to take up a faculty position at the University of Zurich, Merilyn Sleigh to return to the CSIRO Genetics Lab in Sidney Australia, and Mark Zabeau to join the staff at the EMBO lab in Heidelberg.

Newly appointed to head our Mammalian Cell Genetics group is Michael Wigler, who moved here early in December from the College of Physicians and Surgeons of Columbia University. Joining him early in 1979 as a Staff Investigator will be David Kurtz. Both did their Ph.D. research at P & S, where they stayed on for postdoctoral research. In James Lab, Bill Topp has moved up to become a Senior Staff Investigator, while Ashley Dunn and Dan Klessig have become Staff Investigators. Mike Mathews has moved from James to Demerec, to lead the Protein Synthesis section jointly with Jim Lewis. And in the Cell Biology group Stephen Blose is now a Staff Investigator.

Our first appointment in Neurobiology is Birgit Zipser. Before coming here Birgit was a Research Assistant Professor at Downstate Medical School in Brooklyn, to which she moved after graduate and postdoctoral research at the Albert Einstein College of Medicine.

We acquire our first powerful computer

This spring the National Science Foundation awarded us a major grant of \$120,000 to purchase equipment that will let us quantitatively analyze two-dimensional protein gels. These gels, first developed several years ago by Pat O'Farrell when he was a graduate student at Boulder, have enormous potential for probing cells. They allow us to look at each of the thousands of proteins that a given cell type possesses. So much information, however, is present in each gel that mere visual

observation is inadequate, and quantitative procedures for scanning and subsequent computerization are a requisite for their full utilization. Working them out proved not to be a simple problem, and only recently did Jim Garrells, then at the Salk Institute, make the necessary first steps. Now a member of our Cell Biology section, Jim is further developing these methodologies with the help of a very high resolution optical scanner coupled to a PDP 11-60 computer that we have recently installed in the second floor of our library.

The electrophoretic procedures themselves will be done in McClintock Laboratory, in space that is being vacated as we complete the move of our shops and photo services to their new quarters in the renovated greenhouses. We shall start the necessary construction early in 1979 and plan to have the new biochemical labs ready late in the spring. We hope for a routine operation of this program by fall. Then, with the financial help of the National Science Foundation, we will make this unique facility available also to our neighbors in the New York area.

Starting up a year-round yeast group in Davenport Lab

We now have a very active year-round research group in Davenport Lab focusing on Yeast Genetics. Not only are yeasts extraordinarily interesting in themselves, but the recent discovery that they can be transformed opens up the possibility that they will become a powerful cloning vehicle for eukaryotic genes. Most fortunately we were able to attract Jim Hicks and Jeff Strathern, both former students of Ira Herskowitz, to give us our initial momentum. They were soon joined by Jim Broach, who learned to work on yeast genetics in Bob Mortimer's Berkeley lab before coming to work with Ray Geste-



Jim Garrells works at new computer

land, and by Amar Klar, who first worked with yeast as a doctoral student of Harlyn Halvorsen and then mastered yeast genetics with Seymour Fogel in Berkeley. There is space for this group to grow to six or maybe seven members and its bright future seems already assured. Our only problem is how to help it survive the nine summer weeks when it must coexist with our genetically oriented summer courses.

The Robertson Research Fund remains uniquely important

The existence of a sizable endowment specifically restricted to research is an asset possessed by few independent research institutions. Each year our Robertson research funds let us respond quickly to opportunities for the possibility of carrying out an incisive experiment or attracting highly talented scientists. Most importantly, they routinely let me give guarantees of salary or equipment support where a key grant or fellowship stipend fails to materialize. For example, we assured Jim Garrells that we would back up his request to the National Science Foundation for the scanner and computer needed for his 2-D protein gel program. Happily, NSF came through with almost all the monies he requested, and then we used the Robertson monies to acquire an even more powerful computer than we had originally planned for—in this case most fortunate, since even our new computer seems likely to run at full capacity within a year.

This year we also used Robertson funds to add two most badly needed scintillation counters (in Demerec

Lab), for the stipends of summer research students, for the salaries of key junior staff, and to help start up the new yeast group in Davenport Lab.

The greenhouse renovation nears completion

Long a prominent feature standing between the library and McClintock Laboratory have been the shingled potting sheds to which were attached five separate greenhouses in the 1950s and for many years seemed ripe for the wrecker's ball. Indeed, three years ago we started plans to put a new cell biology lab on their site. Last winter, however, we reversed our course and asked our architects, Moore, Grover and Harper, to draw up plans for a total renovation project which would keep the general outlines of the preexisting structures, yet provide us with a sparkling new facility. In it we proposed to put our receiving space, stockrooms, our mechanical and electronic shops, equipment maintenance, the safety office, photography and other artistic services, as well as offices and a seminar room for many of the scientists who work in Demerec Lab.

We very much liked their plans, and the rebuilding process began in mid-May, with completion targeted for late December. Renovation of old structures, however, is seldom straightforward, and not unexpectedly, we have fallen behind schedule and anticipate final completion by late February. Already we can see that we have a visual triumph, which provides a still further case where the adaptive reutilization of an out-of-date build-



Renovated greenhouse provides new facilities

ing successfully allows an institution to maintain a firm connection with its past heritage.

We must also note that this spring we finished an almost total reconstruction of Cole Cottage. It was built in 1932 as an unheated summer dwelling and enlarged slightly with a furnace in 1953. It was justly famous for its drafts and correspondingly astronomical heating bills. Now it is a remarkably tight structure, to which a tiny porch has been added to strengthen its Colonial Revival-style appearance.

A totally new look was also given this summer to Olney House by repainting it in colors appropriate to its elegant 1885 Queen Anne-style construction. Before painting there was extensive carpentry work to make up for decades of total neglect, and a chimney was rebuilt that otherwise might have soon toppled over in a fierce gale.

A most impressive number of book sales

This year we published four major books as well as the Abstracts for our eight summer meetings. The first book to appear was a most comprehensive volume on the *Replication of Mammalian Parvoviruses*, edited by David Ward and Peter Tattersall. Then our latest Symposium appeared, a 1250-page, two-book set on *Chromatin*. It was embarrassingly late, owing to the combined effects of our unbelievably hard winter, the unexpected resignations of several editors, and the loss by a messenger of the last folios of edited page proofs. Fortunately it is one of our more impressive Symposium

volumes and we trust it will obtain virtually encyclopedic employment. Finally, in December, both our volumes on the *Differentiation of Normal and Neoplastic Hematopoietic Cells* and on *The Operon* appeared. In these books we have much to be proud of, and we remain most fortunate that so many outstanding scientists have written well in our behalf.

Our total sales this year will come to more than 16,000 volumes and abstracts, 3000 more than the 1977 sales of 13,000. So far we have already sold over 4500 copies of *Origins of Human Cancer*, which came out just as 1977 ended. Its sales are continuing to be strong, confirming our belief that it contains the best overview now available of the incidence and causation of human cancer.

The working conditions of our publications shop are very cramped and Ms. Nancy Ford and her editorial staff—comprised of Annette Zaninovic, Roberta Salant, Chris Nolan, Mary-Teresa Halpin, and Barbara Cowley-Durst—deserve much credit for their continued high level of performance in the face of seemingly never-ending telephonic interruptions. We have tried to improve their state by a total remodeling job on their two tiny rooms, but the only real long-term answer will be the acquisition of more working space.

Over 1900 attendees at our summer meetings

Seven major meetings were held this summer in addition to the Symposium. We started with a small gather-



Cole Cottage, restored

ing devoted to "Phorbol Esters," still very mysterious compounds that promote the growth of newly created cancer cells. It was followed by a much larger meeting on "Cytoskeleton and Cytomusculature," which brought together many leading cell biologists who work on microfilaments, 100Å filaments, and microtubules. Then we hosted a truly gigantic "RNA Tumor Virus Meeting" whose afternoons were crowded with poster sessions to keep the formal talks to a manageable level. In late July our by now longstanding "SV40, Polyoma, and Adenovirus Meeting" was attended by almost 300 participants. Then, in August, came our long-traditional "Phage Meeting," somewhat smaller than normal because of overlaps with this year's Symposium. Following it was a very concentrated "tRNA Meeting," which will form the basis of a new volume in our Molecular Biology monograph series. Concluding the summer was our sixth annual "Conference on Cell Proliferation," most ably organized by Gordon Sato and Russell Ross and centered on hormones and cell culture. It was dedicated to the memory of Gordon Tompkins and uniquely marked by two concerts, one of chamber music, the other of jazz, of which Gordon was a saxophone master.

That so many massive meetings could occur so smoothly owes much to our ever versatile Meetings Office staffed by Gladys Kist, Winifred Modzeleski, and Barbara Eggers.

Continued expansion of our Neurobiology efforts

Two new Neurobiology courses were added this past summer. The first, on "Basic Neuroanatomical Methods," was taught with enthusiastic vigor by Matt and Jenny LaVal. The large number of excellent applicants reflected the fact that this material is not yet available to graduate students, even in most advanced universities. Equally successful was our lecture course in "Developmental Neurobiology," taught at the Banbury Conference Center by Dale Purvis and Paul Patterson. All the students lived in Robertson House and totally enjoyed the ambiance.

Banbury was also the site of a far-ranging workshop on "Artificial Intelligence" organized by Dave Zipser of our staff and David Marr of MIT. It was supported by the Cognitive Sciences Program of the Sloan Foundation and brought together a number of leading experimental neurobiologists with their computer-oriented equivalents. Virtually all the participants left satisfied, and we plan a related workshop this coming summer.

As our summer program continues to grow, the problems involved in organizing it have correspondingly increased, particularly in view of our virtually chronic difficulty in obtaining even limited Federal support to help cover the very substantial costs. So, most vital to this past summer's success was help from the Sloan and Eppley Foundations, and from the Marie H. Robertson

Fund for Neurobiology. In particular, these private monies allowed us to run advanced workshops on the "Synapse" in July and September, and on "Visual Perception" in August. All these workshops strongly complemented our more formal courses and we are planning two more this coming summer, one on the "Neurophysiology of Pain Receptors" and the other on the "Use of Computers in Processing Neurophysiological Signals."

Given the superb equipment for neurobiology which we now possess, a serious year-round research program could be quickly started if we were to obtain the necessary financial support. A first move in this direction was the appointment this fall of Birgit Zipser as a Staff Investigator, with her support coming from the National Science Foundation and the National Institutes of Health. Her research focus is the neurobiology of the leech, an interest which commenced when she took our leech course in the summer of 1975.

By now we feel most proud of our contributions to neurobiology, noting the impressive number of our former students who now hold teaching positions in major university centers. Hopefully the long-term future of our program will be assured by the finding of new endowment monies both to support our courses and to allow us finally to make long-term salary commitments to scientists whom we would like to do year-round research here.

A very high-level course on "Cellular Immunology"

The significance of the immune response in the control of cancer is still very unresolved, and current attempts to generate clinical responses suffer strongly from deep ignorance concerning the basic interactions between lymphocytes which lead to immune responses. We therefore decided to use our NCI funds committed toward the teaching of tumor immunobiology for the holding of an intellectually demanding two-week lecture course that emphasized the major conceptual problem in cellular immunology. We were very lucky to have Harvey Cantor and William Paul as the organizers, and the leading immunologists they brought to lecture here provided a unique opportunity for the students to see how our best immunologists try to stay one step ahead of their data. All in all it went very well and it will be repeated in 1979 in basically the same form.

Transfer of the last remaining Carnegie lands to the Laboratory

When, in 1963, the Carnegie Institution of Washington closed down its Department of Genetics and conveyed its buildings to the Cold Spring Harbor Laboratory, it retained ownership of two separate land parcels that had been acquired as a buffer to help preserve the relative solitude of science along Bungtown Road. One

piece of some 10 acres, lying along Route 25A and extending up Moore's Hill Road to Stewart's Lane, had been acquired in 1924 with the help of Mrs. E. H. Harriman from the Estate of Townsend Jones, to whom it had been deeded by his uncle, John D. Jones, the Founder of this Laboratory. The second parcel, lying south of Olney House, with a border along Bungtown Road and running well up to land owned by the Smoot Family, was acquired in 1953 from the legendarily erratic Rosalie Jones, daughter of Oliver Livingston and Mary Elizabeth Jones, and long known for her possession of a goat herd.

With the gradual development of most of the vacant land around us, the value of this land as a buffer is even greater than when it was purchased, and for several years we have been attempting to have its title transferred to us. Last year a sense of urgency developed when we realized that the most desirable site for a badly needed new animal facility was across Bungtown Road from Demerec Lab, partly on Carnegie-retained land. Happily, Jim Ebert's assumption of the Carnegie presidency in July helped bring a speedy agreement whereby all the remaining Carnegie land would come to us for a purchase price of \$200,000, to be payable over five years without interest. It then became a matter for the lawyers, and with the invaluable help of our neighbor, Jim Eisenman, the final papers changed hands just before the New Year arrived.

Downscaling our "super mouse house" plans

The very limited quarters (~ 1000 sq. ft.) we have for holding small rodents in our "mouse house" means that we have no choice but to build more such space if we are to stay in the forefront of modern molecular and cellular biology as it relates to cancer research. Upon his arrival here three years ago, Jim McDougall commenced plans for a new 11,000 sq. ft. building that would give us the most advanced air handling, ten large rooms expressly designed for animals, and many well-equipped auxiliary research rooms. The then-estimated cost of this facility was some two million dollars, and we applied to the National Cancer Institute for 1½ million dollars—the maximum allowed under their 75:25 matching provisions. For a site we decided on land that we hoped soon to acquire from the Carnegie Institution. We had been forewarned that we might have to wait some two or three years before our proposal, if approved, might be funded. But, in fact, we were awarded a construction grant in August 1977, which meant that ground could be broken by the spring of 1979. In our excitement we then expanded our plans to include laboratories for advanced mouse embryology in what otherwise would have been vacant basement areas, thereby bringing the total estimated cost to just over three million dollars!

Early this year progress ground to a halt as our negotiations with the Carnegie Institution stalled, we hoped temporarily. At the same time Jim McDougall, the proposed principal user of these facilities, made the decision to move his research to the Hutchinson Cancer Center.

We thus began to reexamine what form of animal facility we should build when we did acquire the Carnegie land. In particular, we began to worry that Federal monies for cancer research were reaching a plateau, and that once our "super mouse house" was built it might become increasingly difficult to find the operating funds for it. After we knew for certain that we would soon have the Carnegie land, we scaled back our design to a building of some 4500 gross sq. ft., with a projected total cost of \$750,000. Schematic architectural plans for this more-economical building are in preparation and by the spring of 1980 we should have at last broken ground for a building to meet our animal needs for at least the next decade or two.

LIBA holds its first Dorcas Cummings Memorial Lecture

Beginning in 1924, community support for us has been channeled through a marvelous organization, the Long Island Biological Association (LIBA), which exists solely for seeing that we prosper. Over the past three years they have been carrying on a special appeal for funds to cover the cost of our newly rebuilt Williams House. Happily this drive, for a total of \$225,000, came to a successful conclusion early this year when the final \$15,000 was conveyed to the Laboratory. We remain uniquely indebted to Mr. Edward Pulling, Chairman of LIBA, for overseeing this most appreciated fund drive.

For many years one of the Association's most devoted members was Dorcas Cummings who, with her husband Robert and family, lived close above us on Ridge Road. Upon her most untimely death two years ago, many of her friends gave special gifts to LIBA to create the Dorcas Cummings Memorial Fund, which now totals some \$12,000 in assets. The income from the fund will henceforth be used to support an annual spring lecture, the Dorcas Cummings Lectureship. The first of these lectures was given on Sunday, May 14, by Professor John S. Kopper of the Department of Anthropology of C. W. Post College. Entitled "Human Evolution and Geomagnetism," it related climatic changes induced by reversals in the earth's magnetic field to sudden bursts of human evolution.

The Annual Meeting of LIBA, held on Sunday, December 10, attracted an unusually large and enthusiastic audience. The main speaker was Professor Fernando Nottebohm, a noted authority on bird song, who does his research at the Millbrook Field Station of The Rocke-

feller University. His talk, on the organization of the nerve cells that control singing, amazed all of us, who had never imagined that canaries could be used for profound science.

Departing from the LIBA Board of Directors at the conclusion of the maximum six-year intervals were Mr. Ralph Maffei and Mr. Gerard Piel. Each has served us well, and we trust we can continue to count on their loyal assistance. We also lost the long-invaluable services of Joan Olney, who, because of her remarriage to Robin Tilney, moved to Far Hills. Newly elected to the LIBA Board were Mr. Joseph C. Dey, Jr., Dr. Alfred Azzoni, and Mr. Edmund Bartlett.

On Sunday afternoon, November 14, there was a special meeting of the LIBA Directors in the James Library, at which Jim I licks and Jeffrey Strathern of our staff explained the use of recombinant DNA to study fundamental problems in yeast genetics. Then everyone went down to Davenport Lab to observe how, in practice, such experiments are carried out. The gathering then moved to Blackford, where they joined other members of our scientific staff for an informal supper. All the assembled Directors and spouses found the occasion most worthwhile and we shall plan similar occasions for succeeding years.

Regretfully we note the death this December of Louise Ford, for over 25 years one of our staunchest supporters. Our deepest condolences to her husband Nevil, LIBA Chairman from 1957 to 1963.

Extraordinary efforts to survive a very ferocious winter

Jack Richards and his Buildings and Grounds department performed far beyond the ordinary call of duty to prevent the massive ice storm of January 14th from wounding us severely. Electricity from LILCO was off for almost three days in subfreezing weather, and without

their nonstop efforts so many pipes would have become frozen that we might have lost months of research. Particularly to be singled out are our plumbers, Lane Smith and Doug Haskett, and our electrician, Owen Stewart, whose quick collection of several small gas-fired generators and seemingly miles of temporary lines kept some hours of electricity in all our key buildings. This blow was soon followed by two of the largest snow storms ever to hit Long Island. In all, some 55 inches came down, and without Hans Trede's and Vincent Carey's devoted snow-removal efforts we would have been effectively closed down for most of February.

As we do still more and more maintenance and renovation, Jack Richards' life correspondingly has gotten that much more hectic. We note with satisfaction that Jack now has the assistance of Charles Schneider, who comes to us with some 30 years of experience as a successful Long Island contractor.

More invaluable help from our Board of Trustees

The inevitable challenges that necessarily accompany our desire to act as a major research institution demand responses that frequently are not obvious. I remain thus most fortunate that I have a powerful Board of Trustees, whose diversely successful backgrounds provide an enormous potential for incisive counsel. Harry Eagle leads this remarkably helpful body in an optimistic non-nonsense fashion that may have no equal. Now joining him on our Executive Committee are Edward Pulling, Clarence Galston, Bayard Clarkson, Robert Cummings, and Vittorio Defendi.

Our spring meeting, once postponed because of threats of still another devastating storm, inaugurated what we hope will be a yearly event—a preceding dinner at Robertson House where our scientific staff



Blackford Hall during 1978 ice storm

informally talked and dined with the scientists on our Board. It was a most enjoyable occasion, illustrating well the great advantages of having a strong scientific contingent on our Board itself.

Departing from the Board as of our Annual Meeting on October 25 were Mr. William Grant, Dr. Julian Davies, Dr. Howard Hiatt, Mrs. Joan Olney Tilney, and Mr. Walter Page. All have been invaluablely helpful and we shall much miss them. In particular I must single out our neighbor, Walter Page, for the crucial help he gave in the setting up of the Robertson Research and Maintenance Funds. The question was bound to arise whether we, as a then relatively small institution, could responsibly handle the proposed endowment monies. Walter's coming back to our Board in 1972 left no doubt as to our intelligent probity.

Following the Annual Meeting was a dinner held at Robertson House to honor our retiring members and to let them know how much we want them to remain our active friends.

Guarantee of senior scientists' positions through "Rolling Five" appointments

Like most private research institutions with limited endowments, we face the problem of how to guarantee long-term salary security to our key senior staff. We can't expect them to have the peace of mind necessary for real creativity if they fear they might soon have to leave if their current grants are not renewed. At the same time it is far from clear how long we should continue salary support for scientists who cease doing the high-quality science for which they were appointed.

To try and satisfy these conflicting imperatives, our Board of Trustees at their May meeting approved the creation of the rank of Senior Scientist, whose holders would have a continuously rolling commitment of five more years of salary support. It is our intention to appoint to this rank only individuals who have demonstrated exceptional scientific promise; once so appointed we would hope that they spend a large fraction of their scientific careers here.

Appointed to this rank as of July 1 were Guenter Albrecht-Buehler, Michael Botchan, Ahmad Bukhari, Richard Roberts, and David Zipser.

Adoption of a mortgage program to help the senior staff live in our community

Most of our scientists, when they arrive, live in one of our apartments or small houses along Bungtown Road. Virtually none of these units, however, is large enough for their needs when, as their families grow, they accumulate more and more possessions. The natural course for our senior staff is to move into an already existing house or build a new one within the boundaries of our local school district, where their children already

may have started grammar school. For the most part, however, our staff do not have the savings necessary to make the required down payments for such houses. So if they are to have the opportunity of moving off the grounds, they must be provided with specially tailored mortgage help. To this end, our Board of Trustees has given its provisional consent to a program similar to that now available at Princeton, which provides low-down-payment, long-term (30-year) mortgages to its permanent faculty.

The role of the Director needs reexamination

This year over six million dollars were spent for our operating and capital programs, an increase over the past decade of more than fivefold when corrected for inflation. Our day-by-day duties are correspondingly that much more complex, and I cannot take so lightly those who question me as to whether I regret leaving science to become an Administrator. Until about two years ago I still easily found the free time to talk or read science, and my life was not greatly different from when I was full-time at Harvard running a large research group. Now, however, I fear I administer more than is compatible with a daily infusion of new scientific facts into my head. Some form of long-term administrative reorganization must thus occur. This necessity only partly reflects my boredom with tasks that several years ago might have seemed novel. The more important consideration is that the Director of this Lab must be actively in science to insure that we look ahead, not backward, in making new staff appointments, organizing new courses or meetings, and choosing topics for books which will be widely read.

That this situation is not yet totally out of control owes much to the first-class job done by Bill Udry in his capacity as Administrative Director. To my relief, he and his family have just moved to a nearby house on Stewart's Lane and he becomes even more available to help me out on short notice. And we now also benefit gladly from the appointment of Pat Hickey to direct our Personnel efforts, including staff benefits, and to oversee our hotellike aspects.

Equally important, at this year's beginning Joe Sambrook agreed to take on the role of Assistant Director. Together we can swiftly solve many problems, but given his major role of running James Lab, he is doing more than we can rightfully ask for the long term.

Extensive delegation of many more duties to our scientific staff thus appears to be a prerequisite for our long-term health. Up till now, I have been reluctant to move this way, feeling that our scientists needed all the time they could get for their research. This still remains true for our younger staff, but now it seems inescapable that our senior members become more equal partners in our universitylike operations.

We should not be afraid to try for the best

When I first took over, I assumed that we would grow to a proper size and then stop, satisfied that we had made it. Now, after 11 years of steadily increasing budgets, I realize first-class institutions don't operate that way. It is not that we want growth per se—for necessarily that much more money must be raised, and this coming year we already shall be spending some \$20,000 per day. More to the point is that it is impossible to have an outstanding staff which does not generate new ideas or set about to fill vacuums that should not exist. Whenever we see a way to solve a classic problem we should get on with it, even if we may have to totally reinstrument ourselves. We naturally have to weigh carefully our chances for success, since once financially overextended, climbing back to safety is not always straight-

forward. The best defense against such mistakes, of course, is the possession of the highest quality scientific staff, and we must always work hard to remain competitive with the very best research institutions and universities. Here we have no more powerful tool than a firm reputation for pushing first-class ideas with the greatest possible vigor.

To do this we shall need the continued help of all the wonderful friends who in the past have helped us raise so well the unrestricted monies that give us the real leverage for the unusual. As long as they remain behind us, and I believe they will, our inherent future remains indeed bright.

January 6, 1979

J. D. Watson

I must now note our great sorrow at the death on February 15, 1979 of Lucy Lettingwell Pulling.

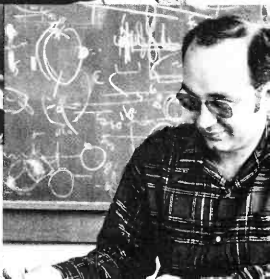
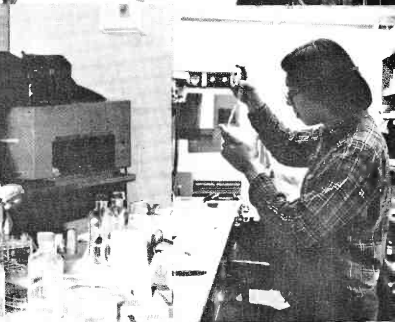
Her interest in our Lab dates back over fifty years. Her father, Russell C. Lettingwell, a leader in the New York banking community, helped found IIBA in 1924 and was one of its original directors. In 1928 Lucy married Edward Pulling, then a master at Croton. This union was to last happily over fifty years. In 1931 they founded the Millbrook School, which, under their devoted, wise direction, became a leading private preparatory school. Upon her husband's retirement as headmaster in 1965, they came back to Oyster Bay to settle in the wonderful

rambling farmhouse on Yellowcote Road in which her parents had lived.

Quickly Lucy became one of our more important supporters, and, in particular, I want to acknowledge the warm friendship which she extended to Liz and me when we came down from Harvard to begin our marriage. She gave to her home and to all who were privileged to know her a sense of gentle compassion, sensible loyalty, and open-minded intelligence. Our lives can never be the same without her, and we shall long remember how firmly she stood with us.



Lucy Pulling and Nina Galston at Airste, June 1978



YEAR-ROUND RESEARCH

MOLECULAR BIOLOGY OF TUMOR VIRUS

J. SAMBROOK, B. Ahrens, M. Botchan, J. Dorso, A. Dunn, R. Frisque, L. Garbarini, Y. Gluzman, A. Graessman, M. Graessman, R. Greene, T. Grodzicker, S.-L. Hu, R. Kelch, D. Klessig, D. Lane, M. Lazarus, M. Mathews, R. McGuirk, R. McKay, R. Pashley, C. Paul, A. Robbins, W. Schaffner, M. Sleight, D. Solnick, N. Stow, J. Stringer, R. Tjian, W. Topp, S. Weirich, I. Wendel, J. Wiggins, S. Young, W. Keller

ADENO AND ADENO-SV40 HYBRID VIRUSES

Genetics

In general, we are interested in the way in which the genomes of DNA tumor viruses are organized and expressed both during the lytic cycle and in the integrated state. During the last year work has progressed on several problems. Previously we have reported the characterization of host-range mutants of the non-defective Ad2-SV40 hybrid virus Ad2⁺ ND1. Unlike Ad2, which grows poorly in monkey cells, Ad2⁺ND1 grows equally efficiently in human and monkey cells. This extended host range is presumably due to the expression of the integrated SV40 sequences that map between 0.28 and 0.11 fractional units on the SV40 map. The seven host-range mutants that grow well in human cells comprise one complementation group, grow poorly and are defective in the synthesis of late proteins (especially fiber) in monkey cells, and, like Ad2, are complemented for growth in monkey cells by SV40. Thus, they behave as if they have lost the enhancement or "helper" function provided by SV40. Ad2⁺ND1 expresses a 30,000-m.w. protein that is coded for in part by SV40 sequences. Whereas four of the host-range mutants synthesize a 30K protein in infected human cells and are probably missense mutants, three of the host-range mutants (71, 140, and 162) synthesize no 30K protein but direct the synthesis of 10K, 19K, and 19K fragments, respectively.

We reported last year that these mutants carried ochre and amber mutations. Thus, in *in-vitro* translation systems, mRNA isolated from mutant-infected cells yielded the 30K wild-type protein as well as the mutant polypeptide fragment in the presence of yeast

suppressor tRNAs (Gesteland et al., *Proc. Natl. Acad. Sci.* 74: 4567 [1977]). This year, in collaboration with Adolf Graessmann, who was visiting from the Institut für Molekularbiologie und Biochemie, Berlin, we assayed the *in-vivo* activity of yeast suppressor tRNAs in mammalian (monkey) cells. We found that microinjection of the appropriate yeast tyrosine suppressor tRNA into mutant-infected monkey cells increased the yield of viral fiber protein and total virus production. We are now interested in seeing whether yeast suppressor tRNA genes can be properly expressed in these cells.

We reported last year that Ad2⁺ND1 grows in monkey cells because it expresses the 3' region of the inserted SV40 sequences. At that time, we had isolated revertants of the nonsense mutants that had regained the ability to grow on monkey cells and had altered genomes. These mutants either carried duplications of the distal end of the SV40 insertion fused to the Ad2 fiber gene or had deletions of the proximal end of the SV40 sequences. Probably they arose by a process of unequal crossing-over. This is important to what follows. Several years ago Dan Klessig had isolated mutants of Ad2 and Ad5 that had gained the ability to grow on monkey cells without acquiring SV40 sequences. In collaboration with him, we have been using marker-rescue and DNA transfection techniques to map the sites of the mutations in these viruses. Our first results placed the location of the mutations between 0.62 and 0.70 on the adenovirus genome, a region that includes sequences of the genes for both the early and the late 100K protein. More recent mapping, using smaller DNA fragments for marker rescue, has narrowed the limits of the lesion in one mutant so

First row: S. Zain; P. Reichel and B. Hoffman; second row: T. Gingeras; H. Khatoun; R. Tjian; third row: D. Zipser; W. Schaffner; fourth row: G. Wong; A. Bukhari; Y. Gluzman

that we are fairly confident it is located in the early adenoviral gene that codes for the 72K binding protein.

Because the Ad5 host-range mutant was isolated without mutagenic treatment and because revertants of Ad2⁺ND1 appeared to arise by a process of aberrant recombination, we set out to see whether host-range mutants of Ad2 or Ad5 could be obtained that had altered genomes and might have arisen by recombination. Such mutants would be recognized by differences in the restriction patterns of their DNAs. Human cells were infected at moderately high multiplicities with Ad5 or Ad2 for several cycles and then plated on monkey cells to isolate mutants. Several nondefective mutants that now grew well were obtained. Analysis with restriction enzymes showed that they had, indeed, rearranged genomes. However, the majority of the alterations were not found in the region that codes for the binding protein, but at the right end of the genome in and around the fiber gene, whose expression is severely reduced in monkey cells. We are presently analyzing these mutants in detail to see how the control of gene expression has been altered.

A new Ad5-SV40 hybrid virus

A new Ad5-SV40 hybrid (Ad5⁺D1) has been cloned from a stock of Ad5 that, many years ago, had been "adapted" by Hartley et al., (*Proc. Soc. Exp. Biol. Med.* 92:667 [1956]) to replicate in rhesus monkey kidney cells. The hybrid is defective and requires helper Ad5 for productive infection of simian cells. Infection of monkey cells with the hybrid virus at low multiplicity gives rise to infectious SV40 virions.

The structure of the genomes in an Ad5⁺D1 hybrid population has been determined by analysis with restriction enzymes, hybridization, and electron microscopy. They comprise a family of hybrids that differ from each other by one copy of SV40. The structure of the largest Ad5⁺D1 hybrid genome is shown in Figure 1.

This molecule is divided into two identical halves, which are joined together as inverted repeats. Each half of the hybrid DNA molecule contains 2.7 SV40 genomes, organized as a tandem head-to-tail repeat and joined to 10% of the Ad5 genome located between map positions 0 and 10. We are presently de-

termining the DNA sequence of the junctions between the adenoviral and SV40 sequences and at the reflection point in the center of the molecule.

Transcription and splicing

Within the past 18 months a great deal of evidence has accumulated that suggests that the transcription of eukaryotic genes is fundamentally different from that observed in prokaryotic systems. The unprecedented observation that many mature viral mRNAs are composed of sequences (leaders) derived from noncontiguous regions of the genome was first demonstrated with the Ad2 system by groups working independently at Cold Spring Harbor and MIT. More recently, analysis of eukaryotic genes in several other systems has revealed the presence of intervening sequences that are not represented in mature RNAs. In the case of Ad2, mRNAs transcribed from the viral *r* strand contain a common leader derived from three noncontiguous regions of the genome around map positions 16.6, 19.6, and 26.6. Until recently it was believed that functional late Ad2 mRNAs contained only these three leader segments. However, it is now clear that some late mRNAs contain additional leaders, which are thought to be intermediates in processing pathways that lead to mature mRNAs.

Within the past 12 months we have made a detailed study of the mRNA encoding the structural polypeptide—fiber, the most distal of all the late genes. Frequently, fiber mRNAs containing the tripartite leader also carry the γ leader encoded 2000 nucleotides upstream from the fiber gene (Dunn et al., *Cell* 15:511 [1978]; Chow and Broker, *Cell* 15:497 [1978]). Our analysis of fiber mRNA has been carried out, in part, using the nondefective adeno-SV40 hybrid virus Ad2⁺ND1 dp2. A convenient feature of this hybrid is the presence of an insertion of SV40 DNA immediately distal to the start of the fiber gene. During the course of productive infection with Ad2⁺ND1 dp2, a viral mRNA is synthesized whose 5' end includes the 5' fiber mRNA sequences and whose 3' end contains SV40 sequences. Biochemical and electron microscopic heteroduplex analyses reveal that two forms of this hybrid mRNA exist in the cytoplasm of cells at late times in infection. Both forms contain the conventional tripartite leader present on late Ad2

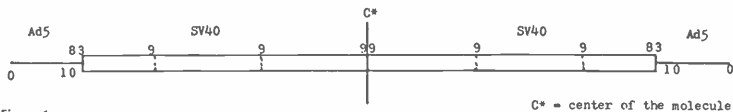


Figure 1
Structure of the largest defective Ad5-SV40 hybrid.

mRNAs but they differ in that the γ leader component, consisting of 180 nucleotides of Ad2 sequences, is either present or absent. This relative difference in size has allowed us to separate and isolate the two forms of hybrid mRNA by electrophoresis through denaturing agarose gels. By introducing highly purified preparations of both forms of the hybrid mRNA into a cell-free translation system, we established that the presence or absence of the γ leader sequence has little or no influence on the ability of the messenger to direct the synthesis of the hybrid protein. It seems unlikely, therefore, that the 4th leader sequence itself plays a direct role in the messenger translation process. We considered the alternative possibility that the ribosome is the site for a final splicing event in the maturation of the hybrid mRNA. Specifically, we wondered whether the 4th leader sequence of the hybrid mRNA is removed during the translation process. Highly purified ^{32}P -labeled 4th-leader-containing RNA was incubated in both the reticulocyte lysate and wheat-germ protein-synthesizing systems under conditions that yielded efficient synthesis of the hybrid protein. The RNA recovered from these reactions and assayed in methyl mercuric hydroxide-agarose gels appeared unaltered in electrophoretic mobility.

As an extension of this work, we have fractionated preparations of adenovirus "late" mRNA by electrophoresis through agarose gels containing methyl mercuric hydroxide and examined the coding capacity of each fraction by cell-free translation. The results have allowed us to make accurate size estimates of the RNAs coding for each "late" polypeptide and thereby confirm or justify the assignments arrived at by other means. This information is complemented by data arrived at by hybridization-selection and hybrid-arrested translation techniques, in experiments carried out with Bryan Roberts at Brandeis and Bruce Paterson at NIH, to build up a detailed picture of the arrangement of the translated sequences on the viral genome. The combination of methods has been particularly useful in resolving the species within families of mRNAs that share common 3' termini (about five such families exist in the major "late" transcription block). For example, in one block we have substantiated the placement of the sequence coding for polypeptide pVI as an adduct on the 5' side of the sequence coding for hexon, and in another we have clarified the order as (5' to 3') penton, pVII, and core-protein V. Having set the methods on a firm footing, we are now turning to the problems posed by the "early" mRNAs, where more extravagant sequence translocations (splices) and lesser mRNA availability combine to redouble the difficulties.

How adenoviral mRNAs are fashioned is unknown. It is generally accepted that posttranscriptional pro-

cessing of short-lived precursor RNAs is involved, but the mechanism by which leaders become covalently linked to each other and to the sequences coding for mRNAs is undiscovered. Presumably, enzymes exist that recognize specific sequences within the precursor molecule and cause the removal of intervening sequences by endoribonucleolytic cleavage followed by RNA ligation. To determine whether common features exist between leader segments and corresponding intervening sequences, we have prepared recombinant plasmids carrying reverse transcripts of the purified fiber mRNA of Ad2. In collaboration with Rich Roberts, Sayeeda Zain, and Walter Keller the complete DNA sequence of the leader region has been determined and, where possible, the junction between conserved and intervening sequences has been defined by reference to the DNA sequence of the Ad2 genome. Although the presence of short, repeated sequences of the splice junctions make it impossible to locate the splice point unambiguously, the borders between conserved and intervening sequences can be arranged in a configuration that reveals several striking features (Fig. 2). First, the dinucleotides GT and AG are found at the respective 5' and 3' ends of each intervening sequence. Second, the nucleotides within the leaders immediately adjacent to intervening regions are considerably similar. The prototype sequence derived from our analysis of the junctions between conserved and intervening regions of adenoviral mRNAs (Fig. 2) is closely related to that obtained previously for several other eukaryotic RNAs. In seven mRNAs of the chicken ovalbumin gene, in the early and late SV40 mRNAs, in the small and large introns of mouse and rabbit β -globin, and in the small and large introns of IgG, the terminal dinucleotides at the 5' and 3' ends of the intervening sequences are GT and AG, respectively. Furthermore, in all of these cases, as in Ad2, only limited divergence of the nucleotides adjacent to the invariant nucleotides is observed. From these data it seems likely that the common nucleotides at the 5' and 3' ends of the intervening sequences serve as recognition signals for excision-ligation enzymes. It is possible that specific folding of the primary transcript could arrange the prototype sequences in a configuration conducive to action of these enzymes.

Adeno-SV40 hybrid proteins

A protein related to SV40 T antigen has been isolated from cells infected with the adeno-SV40 hybrid virus, Ad2⁺D2, and shown to bind to DNA (see last year's report). We discovered that the 107,000-dalton D2 hybrid protein binds sequentially to three tandem recognition sequences located within a stretch of 120 nucleotides which encompasses the origin of SV40

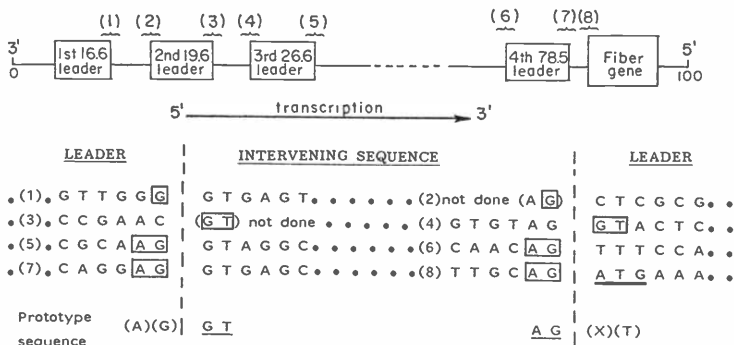


Figure 2

Comparison of DNA sequences of adenoviral leader-intervening sequence junctions. A map of the Ad2 genome (not to scale) showing the four leader sequences of fiber mRNA and their map coordinates. Numbers in parentheses indicate specific leader-intervening sequence junctions. The leader sequence presented is of the RNA strand (5'→3') where T has been substituted for U. DNA sequence of the intervening region follows the same polarity (5'→3'). Vertical dashed lines indicate junction of conserved and intervening sequences. Boxes indicate short direct repeated sequences at the splice junctions. This feature does not allow precise definition of junction points between leader and intervening sequences. Letters in parentheses (regions [2] and [3]) indicate predicted nucleotides. The line beneath the sequence ATC (AUG) (line 8) indicates the translation initiation codon within the main body of fiber mRNA.

DNA replication. Recently, we carried out similar binding studies with T antigen isolated from a different adeno-SV40 hybrid, Ad2*D1, which encodes an "authentic" large-T- and small-T-antigen protein. Not surprisingly, the 96,000-dalton D1 T antigen binds to the origin of SV40 replication in precisely the same manner as the D2 hybrid protein. To investigate further the interaction between these viral DNA-binding proteins and regulatory sequences at the origin of replication, we have utilized the reagent dimethyl sulfate to methylate adenine and guanine bases which are not shielded by the binding protein. By sequencing the methylated DNA, we were able to identify specific purine residues which are in close contact with the D2 hybrid protein in the DNA-protein complex (Fig. 3). Our findings indicate that the D2 hybrid protein contacts the DNA exclusively in the major groove of the double helix and that each of the three binding sites encompasses approximately 20–25 nucleotides, with a space of about 20 bases between each site. Moreover, the binding protein appears to make highly symmetrical contacts with the DNA. This feature of the interaction between DNA and protein is most dramatic within binding-site II, where the sequence of nucleotides can assume a conformation consisting of a perfect 27-nucleotide hairpin-loop structure (Fig. 4). In addition to obtaining a detailed picture of the way in

which the D2 hybrid protein embraces the sequences, at the origin of SV40 DNA replication, we have also discovered that this binding protein interacts in an analogous fashion with the origin sequences of two closely related papovaviruses, the murine polyoma and the human BK viruses.

A logical extension of our efforts to understand the mechanism of action of SV40 T antigen is to determine whether the product of A gene is able to catalyze certain enzymatic reactions that might play a role in DNA replication or cellular transformation. Our most recent studies reveal that the D2 hybrid protein is associated with both an ATPase and a protein kinase activity. Both of these activities copurify with the T-antigen-related protein during several rigorous chromatographic steps. By improving the purification procedure, we were able to resolve two chromatographic forms of the D2 hybrid protein. Form-I protein contains only an ATPase activity, whereas form II is able to catalyze the hydrolysis of ATP as well as transfer the γ -phosphate of ATP to either the D2 hybrid protein itself or to exogenously added phosphoproteins such as phosphovitin. During glycerol gradient sedimentation in the presence of SV40 DNA, both forms bind DNA, but the ATPase activity appears to cosediment with SV40 DNA more efficiently than the associated protein kinase activity. Antibody directed

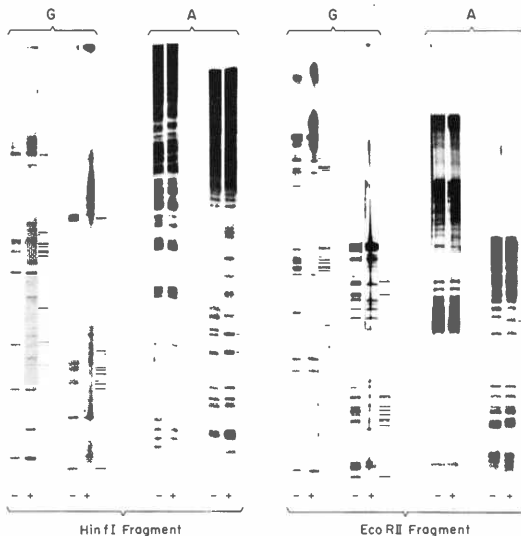


Figure 3

Purine bases in the T-antigen binding site specifically protected from methylation by dimethyl sulfate. Restriction endonuclease cleavage fragments of SV40 DNA were generated by digestion with *Hin*fI and *Eco*RII. *Hin*fI fragment A and *Eco*RII fragment G were labeled at the 5' ends with [γ - 32 P]ATP by T4 polynucleotide kinase and subsequently cleaved with *Mbo*I and *Hin*fI, respectively. The end-labeled fragments containing the T-antigen binding sites were purified by agarose gel electrophoresis and subjected to methylation by dimethyl sulfate in the absence (-) and presence (+) of the D2 hybrid protein. The methylation reaction was performed in 200 μ l of buffer containing 1 μ g end-labeled DNA, 10-20 μ g purified D2 hybrid protein, 0.1 M cacodylate (pH 7.1), 0.1 mM EDTA, and 10 μ g/ml of BSA. After methylation of the purine bases, the DNA fragments were sequenced as described by Maxam and Gilbert (1977). Guanine and adenine residues in the early strand of SV40, which were methylated by dimethyl sulfate, are displayed in the four lanes at the left; purines from the late strand of SV40 are shown in the four lanes at the right. (—) The position in the sequencing gel of specific guanine residues that were shielded from methylation or undermethylated; (- -) the position of adenine residues that were overmethylated as a result of the interaction between the D2 hybrid protein and the T-antigen binding sites on the DNA. The cleavage products for each guanine and adenine reaction are shown in two separate gel loadings so that the pattern of bands near the upper part of the gel are displayed more clearly.

against SV40 T antigen can specifically inhibit the ATPase and protein kinase activities, whereas preimmune sera have no effect. These findings suggest that one and possibly two enzymatic activities are intrinsic to the SV40 gene-A protein. At present, we do not know what structural differences distinguish these two forms of the D2 hybrid protein, nor can we be certain that the associated enzymatic activities are, in fact, catalyzed by the SV40-encoded gene-A protein. However, in the year ahead some of these issues should be resolved, and we may have a better under-

standing of how T antigen functions both in the lytic cycle of the virus and in transformed cells.

Properties of transformed cells

Over the last two years our interests have centered on elucidating the role of the two early gene products of SV40 (small T, m.w. 17K, and large T, m.w. 96K) in the maintenance of the transformed state. Our approach has been to isolate and characterize cell lines transformed following infection with mutant virus strains

Purines Affected by T-antigen Binding

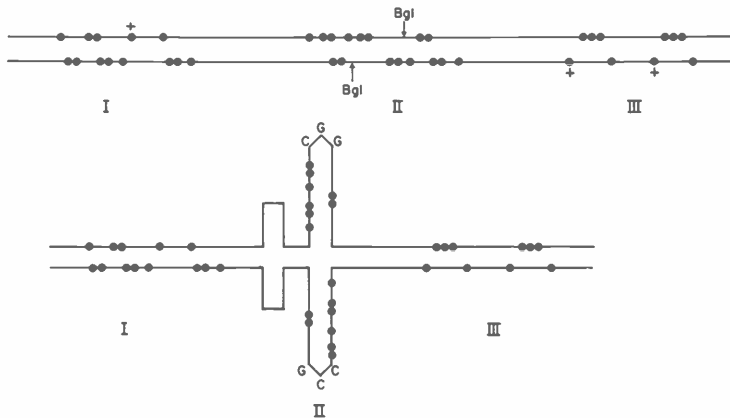


Figure 4

Points of contact between the D2 hybrid protein and SV40 DNA. The parallel solid lines represent the sequence of nucleotides in SV40 DNA which encompasses the origin of DNA replication and the three tandem T-antigen binding sites. (•) Purine residues that are in close contact with DBP; (+) the location of specific adenine residues that are enhanced for methylation to dimethyl sulfate in the presence of the D2 hybrid protein. (I) Strong binding site; (II) medium binding site; (III) weak binding site. The top diagram illustrates the arrangement of contacts made between the D2 hybrid protein and the SV40 DNA in a conventional duplex conformation. The bottom diagram depicts the pattern of contacts made between DBP and DNA represented in an alternate configuration containing a hairpin loop formed by a perfect 27-nucleotide palindrome centered around the BglI cleavage site. The nucleotides CGG and GCC shown at the loops of the hairpin structure indicate the location within binding site II that fails to contact the binding protein.

defective in one or the other of the two "early" proteins. Conditionally lethal (*ts*) mutants of SV40 were isolated several years ago by Peter Tegtmeier (*J. Virol.* 10:591 [1972]) and others. The *tsA* mutants are defective in an early function, failing to induce viral DNA synthesis at restrictive temperatures, and, as shown by Alwine et al. (*Cell* 6:529 [1975]), produce a thermally labile large-T protein. Deletion mutants of SV40 that have lost DNA sequences within the region transcribed early were isolated in 1976 by the Berg group (Shenk et al. *J. Virol.* 18:664 [1976]), and several more mutants of this class (dl54/59) have been isolated in our lab. Last year we showed that these dl54/59 SV40 mutants in many cases produced a small-T protein with an altered electrophoretic mobility, and that the decrease in protein size corresponded closely to that predicted from the approximate number of DNA base pairs deleted. We have isolated a large number of rat embryo fibroblast (REF) cell lines transformed both by wild-type (wt) SV40 and by the dl54/59 (small-T-

defective) and *tsA* (conditionally large-T-defective) SV40 mutants. These lines have been characterized extensively during this past year. We have been assisted in these studies by collaboration with Dan Rifkin and Ruth Crowe at NYU on plasmidogen activation of the lines and with Chungming Chang at NIH on characterizing the virus-specified proteins present in these lines.

The specific properties of the REF transformants we have chosen to study carefully are those described by Pollack and coworkers (Pollack et al., *Proc. Natl. Acad. Sci.* 71:4792 [1974]) at Cold Spring Harbor in 1974: namely, the ability to produce clonal lines, the ability to proliferate in culture medium supplemented with only low levels of fetal calf serum, the ability to grow without anchorage, the ability to activate plasmidogen to plasmin, and the loss of cytoplasmic actin-containing fibers. Pollack found that, as a rule, only two classes of SV40 REF transformants existed. Their properties are listed in Table 1. In our previous

Table 1
Two classes of SV40-transformed REF

	<i>Minimal</i>	<i>Maximal</i>
Properties	clone on plastic grow in low serum	clone on plastic grow in low serum clone in methocel or agar activate plasminogen lose actin cables tumorigenicity in nude mice
Apparent phenotype	T*t ⁻	T*t ⁺

work we had shown that the d154/59 transformants cloned with high efficiency on plastic cell-culture dishes and grew well in low-serum medium. On the other hand, these transformants failed to grow when suspended in methocel.

More recently we have found that the majority of d154/59 SV40 REF transformants fail to activate plasminogen to plasmin. Although many of the d154/59 viruses do induce loss of actin cables, we have identified several viruses of this class that we feel are particularly "tight" for the small-T defect. A large number of lines were isolated following infection with these "tight" mutants (e.g., SV40), and 90% of these lines retained actin cables. Thus, we feel that actin-cable loss and plasminogen activation, like growth in methocel medium, require the presence of small-T activity.

The classification of mutants as "tight" or "leaky" for small-T function is not totally ad hoc. We had previously shown that, by some criteria, several of the d154/59 mutants (e.g., d1890 and d12001) transform with efficiencies intermediate between those obtained with wild-type SV40 and the residue of the mutants. Furthermore, when relative viral burst sizes on CV1 and SV40-transformed (presumably small-T complementing) CV1 cells were compared, many d154/59 mutants gave the same ratio of bursts as did wild-type SV40, whereas others were ten-fold reduced in the CV1 line. Because the small-T protein is by all accounts not required for viral proliferation or plaque formation on CV1 cells, there was no selection either for or against small-T activity at the time of isolation of these mutants. It is quite reasonable that both "tight" and "leaky" mutants should exist. This is to be contrasted with the situation with the similar polyoma virus mutants in which only tight mutants (which showed a host range) were isolated.

To complete our analysis, we have characterized the tsA SV40 REF transformants. Earlier work by Mary Osborn and Klaus Weber (*J. Virol.* 15: 636 [1975]) had shown that similar transformants were temperature-sensitive for the ability to clone and to grow in low serum, a result we have verified with our lines. In

addition, we find that these lines show only a minor temperature sensitivity of plasminogen activation. We feel that in all probability this is due to some proportion (20–80%) of the cells in the several lines tested totally shutting down viral transcription at the restrictive temperature, an effect that would lead to loss of both viral proteins, and that the decreased activation reflects the loss of small-T activity.

To evaluate the role of large T in the loss of actin cables, we turned, through a collaboration with Adolf and Monika Graessmann who spent six months at Cold Spring Harbor on sabbatical leaves, to microinjection of SV40 species into single cells in culture. We chose this approach rather than using the tsA transformants both because of the partial shut down of viral transcription alluded to above and because the morphology of most cells is itself temperature-sensitive. We found that microinjection of wild-type SV40 DNA into either the cytoplasm or the nucleus produced a disappearance within 24 hours of actin cables (as assayed by immunofluorescence) in approximately 90% of the cells injected. Microinjection of a restriction-endonuclease-generated DNA fragment encoding only small T (*Hpa*I+II B) produced a similar loss, whereas microinjection of d1884 SV40 DNA (presumably encoding only large T) or a purified SV40 large-T-antigen-related protein (the D2 protein) failed to affect the actin network in any way, although the bright nuclear fluorescence characteristic of large T was obtained. We think that these experiments show that the loss of actin cables depends only on small-T activity.

Thus, we now feel that those properties characteristic of the minimal transformants (Table 1) depend only on large-T activity and those properties unique to the fully transformed cells (Table 1) depend only on small-T activity (Table 2). In this analysis we would characterize the minimal transformants as phenotypically T*t⁻ and the full transformants as T*t⁺. The logical explanation for this difference would be the presence or absence of small-T protein.

Now that we have identified those properties of transformed rat-embryo cells that depend on each of

Table 2

Dependence of the properties of transformed REF cells on large T and small T

Property	Depends on	Independent of
Clone on plastic	large T	small T
Grow in low serum	large T	small T
Clone in methocel or agar	large and small T	—
Activate plasminogen	small T	large T
Lose actin cables	small T	large T

the two proteins, we are searching, both in vitro and in vivo, for other factors (hormones, phorbol esters, etc.) that act similarly in this system as a step towards elucidating the mechanism of viral transformation.

It is interesting to note that those properties that correlate well with cellular tumorigenicity of SV40 REF transformants are just those that depend only on the small-T protein, and thus we would expect that small T would play an important role in viral tumorigenicity. This is apparently the case. In parallel studies we have undertaken to assess the tumorigenicity of the small-T-defective viruses. Normally, SV40 is tumorigenic if injected subcutaneously into 6–12-hour-old hamsters. Using an inbred line of Syrian hamsters (LHC), we find that wild-type SV40 produces tumors in greater than 90% of the animals within 8 months, with an average latency of 4–5 months. At the time of writing this (11 months) the leaky DI54/59 mutants have produced tumors in 10–20% of the animals injected, with an average latency of 10 months, the tumors have yet to appear from the tight mutants. Although these tumors are just now being established into culture to analyze their pattern of growth in vitro and have not yet been examined histologically, it is already clear that the small-T protein plays an important role in viral tumorigenicity.

We still have not been able to evaluate fully the role of SV40 large T in viral tumorigenicity. At present we still lack that class of transformants that we would characterize as T⁺, and we are currently undertaking to isolate such cell lines.

The organization of integrated SV40 DNA sequences

It has been established that the large T (or 96K) early gene product of SV40 is involved in the maintenance of the phenotype of cells transformed by this virus (see above). On the other hand, the roles of large-T and small-T proteins in the early events, or the "initiation" steps, of the transformation process are unclear. One particular early event that has been a focus of our work is the recombination that occurs between SV40 and chromosomal DNA. Previous work from our group has shown that the cell contains a large number of

chromosomal DNA sites for the attachment of the viral DNA; and furthermore that the viral DNA does not contain a unique DNA site that mediates this attachment. In this past year we have learned that the SV40 viral DNA frequently can be inserted in the chromosome as a partial or complete tandem repeat of the viral DNA sequences. A simple assay for a tandem insert that we employ is the detection of unit-length viral DNA fragments after hydrolysis of total cellular DNA with restriction enzymes which clear viral DNA once. In general, transformed cell lines that contain multiple copies of viral DNA usually have at least one insert that appears to be a tandem array. For example, evidence for tandem insertions of viral DNA was obtained in nine cell lines independently transformed by a sea urchin histone DNA-SV40 DNA chimeric virus. Although our data are incomplete, we suspect that even in cell lines transformed by restriction fragments of SV40, complex tandem arrays of viral DNA are inserted into the chromosome. Our current hypothesis is that these integrated structures arise by a recombination event between an oligomeric replicating SV40 molecule and chromosomal DNA. A requirement for limited viral synthesis prior to integration would be consistent with the need for an active gene-A product in the initiation of transformation steps for both minimal and fully transformed lines. A simple origin for these tandem insertions of SV40 DNA, namely, a recombination between a nonintegrated viral molecule and a chromosomal copy, seems to be ruled out by the following experiment. We have previously characterized revertant cell lines that still maintain an integrated copy of viral DNA (Steinberg et al. *Cell* 13:19 [1978]). These lines can be superinfected with SV40 and subsequent retransformants picked from cultures grown under appropriate selective conditions. New tandem arrays of viral DNA could be detected in 8 of 15 such retransformants studied. However, in no case was the parental or primary insertion of the revertant cell line perturbed. This experiment shows that tandem insertions arise early in the transformation process and are independent of preexisting chromosomal copies.

With the relaxation of the NIH guidelines for recombinant DNA research, we are looking forward to

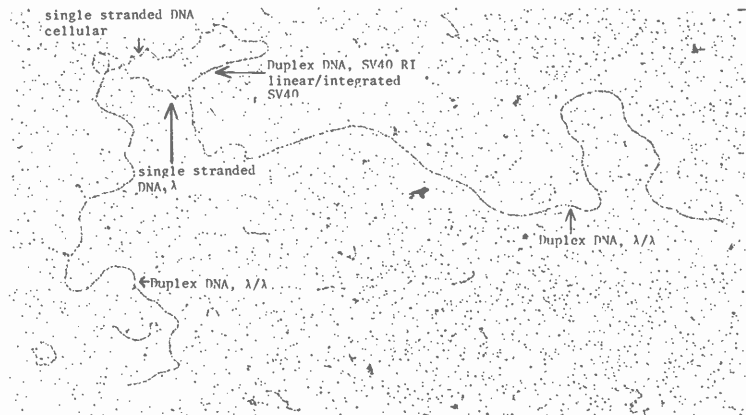


Figure 5

Heterotriplex DNA consisting of (1) a λ Charon 4A DNA strand, (2) a strand of λ Charon 4A DNA into which the cellular DNA *EcoRI* restriction fragment that contains integrated SV40 DNA has been inserted, and (3) a strand of SV40 DNA linearized by cleavage with *EcoRI*.

applying recombinant DNA methods to analyze the molecular details of the junctions between viral DNA and chromosomal DNA in transformed cells. We have recently cloned in the lambda phage Charon 4A, from the cell line 14B, an *endoRI* restriction fragment that contains the entire viral insert of this cell line along with flanking chromosomal sequences from both the 5' and 3' ends of this integrated DNA. Figure 5 shows a picture of a heterotriplex molecule constructed by annealing the phage strands of Charon 4A with the recombinant viral DNA and RI linear molecules of SV40. This figure shows that the SV40 sequences in the chromosomal DNA sequences are very asymmetrically placed with respect to the RI restriction sites. We have begun DNA-sequence analysis of the SV40 junction from the side of the insert that contains very little cell DNA. Our preliminary results show that there are 72 nucleotides of cell DNA extending from this *EcoRI* site and that these sequences simply merge into SV40 sequences without any interspersions or mutation of viral sequence at the joint.

We have been particularly interested in this cell line because of the excision products of the integrated DNA that are detected after fusion of 14B cells with permissive simian cells. In contrast to other lines which yield discrete SV40 molecules upon cell fusion, the replicating "rescued" forms detected in hetero-

karyons of 14B cells and simian cells are very heterogeneous. We have also cloned these excision products in the lambda WES phage and have begun an analysis of their structures. It appears that these circular forms are created by an illegitimate recombination between SV40 DNA and cell DNA in some cases and cell DNA from one side of the insertion with cell DNA from the other side of the insertion in other cases.

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PROTEIN SYNTHESIS

R. GESTELAND, J. Atkins, J. Broach, C. Greenberg, V. Guarascio, M. Harter, B. Hoffman, D. Klessig, J. Lewis, M. Mangano, M. Mathews, C. McGill, P. Reichel, L. Silver, J. Smart, L. Smith, J. Strathern, P. Thomas, C. Waldron, N. Wills

Amino-terminal sequence of adenovirus proteins

We have used the citrate synthase treatment devised by Palmiter (*J. Biol. Chem.* 252:2060 [1977]) to synthesize, *in vitro*, adenovirus proteins with unblocked amino terminals that can thus be sequenced by Edman degradations. The sequencing has been done by Carl Anderson at the Brookhaven National Laboratory, using an automated sequencer. Without the citrate synthase treatment, about 90–95% of the material synthesized *in vitro* has a blocked amino terminus, making sequencing impractical. We have been able to synthesize the major late Ad2 proteins in sufficient amounts to permit sequencing using [³⁵S] methionine and ³H-labeled lysine, leucine, isoleucine, proline, serine, alanine, phenylalanine, arginine, glutamic acid, and aspartic acid. Since nearly the entire amino acid sequence of hexon is known (H. Jörnvall et al., pers. comm.), we first used hexon to check the functioning of our techniques. By labeling with methionine and proline, we found that the initial methionine was at least 80% removed, that other methionines occurred at residues 6, 7, and 13, and that proline occurred at residues 4 and 8, exactly as expected. We next tried fiber, since Sayeeda Zain and her colleagues here at Cold Spring Harbor have sequenced the DNA in the region where the 5' end of fiber mRNA maps and have predicted the amino-terminal sequence of fiber. In contrast to hexon, the initial methionine of fiber is not cleaved. So far, we have found the sequence of fiber to be: Met Lys—Ala—Pro—Glu—Phe—Pro—Tyr Pro Tyr, exactly as predicted from the DNA sequence. We consider identification of 10 of the first 17 residues sufficient to establish the exact map position of fiber on the se-

quence of that region of Ad2 DNA. Nevertheless we are currently analyzing samples of fiber labeled with arginine, serine, aspartic acid, and tyrosine to establish the general validity of this method. Also, we have begun to analyze minor core component (V) by the same method.

Comparison of terminal protein with Ad2-coded proteins

The protein covalently bound to the 5' termini of Ad2 DNA has been prepared by disrupting density-gradient-purified, [³⁵S]methionine-labeled virus by heating in SDS and by separating the DNA-protein complex from the virion proteins by chromatography on SDS-sepharose columns. The complex was digested with pancreatic DNase and the 55,000-m.w. terminal protein isolated. Two-dimensional analysis of tryptic peptides was used to compare the terminal protein with the early region 1A 38–50K protein and late protein IVa₂, also [³⁵S] methionine-labeled. These two proteins were chosen for comparison because they are virus-encoded proteins that have molecular weights similar to the terminal protein. The comparison with 38–50K was of particular interest because this protein is encoded by the transforming region of Ad2. However, both comparisons were negative. Terminal protein shares few, if any, peptides with either 38–50K or IVa₂. We are currently comparing terminal protein with the recently reported 55K protein whose gene maps between 4.6 and 11.1 map units; i.e., within the region required for stable transformation but not within the minimal region of the genome required to produce cell lines with some characteristics of transformed cells.

Table 1
Map coordinates on Ad2 DNA of genes for Ad2 proteins.

Region	Strand	Coordinates	Proteins
<i>Early Proteins</i>			
1A	R	1.3-4.5 1.3-2.6/3.3-4.5 1.3-1.7/3.3-4.5	38-50K
1B	R	4.6-11.1 4.6-6.1/9.8-11.1 9.8-11.1	55K ^a 15K IX
2	L	75.2-75.1/68.6-68.4/66.7-61.8 72.0-71.9/68.6-68.4/66.7-61.8 86.6-86.1/68.6-68.4/66.7-61.8	72K
3	R	76.6-77.6/78.6-84.7 76.6-77.6/78.6-82.7 76.6-77.6/78.6-81.3 76.6-77.6/78.6-79.1/81.3-86.1 76.6-77.6/78.6-79.1/81.3-84.7 76.6-77.6/78.6-79.1/81.3-82.7 76.6-77.6/78.6-79.1/82.7-86.1 76.6-77.6/78.6-79.1/82.7-84.7 76.6-77.6/78.6-79.1/84.7-86.1	{ 15.5K } ^b { 14K } { 13K }
4	L	99.3-99.2/96.8-91.3 99.3-99.2/96.1-91.3 99.3-99.2/96.8-94.3/92.4-91.3 99.3-99.2/95.5-94.3/92.5-91.3 99.3-99.2/94.8-94.3/92.4-91.3 99.3-94.3/92.4-91.3	{ 23K } ^b { 21K } { 19K } { 17K } { 14K } { 13K } { 11K }
<i>Late Proteins</i>			
	R	9.8-11.1	IX
	L	11.2-14.9	IVa ₂
	R	29.1-49.5	{ IIIa } ^b { (III, V) } { pVII }
	R	49.5-62.2 41.9-62.2 68.0-78.6	pVI II { 100K } { 38K } { pVIII }
		86.2-91.5	IV

Data from L. Chow, T. Broker, and J. Lewis (unpubl.).

Proteins from Lewis et al. (Cell 7:141 [1976]); Harter and Lewis (J. Virol. 26:736 [1978]).

^aFirst described by D.J. Spector, M. McGrogan, and H.J. Raskas, personal commun.

^bExactly which mRNA species within each region encodes which protein has not yet been determined.

Analysis of heterogeneity of early Ad2 mRNA's

In collaboration with Louise Chow and Tom Broker, we have been mapping the position of Ad2 early mRNAs on the viral DNA by using R-loop analysis (Chow et al., *Cell* 11:819 [1977]). Since the discovery that early Ad2 mRNA is "spliced" (Kitchingham et al., *Proc. Natl. Acad. Sci.* 74: 4392 [1977]), we have reinvestigated the structures of early Ad2 mRNAs by annealing them with Ad2 single-stranded DNA and then characterizing the heteroduplexes in the electron microscope. The picture that has emerged is much more complicated than our earlier investigation indicated. As can be seen from the summary given in Table 1, as many as nine species of mRNA are complementary to one early region. Whether each of these mRNAs codes for a different protein or whether some species are precursors or aberrant forms is not yet known. We are currently using methyl mercuric hydroxide-agarose gel electrophoresis to separate the various species of mRNA complementary to a given early region so that an exact correlation can be made between a given mRNA species and the protein encoded by that species. Preliminary results indicate that 21K is encoded by the smallest of the six region-4 mRNAs.

An intriguing observation that arose from this study is that the 72K mRNA has a different structure at early and late times. Before the onset of viral DNA synthesis, and at later times if cycloheximide is used to block the onset of the late phase, the short 5' leader sequence of the 72K mRNA is encoded at map position 75. At late times, even if cytosine arabinoside is used to block DNA replication, the 5' leader sequence is encoded at position 72. These results indicate that some difference in transcription or processing factors exists between early and late times in Ad2 infection, and that these factors are synthesized after viral infection, such as a virus-encoded early protein.

Additional early proteins

Our original studies (Lewis et al., *Cell* 7:141 [1976]) of early Ad2 mRNA detected only a 15,000-m.w. early protein encoded in the region 4.6–11.1 map units. Subsequently, Persson et al. (*Virology* 90: 67[1978]) demonstrated that component IX (9.8–11.1 map units) was synthesized early as well as late, and D. Spector, M. McGrogan, and H. Raskas (pers. comm.) reported that a 55,000-m.w. protein was also encoded by RNA complementary to this region if the RNA was prepared 16–20 hours after infection from cells in which viral DNA replication had been blocked by cytosine arabinoside. We have confirmed their observation and, in addition, observed a 17,000-

m.w. protein encoded in this region. Neither the 17,000-m.w. nor 55,000-m.w. protein is synthesized if RNA is prepared at 6 hours postinfection from cells in which early protein synthesis has been blocked by cycloheximide. However, we find that the 22S mRNA mapping in the interval 4.6–11.1 map units, which Spector et al. have shown to code for 55K, is seen by electron microscopy in the 6-hour cycloheximide RNA preparation in amounts equivalent to those seen in the 16-hour cytosine arabinoside RNA preparation. We do not yet understand why the 6-hour 22S mRNA does not appear to be translated into 55K. Perhaps there is a difference in structure between the 6-hour and 16-hour 22S mRNAs that is too small to detect in the electron microscope. Conceivably, such a difference could require the action of an Ad2 early protein.

Characterization of mutants of Ad2 and Ad5 which multiply efficiently in simian cells

Human adenoviruses multiply a thousandfold less efficiently in simian cells than in permissive human cells. This block to multiplication can be overcome by coinfection of the simian cell with SV40. Five host-range mutants of human adenovirus have been isolated which no longer require the presence of SV40 for their growth in simian cells. Four are of Ad2 origin (H2hr400–H2hr403) and were selected by passage in simian cells after nitrous acid mutagenesis. The fifth (H5hr404) is a naturally occurring variant of Ad5. These mutants form plaques and multiply with similar efficiency in both simian and human cells. These growth properties are similar to those found for wild-type Ad2 or Ad5 in simian cells when the block is overcome by coinfection with SV40.

The block in wild-type infections prevents efficient expression of many of the late viral genes. These mutations allow full expression of the late genes. With the best characterized mutant, H2hr400, the mutation appears to act in *trans*, enhancing expression of the Ad5 fiber gene when simian cells are coinfecting with H2hr400 and Ad5.

These mutants appear to be point mutations, as no deletions, substitutions, or rearrangements can be detected by restriction-endonuclease digestion or heteroduplex and hybridization analyses. These results exclude the possibility that SV40 sequences, which in certain Ad2-SV40 hybrid viruses (e.g., Ad2⁺ND1) allow adenovirus to multiply efficiently in simian cells, are present in H2hr400.

In collaboration with Terri Grodzicker, the mutations have been physically mapped using a modified marker-rescue technique. Because transfection of simian cells is very inefficient, we have had to use the

permissive human 293 cell line. These cells were co-transfected with intact wild-type adenovirus DNA and purified restriction-endonuclease-generated fragments of the mutant genome by means of the Ca^{++} ppt method (Graham and van der Eb, *Virology* 52: 456 [1973]) followed by glycerol treatment to further enhance transfection (Frost and Williams, *Virology* 91:39 [1978]). The resulting lysate was then passaged twice on simian cells (CV1) to enrich for recombinants between the wild-type genome and the restriction fragment carrying the host-range mutation. After the second passage, the virus yield was titrated on CV1 monolayers to determine which fragment contained the mutation. In these experiments the serotype of the wild-type virus genome differed from that of the mutant from which the fragments were generated so we would be able to take advantage of the difference in restriction-endonuclease patterns between the serotypes. Restriction-endonuclease analysis of the genomes of the resulting interserotypic recombinants provided a convenient check on the identity of the fragment containing the mutation.

The *Bam*HI A (coordinates 59–100), *Eco*RI B (58.5–70.7), and *Kpn*I E (62–72.5) fragments of H2hr400–H2hr403 recombined with Ad5 or Ad2 to yield recombinants with extended host ranges. This places the mutation between coordinates 62 and 70.7. This region contains most, if not all, of the early 72K gene and the 5'-half of the late 100K gene. The *Bam*HI-*Eco*RI double-digest-fragment B (59–76) and *Xma*I D (56–68) of H5hr404 rescued Ad2. Thus, this mutation must lie in the region defined between coordinates 59 and 68. This region contains the 72K gene, although the presence of the extreme 5' end of the 100K gene cannot be excluded. Finally, the 100K gene was excluded by mapping the H2hr400 mutation within the main body of the 72K gene (coordinates 61.5–66) by rescue with fragment *Ba*II K (62.9–65.6).

Since the 72K gene is already fully expressed in simian cells infected by wild-type adenovirus, the mutation must be affecting the activity rather than the synthesis of 72K protein. This protein binds single-stranded DNA and is involved in viral DNA replication. However, since viral DNA replication occurs normally in wild-type-infected simian cells, the mutation must be affecting yet another function of this protein. Since the mutants fully express their late genes, in contrast to their parents, the mutated gene product must be involved in the control of expression of these genes. Perhaps the wild-type 72K can interact correctly only with the human cellular component(s) to turn on the expression of the late genes, whereas the mutant 72K can interact properly with both human and simian cellular components.

Structure analysis of mRNAs from adenovirus-infected simian cells

The inability of adenovirus to multiply efficiently in simian cells results from poor expression of its late genes. Previous experiments, which utilized cell-free translation and C_t analysis (Klessig and Anderson, *J. Virol.* 16:1650 [1975]), suggested that the block to full expression of these genes may reside at the level of RNA processing. These experiments indicated that although the synthesis of Ad2 fiber protein was decreased 200–1000-fold in abortively (Ad2) vs productively (Ad2 + SV40) infected simian cells, the level of the corresponding mRNA (cytoplasmic RNA complementary to *Eco*RI E, the restriction fragment encoding fiber) was reduced only 7–20-fold. Although a nonlinear relationship between concentration of mRNA and amount of synthesis of the corresponding protein could explain the above results, the alternative possibility that the fiber mRNA was defective was considered first.

Since the cap structure (7mG^{5'}ppp^{5'}N-) at the 5' terminus of eukaryotic mRNAs is required for efficient translation of these RNAs (for review, see Shatkin, *Cell* 9:656 [1976]), the fiber mRNA from infected simian cells was analyzed for the presence of this structure. Fiber mRNA from productively infected cells contains the cap, as does 100K mRNA isolated from both abortive and productive infections. The latter was used as a positive control since its synthesis and translation is normal in both types of infections. Fiber mRNA from abortively infected cells also appears to be capped. However, as most of the late mRNAs contain a common cap (Gelinis and Roberts, *Cell* 11:533 [1977]; Klessig, *Cell* 12:9 [1977]) as part of a long, common, 5' terminal leader, the possibility that the cap found in fiber mRNA preparations originates from other contaminating late mRNAs has not been rigorously excluded.

The presence on late Ad2 mRNAs isolated from human cells of this unusual tripartite leader, which is encoded distant from the main body of the late genes (Berget et al., *Proc. Natl. Acad. Sci.* 74:3171 [1977]; Chow et al., *Cell* 12:1 [1977]; Klessig, *Cell* 12:9 [1977]), suggested that the formation of such a structure is a complex process which might be defective in abortively infected simian cells. Two approaches are being used to test this. First, the size and number of cytoplasmic RNAs from each region of the genome are being accurately determined by fractionation of the RNA on methyl mercury-agarose gels, transferring the fractionated RNA to diazotized paper (Alwine et al., *Proc. Natl. Acad. Sci.* 74:5350 [1977]), followed by hybridization with radioactively labeled restriction fragments to detect complementary RNAs. This has

confirmed our earlier findings that there is a considerable difference in amount of viral RNA from productively vs abortively infected cells for each region of the genome. The size and number of species of viral mRNAs from each region, however, appear to be the same regardless of whether the RNA is isolated from infected human cells or productively or abortively infected simian cells.

The second approach (in collaboration with Louise Chow and Tom Broker) involves electron microscopic analysis of the fine structure of viral RNAs from simian cells. The majority of the late RNAs that are encoded between coordinates 27 and 92 and contain the tripartite leader have similar patterns of splicing in abortively (vs productively) infected simian cells. These splicing patterns mirror those seen in permissive human cells. In marked contrast, fiber mRNA aberrantly is spliced in the abortive infection. In addition to the tripartite leader (encoded between 16–27) and the main body of the gene (encoded between 86–92), a large fraction of the fiber RNAs made in Ad2-infected simian cells contain a large heterogeneous segment of RNA encoded between coordinates 68 and 78. Presumably, abnormal splicing results in failure to remove this region during fiber mRNA processing.

This observation, taken together with the location of the host-range mutations within the 72K gene, suggests that the 72K protein may be involved in viral RNA processing. This is the first evidence in any system that a virus may contribute to the presumed host splicing apparatus. Having preliminarily defined an abnormal splicing system and mutations that will overcome this abnormality, we are now in a position to dissect what is likely to be a very complex enzymatic process.

The lysis gene of the MS2 group of phages appears to overlap two other genes

Ribosome binding experiments performed with Joan Steitz from Yale revealed that the main binding site for mammalian ribosomes on MS2 RNA centered on the AUG which exists in the +1 reading frame 15 codons before the coat-gene termination signal. Examination of the nucleotide sequence determined by Fiers et al. (*Nature* 260:500 [1976]) shows that a polypeptide which initiates at this position should contain 75 amino acids. Our previous mammalian cell-free protein-synthesis experiments (Atkins, et al., *J. Biol. Chem.* 250:5688 [1975]) showed that a major MS2-directed product was a polypeptide of approximately this size. Recently, in experiments performed with the help of Carl Anderson, the positions of the proline and methionine residues in this polypeptide have been

determined. The results show that this polypeptide is encoded in the +1 reading frame at the end of the coat gene.

Ten years ago P. Model identified a lysis-defective UGA mutant of the closely related phage f2 (unpubl., but see Horiuchi (*RNA Phages*, Cold Spring Harbor Laboratory [1975])). The polypeptide encoded in the +1 frame is not seen on addition of RNA from this mutant to the mammalian translation system in the absence of UGA suppressor. It is seen when UGA suppressor tRNA from *S. pombe* (courtesy of J. Kohli) is present in the translation system. Similar results were obtained in an *E. coli* translation system, although the relative amounts of the relevant polypeptide were substantially lower. These results suggest that the +1 frame gene may be the lysis gene, and sequencing of the suppressed protein derived from the lysis mutant is required to test this hypothesis, as the nucleotide-sequencing studies of Steitz severely delimit the possibilities for the mutant site.

Effect of tRNA balance on gene expression

The major products of in vitro translation of phage MS2 RNA are the coat protein and synthetase, which is a component of viral replicase. However, in an *E. coli* cell-free translation system, about 5% of the ribosomes that initiate translation at the beginning of the synthetase gene yield a product that is about 3000 daltons larger than the main synthetase protein (Atkins and Gesteland, *Mol. Gen. Genet.* 139:19 [1975]). Heterogeneity of the template RNA is not the explanation for the larger form of the synthetase, as it is not produced in a mammalian translation system that yields the main synthetase. It follows from the nucleotide-sequencing studies of Fiers and colleagues (*Nature* 260:500 [1976]) and from in vitro translation studies with nonsense suppressors that the larger form of the synthetase cannot be derived from in-phase reading past a leaky termination codon. Utilizing purified *E. coli* tRNAs provided by B. Reid and S. Ribeiro, it was shown that the proportion of the larger form of the synthetase is increased by adding Thr ACU/ACC tRNA and decreased by Pro minor tRNA. The nucleotide sequence suggests that a fraction of the ribosomes shift into the -1 reading frame at a proline codon near the end of the synthetase gene and continues in that frame to generate the larger form of the synthetase. Similarly, Ser AGU/AGC tRNA enhanced synthesis of a protein from the coat gene which is larger than the "0"-frame product, and Ala GC A tRNA decreases this misreading. These pairs of tRNAs have also been seen to affect the synthesis of other polypeptides. Thus, the relative amounts of different tRNAs

influence the type of proteins synthesized. The competition between these tRNAs cannot be explained by conventional decoding rules.

Identification and mapping of the transcriptional and translational products of the yeast plasmid 2μ circle

Investigation of the structure and function of 2μ circle has been pursued because of its value as a cloning vehicle for eukaryotic DNA and because it is one of the few eukaryotic plasmids. It is a 6000-base-pair circular species and contains an inverted repeat sequence of 600 base pairs separated by unique DNA.

RNA from *S. cerevisiae* was fractionated on methyl mercury-agarose gels, and the pattern transferred intact to diazotized paper, which covalently binds nucleic acid, following the procedure developed by Alwine et al. *Proc. Natl. Acad. Sci.* 74:5350 [1977]. The immobilized RNA was then probed with labeled 2μ circular DNA and hybridization detected by autoradiography. Using this method, we identified two major and approximately ten minor poly(A)-containing RNA species that arise from in-vivo transcription of the 2μ circular DNA.

The two major species, which are 1325 and 1275 bases in length, are transcribed from the two unique halves of the plasmid and extend into the inverted repeat sequences that separate the unique regions. The map position of the minor transcripts, which range in length from 2600 bases to 350 bases, indicate that, except for a small region of the genome in which no transcription is observed, both strands of the entire 2μ -circle genome are transcribed. We also found that RNA transcribed from 2μ circular DNA is used to program the synthesis of specific proteins in yeast: (1) yeast RNA complementary to 2μ circle can be translated in vitro to produce specific polypeptides of substantial size, and (2) some of the 2μ -circle transcripts are found on polysomes. Finally, the pattern of transcription of 2μ circle suggests the possibility that mRNA species are derived by cleavage of larger transcripts and, in addition, that the intramolecular recombination of 2μ circle that occurs in yeast functions as a genetic switch to allow separate expression of two sets of genes on the 2μ -circle genome.

Suppressors

In collaboration with Terri Grodzicker and Adolf Graessmann we are asking whether isolated suppressor tRNA of yeast can rescue the growth of nonsense mutants of the adeno-SV40 hybrid ND1. Mutant-infected monkey cells are injected with either Su⁻tRNA or tRNA from various suppressor lines and the virus yield is measured. This will tell us whether the

suppression event that we know works in vitro gives an active 30K product in vivo that is capable of providing rescue. Preliminary results suggest that the yeast tyrosine ochre suppressing tRNA will rescue growth of the H71 mutant. We then plan to see if a yeast suppressor gene can function in a mammalian cell.

We have been supplying suppressor tRNA to various laboratories interested in investigating the termination signals at the ends of specific genes. These experiments have led to the conclusion that in at least two cases it is possible that read-through of a normal terminator is a mechanism used to generate longer polypeptides which are precursors for essential functions. Lennart Philipson working in David Baltimore's lab at the Massachusetts Institute of Technology (Philipson et al., *Cell* 13:89 [1978]) showed that the gag protein precursor (78K) is the predominant product of cell-free synthesis and a gag-pol precursor of 180,000 molecular weight is only a minor product. Furthermore, addition of yeast amber suppressing tRNA to this reaction mixture greatly enhances the amount of gag-pol precursor. The implication is that the gag gene is terminated by an amber codon and that partial reading of this terminator results in synthesis of a gag-pol precursor that is further processed to give the polymerase protein.

In parallel experiments (in collaboration with J.M. Bishop's lab, University of California) with the avian tumor viruses, the same situation does not hold. Here, amber suppression results in a partial elongation of the equivalent gag protein, but there is no change in the amount of the polymerase precursor. The conclusion is that amber is the terminator of the gag gene, but that there is a second in-phase terminator that prevents further reading into the message.

It seems unlikely that these two similar viruses would use completely different mechanisms for synthesis of the polymerase precursor and thus a dilemma exists. Of course, these experiments say nothing directly about how the polymerase is made in vivo. Rather, they only tell us what the structure of the genome is and what might be possible. Therefore, although it is possible that the murine leukemia virus (MuLV) polymerase is made by a low-level reading of the amber terminator at the end of the gag gene, there are still a number of other possibilities. Our experiments with the adenovirus mutants (Gesteland et al., *Proc. Natl. Acad. Sci.* 74:4567 [1977]) and Capecchi's experiments with HGPRT nonsense mutants (Capecchi et al., *Cell* 12:371 [1977]) suggest that mammalian cells do not have an appreciable leakage rate through amber terminators. Therefore, perhaps the read-through seen in vitro is purely a fortuitous result and the low level of gag-pol precursor normally seen both in vivo and in vitro in the absence of suppression is due

to spliced mRNA or perhaps even modification of a small fraction of the mRNA such that the terminator is nonfunctional. In the case of the avian virus, the same argument could say that the large precursor comes from a small fraction of spliced or modified message. Another possibility is that MuLV does use the amber read-through mechanism to make the polymerase precursor and it is the context surrounding this amber terminator that makes it genuinely leaky. And that in the avian case, another translation mechanism such as frame-shifting allows production of the large precursor. These various possibilities are accessible to experimentation using in-vitro protein synthesis, messenger structure analysis, and DNA sequencing, and such experiments are underway in various laboratories.

In collaboration with Hugh Pelham (*Nature* 272: 469 [1978]) an amber read-through mechanism has been implicated in the production of tobacco mosaic virus (TMV) proteins. Here, larger precursor protein is produced in increased amounts when amber suppressing tRNA is added to a cell-free system programmed with TMV RNA. Again, the validity of this explanation for in-vivo synthesis is as yet unclear.

These results coupled, with the results described above on frame-shift by *E. coli* ribosomes, suggest that perhaps modulation of translation may, in fact, be a valuable way for cells to increase the availability of information and to control the levels of protein products without necessarily synthesizing new messages.

In collaboration with Ken Cremer and Bill Summers of Yale, nonsense mutants of herpesvirus thymidine kinase have been identified.

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MAMMALIAN CELL GENETICS

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During the past year we have continued with studies on herpesvirus-transformed cells and on the persistence of herpesvirus nucleic acid sequences in human tissues. The collaboration of Dr. Cecelia Fenoglio at the Columbia University College of Physicians and Surgeons has been invaluable.

This work, which was supported by the National Institutes of Health Cancer Research Center grant, will continue at our new location—the Hutchinson Cancer Research Center, Seattle, Washington.

Herpesvirus-2 (HSV2) transformation

We have studied and characterized extensively the properties of cloned cell lines derived from a UV-inactivated HSV2-transformed Syrian hamster cell line. The cloned lines, which came from an extremely heterogeneous cell population, have defined cytogenetic markers, are different in their oncogenicity in newborn hamsters, and have been shown to contain herpesvirus messenger RNA in a portion of the cells, as detected by *in situ* molecular hybridization. We have carried out reassociation kinetics analyses of a number of these clones, using as probes restriction-endonuclease-derived fragments of HSV2 DNA (D. Galloway, C. Copple, and J. McDougall, *in prep.*). Restriction cleavage of the herpesvirus DNA results in the generation of fragments whose molarities are nonuniform. Molar fragments result from restriction cleavage within the unique sequences; half-molar fragments represent the termini of the DNA; and quarter-molar fragments arise from the junctions of the long and short components in the four configurations. Isolation of a complete set of restriction fragments from a single enzyme digest is difficult.

After HSV2 DNA (100 μ g) was digested with an endonuclease, the cleavage products were fractionated through 0.4% agarose, made visible to UV light

with ethidium bromide, removed from the gel by electroelution, extracted with phenol and chloroform, and ethanol-precipitated. The fragments were resuspended in buffer and 0.5 μ g of the fragments were nick-translated with [32 P]deoxytriphosphates to specific activities around 5×10^7 cpm/ μ g. Eight fragments from 4 different restriction-enzyme digests were used, which, although they did not provide complete coverage of the genome, had the advantages that they could be isolated cleanly and represented 80% of the unique sequences as well as the repetitive DNA of the short component.

The extent to which the radioactive probe annealed alone, or in the presence of transformed cell DNA, or in the presence of one, five, or ten copies of HSV2 DNA was measured in a reaction containing 1.8 mg/ml cellular DNA and 4.8×10^{-4} μ g/ml of fragment in 1 M NaCl. The amount of hybrid was measured by hydroxyapatite chromatography, and the fraction of single-stranded DNA and the number of copies of the viral sequences present in the transformed cell DNA were calculated. A summary of our results diagramming the viral sequences found in 333-8-9 and in the cloned transformed cell lines is given in Figure 1.

Our results and the published and unpublished observations of Niza Frenkel and her coworkers support the following conclusions about the presence of viral DNA sequences in hamster cells transformed by UV-inactivated HSV2:

1. All of the lines examined contained HSV2 DNA.
2. Only a subset of the total viral sequences was retained. Some lines contained an extensive set of sequences, whereas other lines retained only a minimal amount.
3. The sequences that were retained were present in relatively few copies per cell.
4. Viral sequences in the cloned cells have segregated

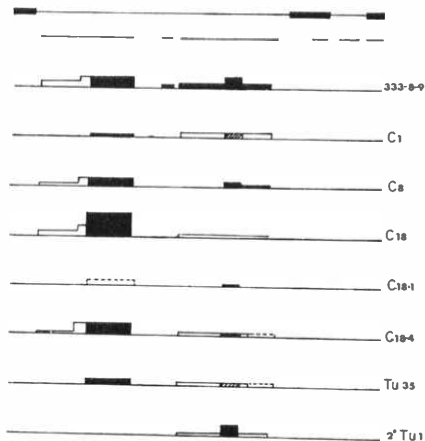


Figure 1

Viral DNA sequences in hamster cells transformed by UV-inactivated HSV2. These results are taken from data obtained by renaturation kinetics studies using as probes various restriction-enzyme fragments of HSV2 DNA. The black boxes indicate that the transformed cell DNA contains sequences homologous to those of the HSV2 fragment; the white boxes also indicate homologous sequences in the transformed cell DNA, but these may be explained by homology to a subset of the sequences; the dotted lines indicate that no data are available; the thatched boxes represent anomalous results that need to be retested.

from the original parental line, 333-8-9, and continue to segregate upon subcloning and by passage in animals.

5. There is no correlation between the content of viral sequences and the extent of tumorigenicity.

Persistence of herpesvirus nucleic acid in human tissues

One of the techniques used previously to demonstrate the presence of herpesvirus DNA in the neurons of sensory ganglia was the hybridization in situ of radioactive virus-specific complementary RNA to sections of ganglia. The state of the virus when latent in the neurons is unknown and could be in any form, from viral DNA (integrated or as free molecules) to incomplete virions. To establish whether any transcription of the latent herpesvirus genome could be detected in neurons, we hybridized ^3H -labeled HSV2 DNA to frozen sections of human sensory ganglia obtained at autopsy. The preliminary results from these studies show that virus-specific RNA can be detected in some neurons. The background level of grains over supporting cells is very low, thereby providing a high degree of confidence in the application of this method for the detection of viral RNA in other

situations where persistence of the viral genome may not have been previously established.

Using the same viral DNA probe we have examined a series of human cervical biopsies taken for pathological diagnosis. The results from these cytological hybridizations indicate that HSV2 RNA can be detected in areas of cells undergoing premalignant changes, autoradiographic grains being associated most frequently with neoplastic cells but also associated in some sections with macrophages and lymphoid cells in the areas of abnormality. The nick-translated HSV2 DNA probe does not hybridize to normal cells or to human DNA bound to nitrocellulose filters under conditions that detect 5×10^{-5} μg of contaminating human DNA in the probe. Hybridization of other viral DNA probes to cervical sections has proven negative. The results from this series of cervical biopsies are shown in Table 1; the negative results are from sections that did not contain any area of abnormality that could be related by pathological examination to malignant or premalignant changes.

The results from the in situ cytological hybridizations described here are at least indicative of a significant presence of HSV2 RNA in cervical tumor tissue. Infection of the human genital area with HSV2 is common and it is a recognized venereal disease; con-

Table 1
Cervical biopsies (42 cases)

Diagnosis	In-situ hybridization with ³ H-labeled HSV2 DNA	
	HSV2 RNA positive	HSV2 RNA negative
CIN I-III	10	4
Squamous metaplasia	2	2
Negative diagnosis	1	23
Total	13	29

sequently, it is possible that we have detected viral RNA synthesized in virus replication. As HSV2 can persist in ganglia, there could be reactivation of latent virus, with consequent replication in the area of neoplastic tissues when all cells are permissive, and we have no evidence to suggest that these patients were undergoing acute infection at the time of biopsy or that infectious virus was present in the tissues. In-situ hybridizations using restriction-endonuclease-derived fragments of HSV2 DNA should identify the regions of the viral genome from which the observed RNA species are transcribed.

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MOLECULAR GENETICS

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

The main projects we worked on during the year were: (1) the mechanism of aberrant protein degradation in *Escherichia coli*; (2) the interaction of Mu repressor with DNA; (3) the expression of cloned yeast genes in *E. coli* minicells; and (4) nuclear injection of DNA into *Xenopus* oocytes.

Mechanism of aberrant protein degradation in *E. coli*

We have completed the purification to homogeneity of protease III from *E. coli*, and have shown it to be a metal-requiring enzyme that can use Zn^{++} , Mn^{++} , or Co^{++} for activity. Protease III greatly favors as substrates small proteins of less than 7000 molecular weight. The cleavage specificity was determined by using the β chain of insulin as a substrate. Protease III cleaves it rapidly at tyrosine-leucine (16–17) and slowly at phenylalanine-tyrosine (25–26). Mutations have been isolated in the structural gene for protease III and they are being mapped. The phenotype of these mutants is being investigated. We are also studying the residual protease activity of protease-III⁻ mutations, which appears to be due to a new protease with quite different properties from protease III. We are purifying and characterizing this new enzyme.

Interaction of Mu repressor with DNA

Using the plasmid pMB9, we have cloned a region of Mu DNA that codes for the Mu repressor and have been able to identify the Mu repressor protein in ³⁵S-labeled minicells. This year we have developed techniques for studying the binding of this Mu repressor to DNA. The binding studies showed that Mu repressor binds to all DNA at salt concentrations of < 0.1 M, but in the range of 0.2 to 0.3 M sodium chloride, it binds specifically to Mu DNA and not to λ or other heteroimmune phage DNAs. By means of

DNA binding assays we have been able to map the position of the Mu operator, which was shown to be on the 1000-base-pair fragment of DNA that carries the repressor. Since operators generally overlap or follow promoters, it is likely that the early Mu promoter is also on this fragment. This means we have clones that carry the Mu repressor, the Mu repressor promoter, a Mu operator, and the promoter for Mu early functions, all within 1000 base pairs of cloned DNA.

Expression of cloned yeast genes in *E. coli* minicells

We have studied in *E. coli* minicells the expression of a large number of plasmids carrying cloned yeast DNA. Those plasmids have been chosen whose inserted DNA is complementary to a major yeast mRNA. Among about 50 such plasmids, five produced very prominent protein bands in labeled minicells. This means they have promoters and protein initiation sites that are recognized by *E. coli* at a high level of efficiency. Several other yeast-DNA-carrying plasmids express proteins at lower or trace levels, and the rest showed no detectable expression. We are continuing this analysis and hope to characterize the features of yeast DNA that allow high-level expression in *E. coli*.

Nuclear injection of DNA into *Xenopus* oocytes

We have been able to set up a nuclear injection system for *Xenopus* oocytes analogous to that developed by John Gurdon of the Medical Research Council, Cambridge. The system functions about as well as the one in England. We have gotten expression of SV40 DNA injected into oocytes. Two proteins, VP1 and the 17K fragment of T antigen, are made in sufficient quantities to be seen easily on one-dimensional acrylamide gels using only one-tenth of an egg per gel slot. The rate of successful injections ranges from 20% to 50%. This is

a combination of the successful rate of hitting the nucleus, which is from 50% to 80%, and the subsequent chance of survival of the oocytes as healthy cells. We are now injecting a variety of eukaryotic cloned DNAs into oocyte nuclei to see whether they are expressed.

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INSERTION ELEMENTS AND PLASMIDS

A.I. BUKHARI, L. Ambrosio, G. Chaconas, F. de Bruijn, M. DuBow, D. Evans, H. Khatoon and N. Sarvetnik

In a series of papers during the past 3 years, we proposed that integration of bacteriophage Mu DNA is intimately linked with its replication and that the process of Mu integration might be a prototype for the integrative recombination of transposable elements. Initial arguments, based on the structure of Mu DNA, underlined the parallel behavior of Mu DNA and that of the known prokaryotic transposable elements (Bukhari, *Ann. Rev. Genet.* 10: 389 [1976]). A characteristic feature of transposable elements is that they generate duplications of 5 or 9 base pairs of host DNA at the site of insertion. (For example, IS1 causes duplication of 9 base pairs and Tn3 and Tn5 cause 5-base-pair duplications.) The nucleotide-sequencing results of R. Kahmann and D. Kamp indicate that Mu also conforms to this rule and causes the duplication of 5 base pairs. Thus, the idea that Mu DNA insertion may be a general model for transposition continues to gain acceptance. However, before this idea can be extended to its logical limit, a clear picture of the molecular mechanism of Mu integration is required.

The process of Mu integration remains a puzzle. It has not been possible to define the steps involved in transposition of Mu DNA from one site to another, because replication of Mu DNA and its insertion at a new site appear to happen simultaneously and Mu DNA intermediates involved in replication or integration have eluded detection.

The Mu paradox

The suggestion that transposition of Mu DNA follows its replication has raised some intriguing questions. The evidence so far is consistent with the hypothesis that prophage Mu DNA replicates in situ and that the products of this reaction are transposed to other locations. We know, however, that the prophage Mu DNA (if the prophage carries an X mutation) can be excised at a low frequency from the host DNA. A model for Mu

transposition must resolve this apparent paradox. That is, although a mechanism for Mu excision exists, excision does not appear to be included in the normal course of events during the Mu growth cycle. We have shown that excision is a Mu-specific function and that it requires the product of the A gene of Mu. We have constructed strains containing the Mu X prophage with an amber (*am*) mutation in the A or B gene of Mu. Mu X *Aam* mutants can be excised only in Su⁻ cells, whereas Mu X *Bam* mutants can be excised in both Su⁻ and Su⁺ cells. (Some excision can be seen with Mu *Bam* mutants even without the superimposed X mutations.)

The A + B hypothesis

The Mu X mutants are defective prophages which generally carry insertions in the B gene of Mu. Both the A- and B-gene products are thought to be required for Mu replication and integration. The realization that elimination of the B-gene function allows Mu excision has led us to propose the following hypothesis. Recognition of the ends of the Mu prophage by the A-gene product can lead to different consequences, depending on the presence or absence of the B-gene function. The A-gene product recognizes the ends of the prophage and initiates the process that would culminate in the replication and transposition of Mu DNA if the B gene were functional. If the B gene is nonfunctional, the action of the A gene at the ends of Mu may leave the prophage in a state susceptible to excision. Thus, in this model the excision of Mu DNA is an abortive transposition event. We are now testing this hypothesis.

Excision products

Hajra Khatoon's studies on excision of Mu DNA from

the *lacZ* gene, involving extensive fine genetic mapping and DNA-DNA hybridization, have revealed some interesting aspects of the excision process of Mu DNA. Imprecise excision is 10 to 100 times more frequent than precise excision. About 80% of the detectable imprecise excision events result in minor changes in the host sequences at the site of Mu insertion. These changes may be further corrected to give rise to wild-type sequences. The rest of the imprecise excisions leave deletions of various sizes. Most of these deletions end at the point of Mu insertion and extend either to the right or to the left of the prophage site. Figure 1 shows the mapping of the excision products of two Mu X prophages located in the Z gene of the *lac* operon. In some cases, deletions remove only a

part of the Mu DNA or no detectable part of the Mu DNA at all. In these cases, the *lacY* gene presumably has come under the control of a Mu promoter.

Nature of insertions in Mu X mutants

We have characterized the insertions in about 50 independent Mu X mutants. These insertions are located at a few specific sites within a 1000-base-pair segment of Mu DNA defining the B gene. All of the insertions have been identified by DNA-DNA hybridization as IS1, IS5, or IS2. These results show that transposition of the IS elements in *E. coli* occurs in the following order: IS1 > IS5 > IS2.

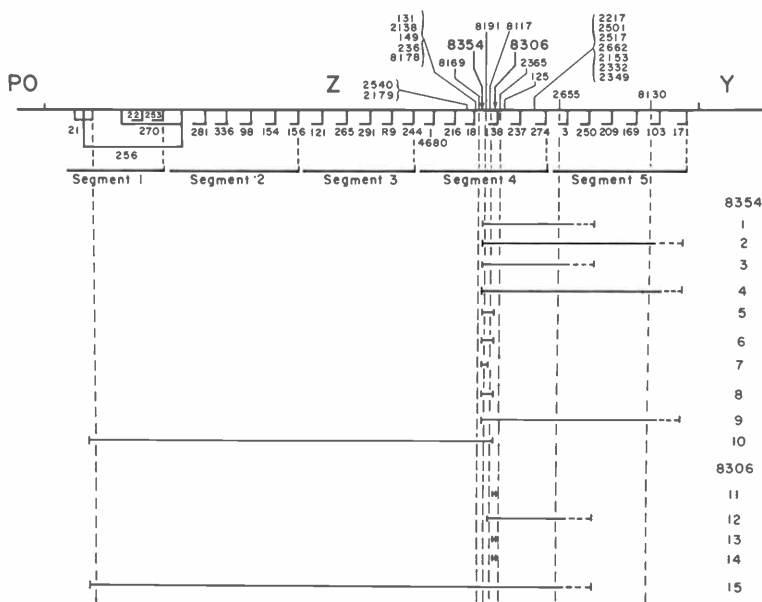


Figure 1

Map of the *lacZ* gene showing some deletions generated by excision of Mu X mutants. The X mutants originated from two Mu cts62 insertions located close to each other in the Z gene. Insertion 8354 carries the *c* end (left) of Mu proximal to the *lacPO* region, whereas insertion 8306 has the opposite orientation. The numbers above the Z-gene line represent nonsense mutations and the numbers below the line show deletions used for mapping. The vertical lines arbitrarily define segments of the Z gene. The horizontal lines show deletions generated by Mu excision. The lines confined within the smallest segment may or may not be deletions (8306, nos. 11, 13, and 14). Only some of the excision products (Z-Y* revertants) mapped are shown. Extensive mapping of such excision products has resulted in the accumulation of a very large number of internal deletions spanning the Z gene.

In vitro and in vivo manipulations of Mu DNA

We have cloned the ends of Mu prophages (Mu located in the *lacI* gene or the *lacZ* gene) in small plasmids such as pBR322. These plasmids are being used as substrates for examining the proteins that bind to the Mu ends, for analyzing the cutting of Mu ends during packaging of Mu DNA, and for sequencing several different sites of Mu insertion. Several other plasmids containing internal deletions of Mu have been manufactured to study the process of Mu transposition and replication. For example, from a pSC101 plasmid containing a Mu insertion (obtained from M. Casadaban of Stanford University), we have removed the middle fragment of Mu by digestion with the endonuclease *Pst* I. The enzyme *Pst* I makes two cuts in Mu DNA; the left cut is within the *A* gene of Mu and the right cut is just to the left of the *G* segment. This plasmid containing the in-vitro-deleted prophage is a hyperproducer of Mu repressor and presumably contains the regulatory region controlling the synthesis of the *A* gene. The *lac* operon of *E. coli* and several drug-resistance markers are being cloned or recombined into the Mu DNA on this plasmid.

In their report Dietmar Kamp and Regine Kahmann describe the studies, involving nucleotide-sequencing and structural analyses, on the small plasmids constructed by them. One of these plasmids, pTM2, containing 1000 base pairs from the left end of Mu joined to 850 base pairs from the right end, is unable to coexist with a wild-type Mu prophage. This plasmid has been found to be compatible only with the *X* mutants (or *B*⁻ mutants) and apparently induces the excision of the *X* mutants. Interaction of this plasmid with the *X* mutants is being examined further.

Prophage Mu induction

We have examined the effect of the induction of prophage Mu located in the *lacI* gene on the expression of the *lacZ* gene. The synthesis of β -galactosidase is not affected after induction when Mu is located in the *lacI* gene in either orientation. Thus, there is no readthrough from Mu into the nearby genes. In strains of the type *lacI::Mu cts O⁺Z⁺Y⁺/lacI O⁺Z⁺Y⁺* (in which there are two *lac* operators and one functional repressor gene) the rate of synthesis of β -galactosidase is not affected after induction. This further shows that, after induction, sequences adjacent to Mu DNA are neither dramatically amplified nor dramatically destroyed. On the other hand, we know that transfer of *F'* *pro lac* episomes containing Mu is depressed beginning about 15 minutes after induction. Thus, there must be some local effect on the Mu-host junction that does not interfere with the functioning of the genes adjacent to the prophage. These studies are now being extended by directly examining the changes in the structure

of small plasmids containing Mu DNA after induction.

Mu proteins

To study the temporal sequence of synthesis of Mu proteins (in particular the *A* and *B* proteins of Mu), we have pulse-labeled proteins with [¹⁴C]leucine at different times during the Mu growth cycle. Mu does not shut off host-protein synthesis. Furthermore, there are minor variations in the synthesis of host proteins during Mu growth (in particular after heat induction of a prophage). Unlike some other bacteriophages, Mu does not grow in UV-irradiated cells. Thus, a system in which host-protein synthesis can be specifically eliminated without affecting Mu-protein synthesis is not available, and it is very difficult to define clearly Mu proteins that are made in small amounts. By running one-dimensional and two-dimensional polyacrylamide gels, we have identified several Mu proteins that are made at different times. Morphogenetic proteins can be clearly seen about 20 minutes after the commencement of the productive cycle, spanning about 75 minutes. The *A* and *B* proteins are made immediately upon induction, but in small amounts, which makes their characterization difficult. Immunological methods are being used to characterize the early proteins of Mu further.

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ELECTRON MICROSCOPY

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

The Electron Microscopy Section has two major research commitments: (a) the characterization of RNA splicing patterns and the pathways of adenoviruses and (b) the analysis of the genome organization of bacteriophage Mu, including RNA transcription patterns, the control and function of DNA G-segment inversion, Mu translocation during replication, and the structures and biological properties of bacterial insertion sequences transposed into Mu. Additional investigations have been made of the replication of adenovirus DNA and of the transcription of 2- μ m circular DNA plasmids from yeast. For many of these studies, we have enjoyed extensive collaborative interaction with colleagues from most of the other research units at the Laboratory, and some of the research and conclusions will be described in other sections of this Annual Report.

Adenovirus RNA splicing: Introduction

The phenomenon of "RNA splicing" was discovered at Cold Spring Harbor Laboratory in early 1977 as a result of a large set of interrelated experiments on adenovirus transcripts (Broker et al., *Cold Spring Harbor Symp. Quant. Biol.* 43:531 [1978]). Briefly, the nucleotide sequences present in mature adenovirus serotype 2 (Ad2) mRNAs are derived from two or more noncontiguous segments of the genome and these become covalently joined by a series of posttranscriptional RNA deletions that occur in long, nuclear precursor transcripts.

Our objectives in the past year have been (1) to extend and complete the identification and mapping of all mRNA species produced by Ad2; (2) to quantify the relative abundances of all early RNA species present under various infection conditions; (3) to begin determining the pathways by which transcripts are spliced; (4) to compare the structures and sequen-

ces of transcripts from different adenovirus serotypes, hybrid viruses, and mutants; and (5) to infer from the RNA splicing patterns some of the biological consequences of this complex form of gene expression. In the course of the electron microscopic analyses, apparent processing intermediates have been identified that should be suitable substrates in the search for splicing enzymes. The structures of the transcripts identified by electron microscopy can be correlated with the Ad2 DNA sequences being determined in R.J. Roberts's and B.S. Zain's laboratories and elsewhere, with the cDNAs to messages prepared by A. Dunn, W. Keller, and J. Sambrook, and with the proteins characterized by J.B. Lewis and M.B. Mathews and their collaborators.

Methodology

Adenovirus-specific RNA samples were prepared under a great variety of conditions. Human KB or HeLa cells were grown either in suspension culture or in monolayers on plates, an important distinction which will be discussed later. Cytoplasmic RNA samples were isolated at early, intermediate, and late times after infection. In some experiments, cycloheximide (CH), an inhibitor of protein synthesis, or cytosine arabinoside (araC), an inhibitor of DNA replication, was utilized. Numerous adenovirus serotypes, Ad2'SV40 hybrids, and other host-range mutants have been examined.

RNAs were hybridized to denatured adenovirus DNA near the melting temperature of the DNA in 80% formamide, conditions that favor RNA:DNA association over DNA:DNA reassociation. The RNA:DNA heteroduplexes were prepared for electron microscopy by spreading in cytochrome c films in the presence of 40–45% formamide, which allows single-stranded DNA segments to remain extended and

traceable. RNA:DNA duplexes can be recognized by their thick appearance relative to single-stranded segments. Splice junctions and the segments of polynucleotide deleted in the composite RNAs are identified by deletion loops constrained in the DNA. Each DNA loop corresponds to one splice in position and length (Fig. 1). Analysis of the RNA structures involved photography and measurement of several thousand samples. Quantitation of the relative abundances of RNAs as a function of time after infection and in the presence of drugs was performed by visual inspection in the electron microscope.

Late adenovirus RNAs

All late Ad2 mRNAs, except perhaps that for peptide IX

(map coordinates 9.8–11.1), are composite molecules with sequences derived from at least two to seven or more separate portions of the genome. A common tripartite leader sequence totaling about 200 nucleotides is complementary to map coordinates 16.5–16.6, 19.5–19.7 and 26.5–26.8 (Chow et al., *Cell* 12: 1 [1977]; Klessig, *Cell* 12: 9 [1977]) and becomes coupled to the 5' end of about 13 different RNAs synthesized from the rightward-transcribed (R) strand (Fig. 2). These 5' ends map at coordinates 29.1, 30.5, 33.9, 37.0, 38.8, 42.8, 45.1, 49.5, 51.2, 66.1, 68.0, 74.0, and 86.3. The transcripts constitute five major families. mRNAs of any one group share 3' proximal sequences and have common 3' ends at 39.0, 49.5, 61.5, 78.3, or 91.3, but they differ substan-

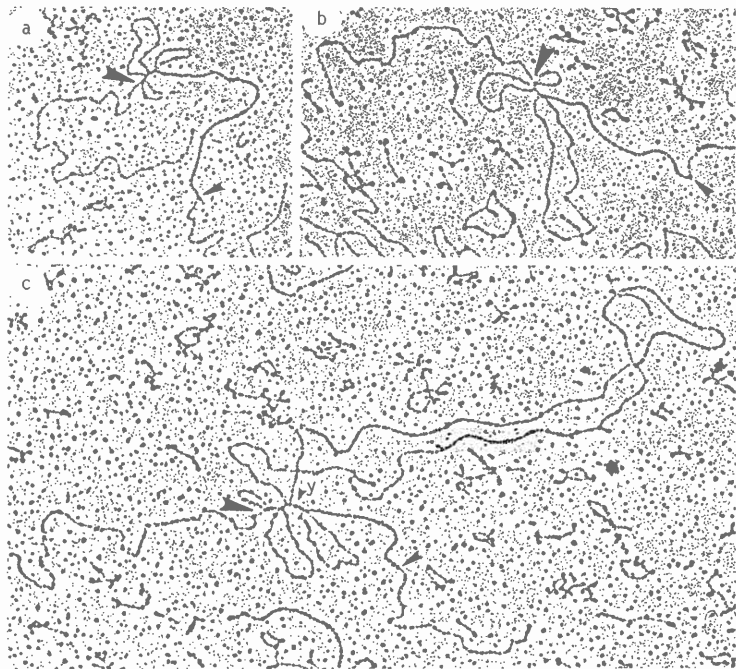


Figure 1
Electron micrographs of Ad2 late RNA:DNA heteroduplexes demonstrating multicomponent 5' leader sequences. (a) IIIa (peripentonal hexon-associated) mRNA; (b) V (minor core) mRNA; (c) IV (fiber) mRNA with the extra leader derived from early-region 3. Large arrowheads and small arrowheads indicate 5' and 3' ends, respectively.

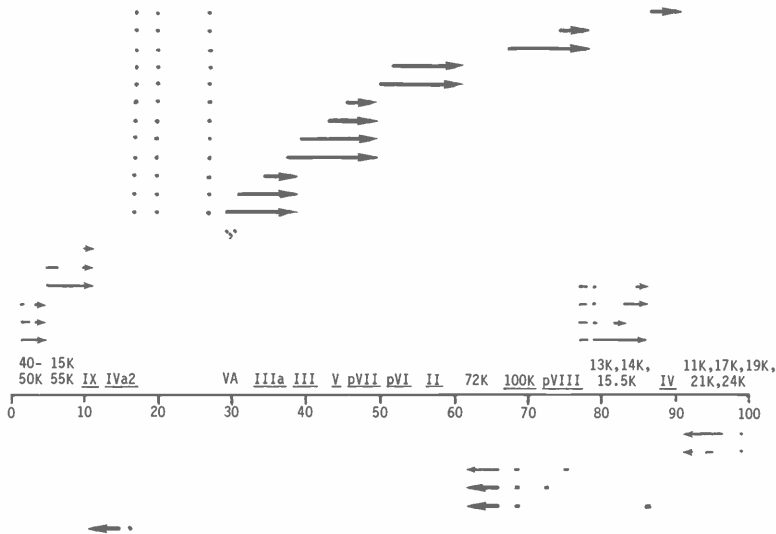


Figure 2
 A simplified RNA transcription and splicing map of Ad2, determined by electron microscopy of cytoplasmic RNA:DNA heteroduplexes. The scale indicates map coordinates in percentages of the 35,000-base-pair genome. Early RNAs are depicted with thin arrows and late RNAs with thick arrows. The arrows indicate the 5'→3' direction of transcription. Gaps in arrows designate splice deletions in mRNAs. Only the major species from early-regions 2, 3, and 4 are shown, and processing intermediates for the late RNAs have been omitted. The proteins translated from various RNAs are designated K (1000 daltons molecular weight) or by Roman numerals to indicate relative mobility during SDS gel electrophoresis. Late proteins are underlined. Protein assignments are based largely on the work of J. Lewis and M. Mathews and their collaborators.

tially in length at their 5' ends and, therefore, in the coordinate at which the leader sequences are attached. Less frequently 3' ends are also seen near coordinates 42, 46, and 74. The longer mRNAs in a family are physically polycistronic. Processing of the primary R-strand transcripts is a complex process that must involve subtle controls to ensure their proper distribution into all the mRNAs.

A late transcript synthesized from the L strand consists of a single-component leader from 16.1–15.7 joined to the main body of the IV_a message, located from 15.1–11.1. The proximity of its 5' end at 16.1 to the 5' end of the major R-strand leader component at 16.5 indicates that late transcription diverges leftward and rightward from a pair of promoters in the interval 16.1–16.5. Other late RNAs are encoded in the intervals 4.6–11.1 (R strand), 76.6–86.1 (R strand), and 72.1–61.5 (L strand) and represent the continued or modified expression of early RNAs.

Early adenovirus RNAs

During the first 8 hours of infection, transcription is largely limited to five "early" regions of the Ad2 chromosome; regions 1A, 1B, and 3 are transcribed from the R strand and regions 2 and 4 from the L strand. Working with Jim Lewis, we have extended previous studies done in other labs and have shown that early RNA from each region consists of a set of composite RNAs with up to four conserved segments spliced together by the deletions of intervening sequences (Fig. 2). Members within each set differ from one another in their splicing patterns and in the lengths and positions of the conserved segments. The derivation of alternative mRNAs from similar precursor transcripts highlights one of the complexities of RNA splicing.

Early-region 1, necessary and sufficient for adenoviral transformation of cells, is divided into two portions, located between coordinates 1.3–4.5 (1A) and

4.6–11.1 (1B). Region 1A has at least three forms of transcript with the same 5' and 3' ends, but internal deletions of various lengths. This splicing pattern is analogous to that found by others in the early transcripts of the DNA tumor viruses, SV40, and polyoma. The relative abundance of the longest internal deletion increases at intermediate times after infection, a transition suppressed by cycloheximide. N. Harter and J. Lewis (*J. Virol.* 26: 736 [1978]) have identified a set of related proteins encoded by RNAs from this region. J. Maat and H. van Ormondt of the University of Leiden, The Netherlands, have sequenced the DNA in this portion of the closely related Ad5 serotype and shown that two, long, open translation frames are separated by termination codons. The internal splice deletions in the early RNA apparently bypass these termination codons to join the open frames and permit the synthesis of larger proteins than would otherwise be possible. Early-region 1B specifies two transcripts; one is continuous, the other spliced. The presence of *araC* results in a relative increase of the unspliced form at the expense of the deleted species.

The transcripts from early-region 2 (75–61; L strand), which encode the single-stranded DNA-binding protein (DBP), consist of either three or four segments. The latter class has a very small internal splice in the main body of the message and was detected by a new type of heteroduplex structure. Surprisingly, the splicing pattern of this message changes with time after infection. At early times the short 5' leader segment of the message is encoded at map

coordinate 75. At late times the short 5' leader is derived from either coordinate 72 (major form) or coordinate 86 (minor variant) (Fig. 3). In the presence of cycloheximide, this changeover does not occur. The alternative 5' leaders probably indicate the utilization of different promoters at early and late times, with activation of the late promoters dependent upon protein synthesis.

Early-region 3 (76.6–86.1; R strand) gives rise to multiple transcripts, which have four different splicing patterns represented in families of transcripts with three different 3' ends. The relative abundance of each species within region 3 is largely unaffected by time or by the presence or absence of *araC* or CH. Harter and Lewis observe three protein products from this region. At late times this region can be transcribed under the direction of the major late promoter at 16.5. Region 3, like region 2, exhibits a splicing change at late times; the transcripts can have the common late tripartite leader coupled to the 5' ends of a set of composite mRNAs similar to that seen at early times.

Early-region 4 (99.3–91.3; L strand) has six or more different species with the same 5' and 3' ends but variable internal splicing. This is reflected in the proteins synthesized during cell-free translation of this RNA; Harter and Lewis find five different proteins, some of which share common tryptic peptides. In agreement with the work of several others, transcription of region-4 RNA is increased dramatically when CH is present during the infection, and the relative abundances of the species with respect to one another

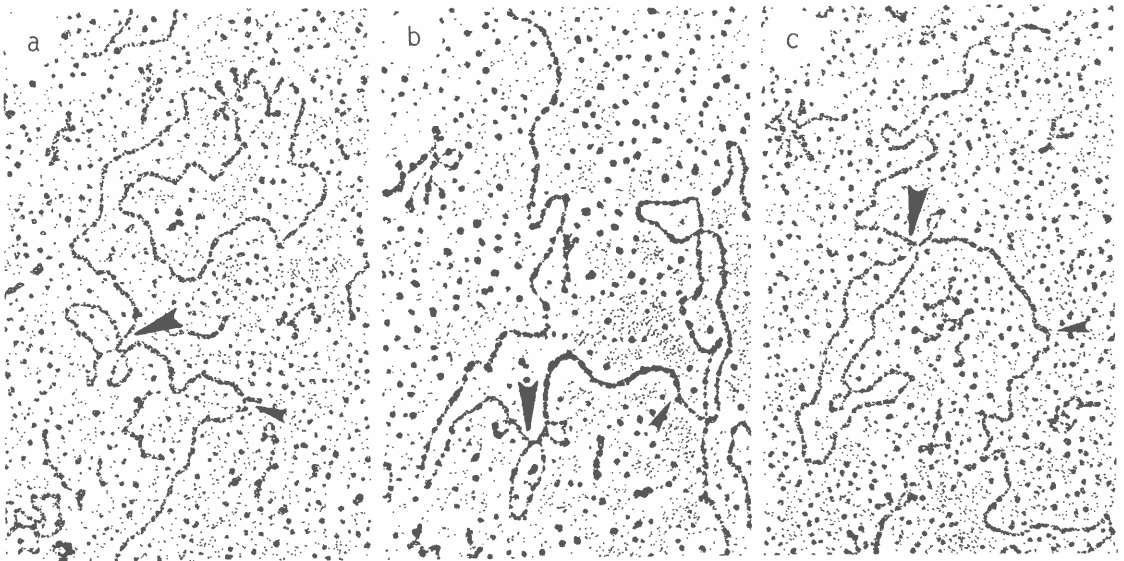


Figure 3
Electron micrographs of Ad2 single-stranded DNA-binding-protein mRNA:DNA heteroduplexes. (a) Early form; (b) major late form; (c) minor late form. Large arrowheads depict 5' ends and small arrowheads point to 3' ends.

are also modified. Again, the species with fewer or shorter splice deletions predominate in the presence of CH.

Other adenovirus serotypes

Several class-C (1, 5, 6) and class-B (3, 7) adenovirus serotypes have been examined, in collaboration with R. Gelinus, for splicing of their late mRNAs. These studies were performed by hybridizing-late polysomal adenoviral mRNAs with single-stranded adenoviral DNA and several of its restriction fragments and examining the heteroduplexes by electron microscopy.

Each serotype studied exhibits splicing of tripartite leaders to the 5' end of many mRNAs transcribed from the R strand. In the newly studied class-C adenoviruses, the tripartite leaders are homologous to those of Ad2 (another class-C virus). The tripartite leaders of Ad3 and Ad7 mRNAs are homologous to one another, but their nucleotide sequences have diverged substantially from those of class-C viruses and do not cross-anneal with Ad2 DNA. Nevertheless, class-B leader sequences map at essentially the same respective positions found for Ad2 leaders, and these are joined to ten or more different R-strand messages, with 5' ends at the same coordinates as determined with Ad2. The spliced structures of Ad3 and Ad7 mRNAs for early-region 1B, IVa₂, and DNA-binding proteins are also very similar to those of Ad2. Apparent Ad3 and Ad7 RNA-processing intermediates with additional 5' leader sequences were observed. The intermediates are analogous to those found in other adenovirus serotypes, as discussed below. These similarities in RNA structure emphasize a strong conservation of processing signals, despite the general divergence in nucleotide sequences between serotype classes, and make comparative sequence analysis of particular importance in the search for features necessary for splicing. The divergence in leader sequences of class-B and class-C mRNAs may explain, in part, the apparent species barrier to recombination between serotype classes if leader sequences must be matched to the remote message sequences for RNA splicing to occur.

Ad2*SV40 hybrids and processing intermediates

Cytoplasmic RNAs isolated from HeLa cells at different times after infection with Ad2 or with the adenovirus-SV40 hybrids Ad2+ND1 and its mutant H71 have been examined in collaboration with T. Grodzicker, J. Sambrook, and J. Lewis. When cytoplasmic RNAs are recovered from infected cells grown in monolayers on plates, presumptive processing intermediates for the tripartite leader segment on all the late R-strand

mRNAs were found as predominant species shortly after the onset of late transcription. The most common form, present in up to 80% of the cytoplasmic RNAs, has an extra segment located between the second and third leaders in the interval 22.0–23.2; others extend continuously from coordinate 22 through the third leader segment, and still others retain variously sized extra sequences between coordinates 24.0 and 26.8. The frequency of observation of the extra sequences decreases gradually as infection progresses, but a substantial number may persist even at 36 hours. For as yet unexplained reasons, splicing is much slower in cells grown on plates than in those grown in suspension cultures.

The joining of the tripartite leader sequence to the main bodies of several of the mRNAs, notably those for fiber (86.3–91.3) and for the IIIa protein (30.5–39.0), are also multistep reactions. The IIIa mRNA seems to be derived by a two-step deletion from a longer member of the same family, extending from 29.1–39.0. In the first deletion, the internal segment 29.6–30.5 is eliminated. Then the internal segment 29.1–29.6 is deleted, and the tripartite leader is thereby transferred to the 5' end of the IIIa message. Interestingly, the segment 29.1–29.6 corresponds to most or all of the sequences in virus-associated RNA (VA) RNA₁, previously characterized by M. Mathews, U. Pettersson, and their colleagues.

One, two, or three leader segments derived from coordinates 77 (x), 79 (y), and 85 (z) can be found situated between the tripartite leader and the main body of the message in some of the fiber transcripts (Fig. 1c). These segments correspond to the three components of one of the early-region-3 transcripts. This coincidence indicates that early splicing signals may continue to be recognized at late times during the processing of the one late gene (fiber) that is separated from its leader region by early-region 3. Eventually, most of these x, y, and z segments become deleted, which occurs in several orders and combinations. Dunn and collaborators (*Cell* 15: 511 [1978]) have studied the y leader in fiber-region transcripts from the hybrid virus Ad2+ND1 dp2 and have shown that it does not affect the efficiency or the product of translation.

The joining of the leaders to many other late mRNAs also appears to proceed by multistep deletions of intervening sequences. In addition to the common tripartite leader, one or more leader sequences can be found preceding the main body of the mRNAs. In each case, the extra leader consists of short sequences derived from the 5' end of an upstream gene. We propose that these segments serve as carriers to transport the common leader to downstream coding sequences in polycistronic precursor transcripts, i.e., that the

coupling of the leader regions to distal messages occurs by multiple small deletions that pass the leaders down the polynucleotide chain to preferred splice targets. These preliminary observations are now being examined in more detail.

Our overall view is that RNA splicing can occur in different patterns within a single region; that deletions need not occur in a single complete step, but rather take place by a series of partial deletions; that the order in which deletions occur can be variable with respect to both time and relative upstream-downstream location; and that multiple pathways can be followed in the conversion of precursor to product. The adenoviruses are superb systems with which to analyze RNA processing and will likely be ideal probes of normal cellular processes.

Adenovirus DNA replication

L. Kaplan, R. Kleinman, and M. Horwitz of Albert Einstein College of Medicine have developed an in-vitro replication system for adenoviral DNA. DNA that has initiated replication in vivo can continue synthesis after extraction (*Proc. Natl. Acad. Sci.* 74:4425 [1977]). The system is dependent upon added deoxynucleoside triphosphates and ATP. We have used electron microscopy to analyze the replication complexes present at the time of extraction and after synthesis in vitro. In agreement with the results of Lechner and Kelly (*Cell* 12: 1007 [1977]), who studied replication intermediates directly after extraction from infected cells, we observed a variety of structures consistent with the following replication model: (a) double-stranded DNA undergoes initiation at either end, with the displacement of the homologous parental chain as a single-stranded branch (type-1 structures); (b) up to four or five initiations can occur at the same end before the first replication fork reaches the distal end; (c) when replication does reach the distal end, the single-stranded branch is released as a free strand; (d) these displaced strands can then undergo initiation at their 3' ends, and they become converted to partially double-stranded structures (type-2 structures); (e) as with double-stranded templates, type-2 replication intermediates can undergo several successive reinitiations so that they may have several single-stranded branches as well as a single-stranded end. Rarely, replication can initiate from opposite ends of the same duplex template.

Replication proceeds for up to 2 hours in vitro with the utilization and formation of the same structures observed in vivo. However, no evidence for initiation of replication in vitro has been achieved. The main intent of future studies is to characterize the defects of temperature-sensitive adenovirus mutants by compar-

ing biochemical and electron microscopic analyses and by attempting in-vitro complementation of defective functions.

Bacteriophage Mu DNA structure

The DNA from the temperate bacteriophage Mu of *E. coli* is a linear duplex about 38 kilobase pairs (kb) in length. A 3-kb segment of its DNA, designated G, can be present in either orientation relative to its neighboring segments to the left (α , 30.7 kb) and to the right (β , 1.7 kb). Inversion of the G segment is catalyzed by the product of the Mu gene, *gin*. Phage Mu DNA integrates at random into the bacterial chromosome during lysogenization and repeatedly transposes to new sites during DNA replication, whether after prophage induction or during lytic infection. The attachment sites on the phage DNA are the ends of its genome. During maturation, integrated Mu DNA is encapsidated into phage heads, starting just beyond the left end within the flanking bacterial DNA, and packaging is from the left toward the right end of the genome (Bukhari and Taylor, *Proc. Natl. Acad. Sci.* 92:4399 [1975]). Because the head can accommodate slightly more DNA than constitutes the Mu genome, rather long segments (1-3 kb) of bacterial DNA flanking the right ends are packaged. Following denaturation and reannealing of mature Mu DNA, these heterogeneous bacterial sequences form single-stranded "split ends" adjacent to the β region.

In last year's Annual Report we described a variety of experiments on the structure and function of the invertible G segment. This year our effort has been directed toward understanding the Mu translocation process as well as the early and late transcription patterns.

Translocation of a mini-Mu phage

The construction of a mini-Mu-containing plasmid pTM2 is described by D. Kamp and R. Kahmann elsewhere in this Report. Briefly, it contains only the end segments of the Mu prophage present in *E. coli* strain DK445 (Chow, Kahmann, and Kamp, *J. Mol. Biol.* 113:591 [1977]). The 1000 base pairs of DNA from the left end of Mu are joined to a portion of the bacterial insertion element IS2 and then to the 850 base pairs of β DNA from the right end of the Mu genome. This mini-Mu DNA and a small amount of *lacZ*-gene DNA flanking the original DK445 lysogen are carried in the vector pBR322. To test the ability of the mini-Mu DNA to replicate, to translocate, and to be packaged, helper functions were provided by superinfecting the plasmid-containing *E. coli* with Mu vir phage. Progeny phage resulted, and their DNAs

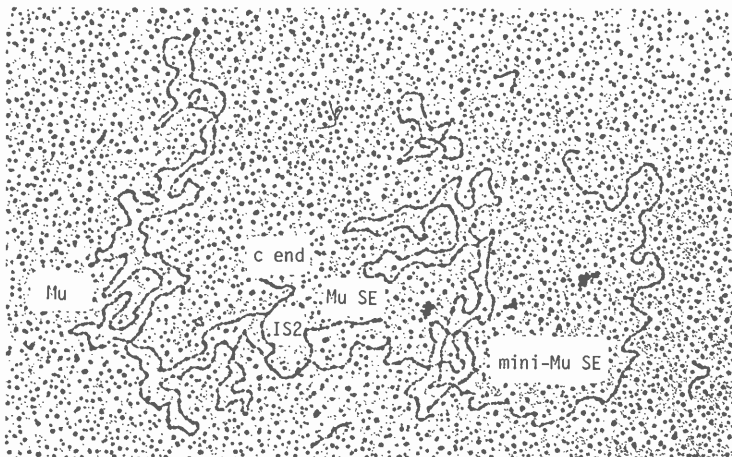


Figure 4
Electron micrograph of a heteroduplex between phage Mu DNA and encapsidated mini-Mu DNA. The c-end (left) 1000 bases and the 850 bases at the right end of the β region are present in both chromosomes and establish duplex pairing. Mini-Mu contains most of bacterial insertion element IS2 and contributes a very long split end (SE) of bacterial DNA. Wild-type Mu forms a large substitution loop between the paired left and right ends and contributes a short split end of bacterial DNA.

were examined by electron microscopy. Following denaturation and renaturation of the DNAs to form heteroduplexes, two types of phage were identified in the lysate. One was the helper phage Mu *vir*. The other was mini-Mu in association with heterogeneous bacterial DNA; therefore, mini-Mu replicated, translocated, and was packaged. The left (c) end of mini-Mu DNA was always found at one end of the packaged DNA, which was as expected since encapsidation starts from the left end. Because the mini-Mu sequences are only 2800 base pairs long and the Mu head can accommodate about 38,000 base pairs of DNA, the heterogeneous bacterial sequences adjoining the β (right) end were very long and formed dramatic split ends in heteroduplexes (Fig. 4). Additional copies of mini-Mu were often found embedded within the heterogeneous bacterial DNA. These mini-Mu genomes could be of either the same or the opposite orientation as the first copy. As a result, the DNA single strands often exhibited a snap-back duplex equal in length to the mini-Mu (2800 base pairs). In some examples of packaged DNA, mini-Mu was found near Mu *vir* helper DNA sequences arranged in either the same or opposite orientation. Work is in

progress to follow the translocation of wild-type Mu after infection or induction.

Electron microscope mapping of Mu-specific RNA transcripts

Electron microscopy was used to map the RNA isolated at various times after heat induction of Mu *cts62* lysogens. The RNA was annealed to self-renatured Mu *vir* or Mu *cts62* DNA to form R loops. The split ends of bacterial sequences and the G-segment inversion loop, when present, served to orient the molecules.

Tetracycline was employed to retard intracellular degradation of the RNA and to block protein synthesis, as well as the early-late switch in transcription. When tetracycline was added to Mu lysogens at a late time (35 min after heat induction) and RNA was extracted 10 minutes later, the samples formed R loops of various sizes along nearly the entire Mu DNA molecule and revealed the presence of early plus late RNA. When tetracycline was added 5 minutes before induction, however, the RNA present was complementary to genome coordinates from 3% to 21% of the left (or c) end, which defined the region transcribed at

early times. RNA synthesized from a cloned segment of Mu DNA (Zipser et al., *Gene* 2:263 [1978]) derived from the immunity (repressor region mapped in the leftmost 3% of the genome, often resulting in a forked structure with one double- and one single-stranded arm.

The middle segments of Mu DNA defined by two *EcoRI* and two *Bam* restriction-enzyme cuts were cloned using the bacterial plasmid pBR322 as a vector. Three isolates were chosen for further study: a 10.5-kb segment defined by the leftmost *EcoRI* and leftmost *Bam* cleavage sites, a 12.5-kb segment defined by the leftmost *EcoRI* and rightmost *Bam* cleavage sites, and a 6-kb segment defined by the rightmost *Bam* and the rightmost *EcoRI* cleavage site. In collaboration with M. Howe and coworkers at the University of Wisconsin, marker-rescue experiments were performed which showed that the leftmost and rightmost *Bam* cleavage sites cut, within the Mu *E* and *F* genes, respectively.

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DNA-PROTEIN INTERACTIONS

D. KAMP, R. KAHMANN

For the last year we have focused our work on two site-specific recombination phenomena: the integration of bacteriophage Mu DNA and the inversion of the G segment of Mu DNA. Both are prominent examples of how, through the specific interaction of proteins with DNA, rearrangements of DNA occur which can alter the genetic content.

Integrative recombination of Mu

We have determined the nucleotide sequence of the Mu attachment sites. Cloned end fragments of Mu phage DNA were analyzed with restriction enzymes and subsequently sequenced using the Maxam-Gilbert method. Sequences obtained for three clones from the left end have shown a precise juncture point between DNA common in all three clones and DNA that is different for every end and is presumed to be bacterial DNA. For the right end we have found an analogous situation. This substantiates a concept that has emerged during the last years, namely, that phage Mu DNA is linked at both ends to heterogeneous host DNA. The precise juncture points suggest an integration mechanism whereby a phage-encoded protein recognizes a specific nucleotide sequence at or near these junctures, commonly referred to as attachment sites. The attachment sites of the left or immunity end (*attL*) and the right or variable end (*attR*) are not identical. They do show similarities, however, that could represent contact points of proteins that interact with the attachment sites. For instance, the first two nucleotides at *attL* and *attR* are the same. A closer inspection reveals that, in fact, the first 30 base pairs in *attL* are almost completely present in *attR*, but they are arranged differently. The first 14 base pairs of *attL* are found 79 base pairs away from the juncture in *attR*, whereas nucleotides 15 to 31 in *attL* seem to correspond to the first 20 nucleotides in *attR* (Fig. 1). The

biological significance of this sequence arrangement remains to be seen. In addition to attachment sites in mature phage DNA, we have sequenced the attachment sites of a Mu prophage, Mu 445, which is inserted in the *lacZ* gene of *E. coli*. From the known amino acid sequence of the β -galactosidase (the product of the *lacZ* gene) and the sequence of the Mu attachment sites as determined for mature phage DNA, we were able to reconstruct the sequence of the Mu 445 insertion site. We found that the Mu 445 insertion is associated with the direct repeat of a sequence of 5 bases of *lacZ* DNA, one copy appearing at each junction. Duplication of host sequences at the insertion site seems to constitute a general feature of recombination systems associated with transposable elements. Integrative recombination is therefore related to the insertion mechanism of transposable elements.

To study the relevance of the host DNA duplication and other features of the sequence for the mechanism of Mu integration, a biological assay is required. We have designed a system whereby we can test fragments containing the attachment sites of Mu for their capability to act as substrates for transposition. End fragments of the Mu 445 prophage were joined together and inserted into the plasmid pBR322 in such a way that the original insertion site of Mu 445 was recreated. This plasmid, pTM2, is lacking 95% of the Mu 445 prophage DNA. We have shown that the remaining Mu sequences, which we refer to as TnM1 and which consist of 1000 base pairs of the left end and 1600 base pairs of the right end of Mu 445, are transposed from pTM2 to the host DNA when a Mu helper phage provides the integration functions of Mu. The helper phage not only transposes the TnM1 sequences, but also will package them together with host DNA into phage particles. Since the pTM2 plasmid is much too small to be packaged into a full-size

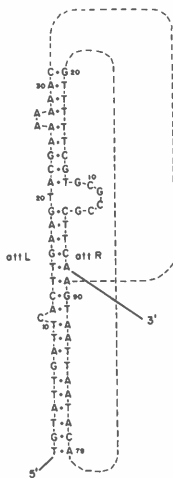


Figure 1
Structural features of the Mu attachment sites. To illustrate the similarities of the two Mu attachment-site regions a single-strand sequence is presented in a stem-loop structure. attL indicates sequences from the left end and attR indicates sequences from the right end of the Mu genome. Numbers indicate distances from the ends of the genome (in base pairs).

phage particle, transposition from the plasmid to the host genome is a prerequisite for packaging of the TnM1 sequences. The presence of the TnM1 sequence in the DNA of mature phage particles has been demonstrated by restriction-enzyme analysis and, in collaboration with L.T. Chow, by electron microscopy. As a first application of this assay, we have replaced the Mu attR fragment in pTM2 with different attR fragments obtained from mature phage DNA such that the TnM1 sequence is restored but the bacterial DNA is different in each case. With three of these new plasmids we have shown transposition of the TnM1 sequence. We consider that the possibility that a duplication of 5 base pairs of host DNA is retained by chance in all three recombinant plasmids is extremely remote and thus conclude that the homology provided by the duplication is not essential for translocation.

Preliminary experiments indicate that excision of the TnM1 sequence can occur when the plasmid is grown in the presence of the expressed A gene of Mu provided by a Mu X mutant or a λ-Mu hybrid. When

the plasmid DNA is isolated from such a strain, its DNA is heterogeneous in size, a fraction of the molecules being smaller than the original pTM2 plasmid. In collaboration with A.I. Bukhari, we are determining whether precise excision occurs in the presence of the host duplication and, if so, whether the duplication is actually needed for excision.

Inversion of the G segment of Mu DNA

In continuing our studies on the invertible G segment of Mu we have characterized the recombination mechanism that promotes G inversion. G inversion does not seem to be related in any way to the integrative recombination of Mu. Functions that are required for Mu integration are not required for G inversion, and vice versa. All functions essential for G inversion are expressed by the recombinant plasmid pGM1, which contains the entire G segment plus 1200 base pairs of the Mu alpha segment and 850 bp of the Mu beta segment. The only function implicated in G inversion thus far, the *gin* function of Mu, has now been mapped precisely within the 850 base pairs of the beta region right next to the G segment. The mapping was achieved by complementation analysis with various λ-Mu hybrid phages and derivatives of plasmid pGM1 constructed in vitro. In collaboration with D. Kwon and D. Zipser, these plasmids have also been used to identify the *gin* protein in an *Escherichia coli* minicell system. We have not been able to demonstrate transposition of the G segment to bacteriophage λ. All available evidence seems to indicate that the G segment, unlike the Mu phage itself, does not represent a transposable element, but rather that recombination promoted by the *gin* protein resembles recombination promoted by the *int* protein of bacteriophage λ. Site-specific "transposition" of the G segment can be observed, however, in the sense that one Mu G segment can exchange with another Mu G segment at high frequency through recombination promoted by the *gin* protein. We have investigated the possibility that the presence of an invertible segment in P1 DNA that is homologous to the G segment of Mu and that furthermore represents the only homology between P1 and Mu could be due to such a site-specific transposition event. In spite of the fact that a Mu G segment can be inverted by a P1 *gin* function, a Mu G segment cannot be replaced by a P1 G segment by means of recombination promoted by either the P1 or Mu *gin* protein. This suggests that the two systems are not the same. The target sites for the Mu and P1 *gin* proteins may not be entirely identical, yet they may be similar enough to allow the complementation of a Mu *gin*⁻ mutant by P1. It is also possible that the *gin* protein is not involved directly in the recognition of the target

G(+) G(+) G(-)
 +
 G(-)

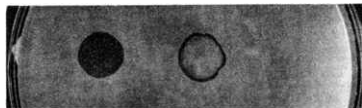


Figure 2
The Pen phenotype: Inhibition of Mu 445-5 G(+) by Mu 445-5 G(-) phage particles. Concentrated lysates (about 10⁹ phage particles/ml) of Mu 445-5 G(+), Mu 445-5 G(-), and a 1:1 mixture of both phages were spotted on a lawn of a Mu-sensitive indicator strain.

sites but is instead part of the enzymatic machinery for G inversion. A second gene product would then be required which is responsible for the site specificity and could be different in P1 and Mu.

As a prerequisite for a rigorous search for additional functions that control G inversion and also for functions that are controlled by G inversion, we have isolated insertions of the transposable element Tn5 (encoding kanamycin resistance) in the G area of the plasmid pGM1. These insertion mutants are being characterized physically by electron microscopic methods and restriction-enzyme analysis in collaboration with K. McElwain and T. Broker. Of 30 mutants that have been mapped thus far, 20 have Tn5 insertions at different locations in the G segment; for every potential gene in the G segment, at least one insertion mutant should be available for further characterization.

One new mutant phenotype of Mu that bears upon

the phenomenon of G inversion has been uncovered accidentally. These mutants, called Pen⁻ (penetration), are unable to inject their DNA into the cell when the same cell is infected by a G(-) phage particle. The Mu Pen⁻ mutants appear to be the result of point mutations, they are *gin*⁻ and *mom*⁻, and they can be rescued from the plasmid pGM1, indicating that the mutations map in or near the G segment. The Pen⁻ phenotype was originally observed with Mu 445-5 G(+) (Fig. 2), which is *Gin*⁻ and *Mom*⁻. Pseudorevertants to Pen⁻ which retain their *Gin*⁻ and *Mom*⁻ phenotype have been isolated from Mu 445-5.

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NUCLEIC ACID CHEMISTRY

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Ad2 sequences

Following our discovery last year of the phenomenon of mRNA splicing in the late Ad2 mRNAs (and in the late mRNAs from other adenovirus serotypes), it has become apparent that transcription of the Ad2 genome at both early and late times is extremely complicated. Studies of Ad2 mRNAs by electron microscopy (L.T. Chow and T.R. Broker) suggest that more than 50 splicing events are required for the production of the complement of mature mRNAs and have indicated that multiple pathways may be present. To understand the molecular events inherent in the splicing process will require the determination of the sequences involved and the characterization of the enzyme(s) responsible. Our initial attempts to isolate an enzyme activity able to perform splicing *in vitro* have been unsuccessful and further efforts have been postponed until an appropriate substrate is available. However, progress has been made in characterizing the sequences involved in the splicing process.

Two kinds of sequence information are required for this purpose, one being the sequences present within individual mRNAs and the other being the genome sequences from which they are transcribed. Consequently, two major projects are underway at the present time, one of which, the determination of the complete sequence of the Ad2 genome, has occupied much of our attention. In view of the size of the genome, 35,000 nucleotide pairs, it was essential that a rapid method be used, and so we have set up the new chain-termination sequencing technique which uses 2', 3' dideoxynucleotides developed by Dr. F. Sanger (MRC Laboratory, Cambridge). The template is prepared by exonuclease-III digestion of either intact Ad2 DNA or isolated restriction fragments and the primers are prepared from subfragments of the genome. Sequ-

ences are determined using all possible template-primer combinations and no fine-structure mapping is being undertaken. The assembly of the complete sequences of the subfragments, followed by the assembly of the complete sequence of the genome, relies heavily upon computer-assisted methods as described below.

One major technical improvement of the dideoxy method has been made and involves the more efficient use of primers. By treating a primer with exonuclease III prior to its hybridization to template, it is possible to use much less primer than usual, as the hybridization now proceeds without the competitive effect of the complementary strand of the primer. In addition, sequences from within the primer are obtained, so that the net result is more sequence from less primer. Also, by using all four [α - 32 P]dideoxyribonucleoside triphosphates, one sequencing reaction can now be accomplished using as little as 0.05 pmole of template and primer.

The determination of sequences present in Ad2 mRNAs has been undertaken and this also uses the dideoxy method. In this case, mRNA, rather than DNA, is used as the template and reverse transcriptase is the source of the DNA polymerase. Although this approach has certain inherent limitations due to the presence of multiple mRNA species and the requirement for appropriate primers, it has been used successfully to study the junction between the main body of fiber mRNA and either the third or fourth leader segments. The results obtained have been useful in conjunction with sequences obtained from a cloned copy of a reverse transcript of fiber mRNA, prepared by J. Sambrook, A. Dunn, and W. Keller. The complete sequence of the leader region present in this plasmid has been determined and, by comparison with genomic sequences, the exact lengths of the four

leader segments are now known. We are currently completing the analysis of the genomic sequences from which these leaders are transcribed so that we can assess the role of primary sequence and putative secondary structure in the excision of the intervening sequences.

Following our success in studying fiber mRNA directly, we have been interested in looking at some of the early mRNAs, and experiments are in progress to define the sequences present in early mRNAs from the extreme ends of the genome—where much of the genomic sequence is now known. In addition, the early mRNA transcribed from EcoRI fragment D is being studied because it too is transcribed from a region of the genome that has been almost completely sequenced. Furthermore, within the limits allowed by electron microscopy, there is some possibility that the splicing events associated with the formation of this mRNA may be related to the existence of the fourth leader component of fiber mRNA.

Computer-assisted methods

In the surge of sequence information arising from our ongoing research, it has become impractical to rely upon manual methods of storage and analysis. Consequently, we have been exploring computer-assisted methods to aid in these tasks. Two main projects have been undertaken, one which is related to the analysis of restriction-enzyme sites and a second which is essential to the determination of the complete Ad2 sequence.

A major problem in the analysis of DNA sequences lies in the recognition of patterns, which may be of biological significance. Although the computer is ideally suited for the rapid identification of specific patterns provided by the operator, it is presently impossible for the computer to identify patterns de novo. Our intuitive human ability to do so is still an essential ingredient in successful computer programming.

Within a DNA sequence, restriction-enzyme cleavage sites provide patterns whose positions are reflected by the length of the fragments produced upon cleavage. We have written a program, in collaboration with J. Milazzo from the Stony Brook Computing Center, that can be provided with the approximate lengths of the fragments resulting from restriction-enzyme cleavage of a DNA of known sequence and can then predict the recognition sequence of the restriction enzyme. This program has proved useful in the analysis of several new restriction enzymes, including BbvI and SfaNI. At present, it is especially useful for enzymes recognizing tetra- or pentanucleotide sequences, as such enzymes usually have many sites within the available substrates of known

sequence. It will be of considerably greater value when much larger, fully sequenced substrates, such as Ad2, are available.

The second use of the computer that we have explored lies in the assembly of DNA sequences from the raw data obtained by performing arbitrary template-primer reactions. Such data contain sequences from within the segment of the Ad2 genome from which the primer was prepared. We have written, again in collaboration with J. Milazzo, a program that can assemble these isolated sequences into a complete sequence. In essence, a file is maintained of all stretches of primary sequence and a search made for regions of direct homology (i.e., exact sequence match) or of complementarity. Where these occur, the primary sequences are merged to produce a new, longer sequence that contains all elements of the two primary sequences. By repeating this process for all available data, the complete sequence is produced for the DNA fragment from which the primers were generated. In this way it is possible to avoid the preparation of fine-structure restriction-enzyme maps, and indeed a comparison of predicted and observed cleavage patterns can serve as an additional check on sequence accuracy.

Restriction endonucleases

The collection of restriction enzymes continues to expand, and of the more than 150 such enzymes now known, over 65 different specificities exist. Although screening of new strains has been limited, one new enzyme, BbvI, has been characterized in *Bacillus brevis* and its recognition sequence deduced (GC(A/T)GC). A second enzyme, FnuAII, which is an isoschizomer of MboI, has been isolated from *Fusobacterium nucleatum* A.

Recognition sequences have been determined for a number of restriction endonucleases, including MstI (TGC/GCA) from a *Microcoleus* strain, KpnI (GGTAC→C) from *Klebsiella pneumoniae*, PvuI (CGATCG) from *Proteus vulgaris*, and SfaNI (GATGC) from *Streptococcus faecalis*.

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CELL BIOLOGY

G. ALBRECHT-BUEHLER, S. Blose, K. BurrIDGE, A. Bushnell, A. Calasso, J. Farrar, J. Feramisco, J. Garrels, W. Gordon, L. Jordon, E.B. Lane, T. Lukralle, M. Schwartz

The main research topic of the Cell Biology group is the motility of animal cells. There are fundamental biological reasons for the choice of this focus, as pointed out in previous Annual Reports. By and large, motility seems to be a matter of the cytoplasm. Therefore, the efforts of the group focus not only on the phenomenology of cell movement and cell migration, but also on the organization of the cytoplasm, and there particularly on the organizations of fibrous structures, such as microfilaments, microtubules, and intermediate filaments. This includes the study of relationships between them as well as to the plasma membrane.

There are two major approaches to the organization of the cytoplasm. One is a more biochemically oriented study of the structural proteins, their interactions, and their expression in various cell types and states. The other is a more micromorphologically oriented study of the spatial distributions of these proteins and of the changes concomitant with movement and changes of cell states. The Cell Biology group attempts to cover both approaches in selected topics, such as the phenomenology of cell movement, the cytoplasmic organizations of actin, tropomyosin, α -actinin, and 10-nm filaments and their role in determining cell morphology and movement, and the study of the overall protein composition of the cytoplasm. Our progress in various aspects of these topics is reported further below.

As of next year, the entire group will be housed in the McClintock building. Remodelling of the building is underway. We are grateful for Dr. Watson's support in this matter, as well as for financial support from the Robertson Research Fund for the construction.

There were several changes in our staff. Ian Bo Chen left us to join the staff at the Sidney Farber Cancer Center in Boston. We regret his leaving, because his

lively and imaginative approach to Cell Biology was a great source of inspiration for us. In July 1978 Stephen H. Blose joined us as a junior staff member. He has discovered an intriguing ring of 100-Å filaments surrounding the nucleus of guinea-pig vascular endothelial cells. This system seems quite promising in elucidating the role of these intermediate filaments in cell motility because the ring-shaped array can be observed in live cells by polarization light microscopy. James Garrels joined us in September 1978. He recently received his Ph.D. from the University of California, San Diego, and will work on a computer analysis of two-dimensional gels. His work will considerably strengthen our efforts to study differences in protein composition between normal and transformed cells. Jim Feramisco, likewise a postdoctoral fellow, who joined us in October, will concentrate on the biochemistry of α -actinin and associated proteins. At the same time, Birgit Lane arrived from England. Her main interest is the study by electron microscopy of 100-Å filaments in cultured cells.

The guidance behavior of 3T3 cells

Animal cells respond to anisotropies on the substrate by orienting or even migrating along them. Various explanations for this phenomenon, called "contact guidance" by Paul Weiss, have been suggested in the past, and its importance for malignant invasion, immune response, and embryonic development has been emphasized.

The guiding substrate we used was designed to be reproducible, to be easy to vary experimentally, and to be compatible with high-resolution light microscopy and the previously described technique of phagokinetic tracks. We used glass coverslips which were covered with a 290-Å-thick homogeneous layer of evapo-

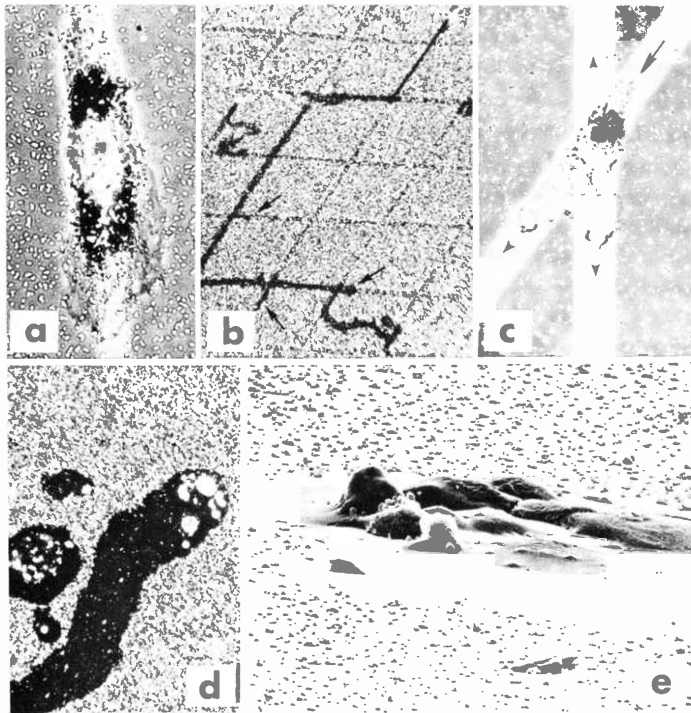


Figure 1

(a) 3T3 cell migrating along a guiding line with the nucleus centered above it. (b) Phagokinetic track of a 3T3 cell (upper right corner) migrating within a network of guiding lines. Arrows point to some of the recordings of a "probing" action of the cell at intersections between guiding lines. Note that the cell can migrate across a gold square as shown in the beginning of the track (bottom). (c) Phase micrograph of a live 3T3 cell at the intersection between guiding lines. The arrow indicates the direction of approach. Arrowheads point in the directions into which the cell probing has already extended (upper arrow) or is extending. (d) Phagokinetic track formed by a migrating group of PtK1 cells during 4 days. (e) Scanning electron micrograph of a migrating group of PtK1 cells.

rated gold. Using a fine diamond tip, thin lines were scratched into the gold layer to expose the underlying glass surface without, however, scratching it. The guidance behavior of cells on such substrates was recorded by their phagokinetic tracks. Therefore, as required by the technique, an additional thin layer of denatured bovine serum albumin, followed by a layer of supracolloidal gold particles, was placed on top of the guiding substrate.

In typical tracks produced by 3T3 cells on the guiding substrates, it was obvious that the cells followed the guiding scratches for some distance (average 280 μm) but were able to leave them by turning at certain angles. Similar behavior could be observed on platinum layers. Live-cell observations showed that cells following scratches generally extended to both sides into the gold area while keeping the nucleus approximately centered over the scratch. Their

method of locomotion involved the usual formation of a leading edge and a retracting tail as is seen on normal substrates (Fig. 1a).

Measuring the angles at which cells moved into or out of the guiding lines yielded essentially identical angular distributions. Therefore, both distributions were combined into one. This angular distribution of directional change shows a peak between 40° and 60° and decreases gradually towards 180°.

In another type of experiment there were two sets of parallel scratches crossing each other at a prefixed angle. A cell following a guiding line and approaching the intersection between two lines had the options of continuing straight ahead, of turning at the prefixed angle w , of turning at the complementary angle $180^\circ - w$, of migrating backwards, or of leaving the guiding lines by moving into one of the diamond-shaped gold areas. Counting in nine independent experiments the percentage of cells which turned at the present angle w or at its complement $180^\circ - w$ in a total of 2208 encounters of cells with an intersection again yielded a curve where one can discern a preference for angles between 30° and 60°.

Live-cell observations showed the intriguing action of cells at intersections whereby they extend into the various possible directions before following one of them (Fig. 1c). Such "exploring" activity can also be seen recorded in the phagokinetic tracks as short thorns extending into various preformed directions at intersections (see arrows in Fig. 1b).

Group locomotion of PtK1 cells

In the animal embryo, single cells can migrate (e.g., primary mesenchyme cells) but also whole sheets of cells can deform and move (e.g., gastrulation, folding of the neurocrest). Single cells can also migrate in tissue culture. Whole sheets of human epidermal cells on top of a feeder layer were recently shown to deform and fold into swirls (Green and Thomas, *Science* 200:1385 [1978]).

One may wonder whether there is an intermediary form of cell locomotion between the two extremes of single-cell migration and collective deformation of entire cell sheets. The following study shows that an intermediary form does exist in the form of small groups of PtK1 cells (rat-kangaroo kidney cells isolated by Walen and Brown, *Nature* 194:406 [1962]), which are able to migrate in unison across glass substrates.

During 4 days of observation, single PtK1 cells migrated very little, although they were alive, as shown by the particle-free rings around them. There were also large groups of cells (Fig. 1a) that hardly displaced themselves, whereas the group shown in Figure 1d left a wide and approximately 700- μm -long particle-free

track on the gold-particle-coated substrate indicating its collective migration. Figure 1e shows one migrating group in scanning electron microscopy. Its phagokinetic track extends further to the right-hand side of the frame.

By observing living cells, we found various ways by which migrating groups propagated themselves. As one would expect, only cells at the periphery of the groups were able to extend the ruffling lamellipodia, which appear necessary for single-cell locomotion in fibroblasts and epithelial cells. The centrally located cells in a group, which could not extend lamellipodia, nevertheless changed their outlines continuously during the migration of the group.

Yet, a quite different type of group migration could be observed as well. In other live-cell sequences, the largest extending lamellae were consistently at the rear end of the group and small, frequently retracting lamellae were located at the front edge of the group.

Consistent with an earlier report by Brecher (*Exp. Cell Res.* 96:303 [1975]), we found an abundance of 100- \AA filaments in electron micrographs of both fast-migrating and nonmigrating groups of PtK1 cells. Besides this conspicuous cytoskeletal feature of PtK1 cells, there was often a striking parallel orientation of actin-containing microfilament bundles across cell border lines.

The orientation of centrioles in migrating 3T3 cells

On the one hand, and as a consequence of their ninefold rotational symmetry, centrioles divide their circumference into portions of $360^\circ/9 = 40^\circ$. On the other hand, it seems that migrating 3T3 cells change direction preferentially at angles of $40^\circ \pm 20^\circ$, as was pointed out above. Of course, the simultaneous appearance of this angle in seemingly so unrelated subjects as centriole structure and angular spectrum of directional changes may be fortuitous. Yet, one may tentatively take seriously what might be a coincidence and test one of its immediate consequences: If an angle related to the structure of centrioles is, indeed, expressed in the movement of 3T3 cells, it seems logical to suspect that the centrioles themselves maintain a certain well-defined orientation with respect to the direction of movement and the plane of the substrate.

We selected and serially sectioned 20 cells that had formed phagokinetic tracks more than 200 μm in length without undergoing cell division, as judged by the fact that their tracks did not branch.

For each cell, we collected the micrographs that showed centrioles and oriented them relative to the light micrographs that showed the cells' tracks and a phase-contrast image of the cells. We consistently

found the centrioles in levels above the substrate where the cells did not contain any microfilament bundles, indicating that the centrioles float above the ventral network of microfilament bundles.

With the exception of two cells, we consistently found the centrioles sectioned either approximately perpendicular or parallel to the substrate.

This finding of a peculiar orientation of centrioles relative to the substrate supports the previous suggestion that the preferential angle of directional change of $40^\circ \pm 20^\circ$ may be related to the 40° angle resulting from the ninefold rotational symmetry of centrioles. The angle of 40° occurs in the plane perpendicular to the centriole axis. If the centriole axis is oriented perpendicular to the substrate, as reported, then this angle could conceivably become expressed in the plane of the substrate.

Polygonal networks and intermediate filaments in nonmuscle cells

In the studies of the organization and function(s) of the microfilament system of cultured nonmuscle cells, we used a cell line derived from a fibroma of a gerbil. These cells were chosen for two reasons: they have numerous uniformly thin stress fibers, and, at a well-defined time during spreading, form transient polygonal networks of microfilaments (Fig. 2).

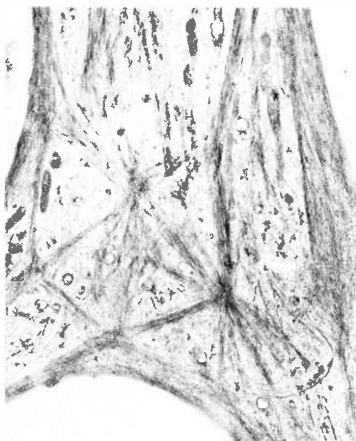


Figure 2
Electron micrograph of a polygonal network near the ventral side of a gerbil fibroma cell.

In the studies of the stress fibers, we have used antisera against chicken gizzard actin, myosin, and filamin and bovine skeletal muscle α -actinin to localize these proteins by means of immunofluorescence. From work by others, it was known that myosin, tropomyosin, and α -actinin are distributed in stress fibers in periodically arranged units. These results led to the suggestion by numerous authors that stress fibers have a sarcomerelike arrangement of contractile proteins. One key piece of information needed to support this suggestion was the relationship between the myosin and α -actinin units; a sarcomeric arrangement requires an alternate spacing. Another important question was whether actin is periodically arranged as in myofibrils. Both pieces of information have been provided by our immunofluorescent and electron microscopic studies. Myosin and α -actinin units are spaced alternately along stress fibers, with α -actinin localized in Z-line-like dense bodies and with myosin in spaces between them. An actin periodicity can be detected along some stress fibers in at least some cells by immunofluorescence (Gordon, *Exp. Cell Res.* 117:253 [1978]).

In immunofluorescent studies of respreading rat embryo cells, Lazarides (*J. Cell Biol.* 68:202 [1976]) discovered an actin-based polygonal network of fibers. He localized actin, α -actinin, and tropomyosin in this network and proposed that the network serves as a structural intermediate during stress-fiber formation. The polygonal networks that form in gerbil fibroma cells are similar to those seen in rat cells. We used immunofluorescence to demonstrate the distribution not only of actin and α -actinin in these networks, but also myosin and filamin. In addition, the ultrastructure of the networks was examined by electron microscopy. They are constructed of foci, condensed meshworks of microfilaments attached to the inner surface of the plasma membrane, and interconnecting microfilament bundles indistinguishable from stress fibers (Gordon and Bushnell, *Exp. Cell Res.* [1979, in press]).

In recent studies we have reinvestigated both the stress fibers and polygonal networks of the gerbil fibroma cells by means of a double immunofluorescent labeling technique. Employing this technique we have been able to demonstrate that in both stress fibers and polygonal networks the units of myosin and tropomyosin occur in exactly the same locations. We have also been able to provide direct evidence for the complementarity of the distribution of these myosin-tropomyosin regions with the α -actinin-containing regions.

In the course of studies on the microfilament system of cultured nonmuscle cells, we discovered a unique autoimmune antiserum reactive with the major pro-

tein (56,000 m.w.) and possibly a minor protein (30,000 m.w.) of the intermediate (10-nm) filaments of most cultured cell types, muscle and nonmuscle. The immunofluorescent results, ultrastructural views of the filaments and the immunochemical characterization of the autoimmune antiserum have been published (Gordon, Bushnell, and Burridge, *Cell* 13:249 [1978]).

In collaboration with Dr. Ian Buckley (John Curtin School of Medicine, Canberra, Australia), we have investigated another aspect of the intermediate filaments in a variety of nonmuscle cells. Buckley and Raju (*J. Cell Biol.* 78:644 [1978]) reported that intermediate filaments of glycerol permeabilized cells after mild trypsin treatment (5 μ g/ml for 1 hr at 20°C) can bind heavy meromyosin (HMM), forming the well-known arrowhead pattern characteristic of actin filaments. This led these authors to conclude that intermediate filaments in primary cultures of chicken embryonic connective-tissue cells have an actin core. Together, we reinvestigated this finding employing a wide variety of nonmuscle cultured cell types viewed in glutaraldehyde-tannic acid-fixed, HMM-labeled, critical-point-dried whole mounts. Apparently, it is a general phenomenon that mildly trypsinized intermediate filaments of nonmuscle cells can bind HMM (Buckley and Raju *J. Cell Biol.* 78:644 [1978]).

10-nm Filaments in vascular endothelial cells

In previous work we discovered that vascular endothelial cells cultured from guinea-pig thoracic aorta and portal vein contain a ring of 10-nm filaments that encircles the nucleus (Fig. 3). Currently, we have observed that intracellular organelles—mitochondria, lipid vacuoles, lysosomes, and phagosomes—are closely associated with the ring and the attending 10-nm filaments. When the ring is induced to undergo cap formation by colcemid, these organelles become dispersed throughout the cell. Reversal of this experiment by washing out colcemid causes the ring to re-form with the reassociation of organelles. These observations have led us to the hypothesis that the 10-nm-filament ring in endothelial cells operates to organize and/or transport intracellular organelles. Utilizing time-lapse video-tape microscopy on living endothelial cells, we are in the process of determining whether 10-nm filaments are involved in the orderly movement of organelles.

We have also studied the fate of the 10-nm-filament ring during mitosis. By indirect immunofluorescence utilizing antibodies against gizzard 10-nm-filament protein or the 10-nm-filament autoimmune antisera (Gordon, Bushnell, and Burridge, *Cell* 13:249 [1978]), endothelial cells at various stages of mitosis were stained. During late metaphase, the ring is largely

distorted, though still a closed structure. In anaphase, the major axis of the ring enters the daughter cells preceding the chromosomes. In late telophase, cytokinesis cleaves into crescents the 10-nm filaments at the site of the contractile ring. Inhibition of cytokinesis by treatment with cytochalasin B causes the daughter nuclei to remain surrounded by the parent 10-nm-filament ring. At no point during mitosis does the array of 10-nm filaments disassemble into molecular components. This is in contrast to the other major cytoplasmic structures that disassemble. We are presently investigating the intriguing possibility that during mitosis the 10-nm filaments carry topological information about the distribution of organelles and structural proteins into daughter cells.

Computerized two-dimensional gel analysis of proteins

A two-dimensional gel scanning facility has been set up on the second floor of the Carnegie library. The equipment includes a PDP 11/60 computer, an 88M byte disk drive, an Optronics film scanner, and an interactive TV display system. The computer will process the data from each gel scan to detect and quantify the spots that represent proteins. The TV system will be used to display the data and to match patterns from separate scans. The position of each detected protein will be expressed in terms of a standardized coordinate system and the list of protein coordinates and intensities will be stored on the disk for future reference. Because the data will be standardized, it will then be possible to compare the types and amounts of the proteins that occur in any or all of the samples that have been analyzed previously.

A special laboratory facility for two-dimensional gel electrophoresis is being constructed in the McClintock building. This facility will make possible the routine production of large numbers of two-dimensional gels, which will be required for many of the experiments that are planned. A number of labor-saving features have been incorporated into the design, as well as features to assure a high degree of reproducibility.

When the electrophoresis and gel-scanning facilities are in operation, we plan to begin studies on the basic mechanisms of cellular differentiation. We will first examine briefly a large number of different cell types and animal tissues to determine how much heterogeneity of gene expression does exist and to find some of the basic tissue-specific patterns of gene expression. This survey will establish a data base that will be frequently referenced and expanded in future experiments. We plan then to concentrate our studies primarily on the molecular mechanisms of muscle differentiation, using cloned cell lines that can be

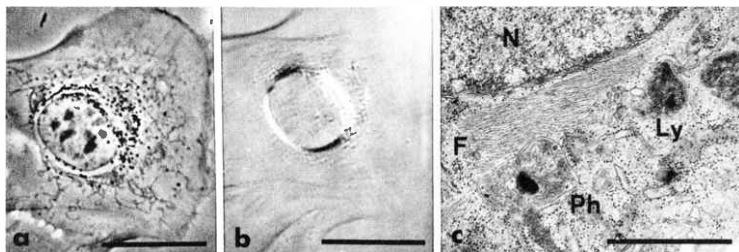


Figure 3

Phase (a) and polarized light (b) micrographs of the same living endothelial cell, demonstrating the perinuclear ring of 10-nm filaments surrounded by lipid vacuoles, lysosomes, and mitochondria. (Bars = 25 μ m) (c) An electron micrograph of the ring, F, shows association of a lysosome, Ly, and a phagosome, Ph, with the 10-nm filaments. N, nucleus. (Bar = 1 μ m.)

induced to undergo myogenic differentiation in cell culture.

The association of α -actinin with the plasma membrane

A major unresolved question in the movement of cells is how the presumed force-generating elements, such as the actin and myosin, are linked to cell membranes or membrane components. During this last year we have concentrated on how actin filaments may be linked to the plasma membranes of motile cells, such as fibroblasts and lymphocytes. A possible candidate which may link actin filaments to membrane components is the protein α -actinin. Originally, this protein was considered to be specific to muscle and to function in the attachment of actin filaments to muscle Z lines. The discovery of this protein in nonmuscle cells suggested that it might have a similar function in attaching actin to membranes. Suggestive indirect evidence supporting this possibility has come from immunofluorescent antibody studies. Since ultimately the mode of actin attachment will have to be resolved biochemically, we have asked the question whether α -actinin is present in pure preparations of the plasma membrane and, if so, can it mediate the attachment of actin filaments. α -Actinin is a difficult protein to assay as it has no known enzymatic function. As a result, we have identified α -actinin by means of the technique we developed two years ago for detecting antigens directly in gels by their binding of specific antibodies. When purified plasma membranes are analyzed on gels, a band at 100,000 daltons is revealed that reacts with specific anti- α -actinin antibodies. We have obtained this result by means of two different procedures

to purify plasma membranes from HeLa cells, lymphocytes, and cultured fibroblasts. Because the α -actinin could be present in these preparations for the trivial reason that it is attached to the actin which is bound to membrane components via some other molecules, we have tried various extraction procedures to find out how the α -actinin is associated with the membranes. Whereas myosin associated with these membranes can easily be extracted in the presence of ATP and high ionic strength, much of the α -actinin is difficult to extract and can only be removed from these membranes when conditions are used to depolymerize and dissociate the actin. This fraction of the α -actinin that is tightly associated with the plasma membrane may still be present as a consequence of its interaction with actin, but the necessity to depolymerize the actin in order to remove the α -actinin from these membranes is also consistent with the α -actinin mediating the actin attachment. Further work will be needed to distinguish between these two alternatives. One possibility is to do reconstitution experiments with membranes depleted in both actin and α -actinin and to determine whether either protein can interact directly with specific membrane proteins in the absence of the other component.

In studies of membranes, the red blood cell has provided a popular, albeit specialized, model system. Since actin has been found peripherally associated with the red-blood-cell membrane, we decided it would be interesting to look here also for the presence of α -actinin. We were unable, however, to detect a gel band reactive with our anti- α -actinin antisera in gels of red-blood-cell membranes. Since our antiserum has quite a broad specificity, it seems unlikely that the red-blood-cell membrane contains any α -actinin; but

given the unusual properties of red blood cells, it could still be that a related molecule is present though sufficiently different not to cross-react with our antibody.

Whether or not α -actinin does mediate the attachment of actin to membranes, it is clearly an interesting protein both in muscle and nonmuscle cells. Yet, it is a protein about which surprisingly little is known when compared with many of the other proteins of the contractile complex. We have, therefore, undertaken a biochemical study of this protein in order to compare muscle and nonmuscle forms and also to "dissect" the regions of the protein responsible for its different functions. Just as a decade ago the myosin molecule was dissected into a "head" region which carried the catalytic and actin-binding sites and a "tail" region whose properties were responsible for its association into filaments, so we hope to dissect the α -actinin molecule into various functional regions. In this study, it will be particularly interesting to identify major differences between the muscle and nonmuscle proteins, possibly indicating different potential interactions or functions.

Changes in glycoproteins during muscle development

The cell surface is a critical region in muscle-cell differentiation. Some of the stages of muscle-cell development can be studied conveniently in tissue culture using either primary cultures or myoblast cell lines. We have been analyzing the glycoproteins of a clonal rat myoblast line that is a derivative of Yaffe's L8 line. The glycoproteins of the prefused and the post-fused myotubes have been compared with each other and with those from a nonfusing clone by direct lectin-binding analysis to SDS gels of whole cells. We have used several lectins with different carbohydrate-binding specificities. Some glycoprotein changes are very prominent, but comparison with the nonfusing clone has enabled us to show that some of the changes do not relate to myoblast fusion itself but correlate more with actively growing cultures or cultures that have become quiescent. However, some bands are specifically lost as myoblast fusion occurs, whereas others appear. The bands that appear may represent glycoproteins involved in the fusion event or they may represent specialized glycoproteins of the differentiated myotube. Certainly, the mature muscle

does have specialized membranes and membrane functions not present in myoblasts, such as the sarcoplasmic reticulum and the transverse tubular system. It will be interesting to identify any of the specific glycoproteins that we detect on gels with proteins whose functions in the mature muscle are known.

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POSTGRADUATE TRAINING PROGRAMS

SUMMER 1978

Since its inception, the postgraduate program at Cold Spring Harbor Laboratory has been aimed at meeting the rather special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our intention is to provide intensive training in the most recent developments and techniques in each of the subjects so that, upon completion, the students will be able to enter directly into research in the particular area. To ensure this up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

THE TRANSFORMED CELL, June 11—July 1

INSTRUCTORS

Rifkin, Daniel, Ph.D., New York University Medical Center, New York
Ozer, Harvey, M.D., Hunter College, City University of New York, New York

ASSISTANT

Crowe, Ruth, B.A., New York University Medical Center, New York

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



Special thanks for their contributions of time and material to these exercises go to Bill Topp, Keith Burrige, Seung-II Shin, Richard Hynes, and Robert Pollack.

PARTICIPANTS

Aimtmann, Eberhard, B.A., Deutsches Krebsforschungszentrum, Heidelberg, Germany
Babson, John R., B.S., Oregon State University, Corvallis
Canary, Patricia K., M.S., Albert Einstein College of Medicine, Bronx, New York
Coutino, Rocio, B.S., Instituto Politécnico Nacional, Mexico City, Mexico
Dittmar, Kurt, Ph.D., Max-Planck-Institut, Berlin, Germany
Frantz, Christopher N., M.D., Sidney Farber Institute, Boston, Massachusetts
Gebhardt, Rolf, B.S., University of Tübingen, Germany
Hill, David J., B.S., Tufts University, Medford, Massachusetts
Izzo, Paola, B.S., University of Naples, Italy
Klarlund, Jes K., B.S., Fibiger Laboratory, Copenhagen, Denmark
Lis, Martin, Ph.D., Clinical Research Institute of Montreal, Canada
Mizunaga, Takemitsu, Ph.D., Waksman Institute, Rutgers University, New Brunswick, New Jersey
Montarras, Didier, M.S., Institut Pasteur, Paris, France
Mullins, Deborra E., B.S., University of Florida, Gainesville
Nadakavukaren, Karen K., B.S., University of Oregon, Eugene
Reyès, Gregory R., B.A., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Reich, E., Rockefeller University. *General properties of malignancy.*
Vogt, P., University of Southern California Medical School. *Transformation by RNA viruses.*
Tegtmeyer, P., State University of New York, Stony Brook. *Transformation by DNA viruses.*
McCulloch, E., Ontario Cancer Institute. *Hematopoietic cells in culture.*
Green, H., Massachusetts Institute of Technology. *Epithelial cells in culture.*
Barrett, C., National Institute of Environmental Health Sciences. *Chemical carcinogenesis.*
Rifkin, D., New York University Medical Center. *Proteases and transformation.*
Gospodarowicz, D., Medical Center, University of California, San Francisco. *Hormones and growth factors in culture.*
Hochstadt, J., New York University Medical Center. *Transport changes and transformation.*
Pollack, R., Columbia University. *Cytoskeleton changes with transformation.*
Hynes, R., Massachusetts Institute of Technology. *Surface changes and transformation.*
Martin, B., National Institutes of Health. *Regulation of DNA synthesis in transformed cells.*
Nicholson, G., University of California, Irvine. *Metastasis.*
Seung-II, S., Albert Einstein College of Medicine. *Tumor growth in the nude mouse.*
Ozer, H., Hunter College, City University of New York. *Transformation and somatic cell hybrids.*
Basilico, C., New York University Medical Center. *Conditional viral transformants.*

CENTRAL NERVOUS SYSTEM OF THE LEECH, June 11–July 1

INSTRUCTORS

Nicholls, John, Ph.D., M.D., Stanford University Medical School, California
Muller, Ken, Ph.D., Carnegie Institution, Baltimore, Maryland
Wallace, Bruce, Ph.D., Stanford University Medical School, California

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

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

The final phase of the course consisted of devising and performing original experiments, some of which proved to be of sufficient interest to be pursued in greater detail. For example, the nervous systems of various hitherto unexplored leeches were studied. In addition the properties of a new fluorescent dye, Lucifer yellow, were tested by intracellular injection into identified cells. A systematic study was made for the first time of the branching patterns of homologous neurons throughout the length of an animal.

PARTICIPANTS

- Blackshaw, Susanna E., Ph.D., University College London, England
Blair, Seth S., B.S., University of California, Berkeley
Feder, Ned, M.D., National Institutes of Health, Bethesda, Maryland
Feinberg, Andrew P., M.D., University of California, San Diego
Fuchs, Paul A., B.A., Stanford University, California
Payton, Brian W., Ph.D., Memorial University of Newfoundland, Canada
Reiser, Georg, Ph.D., Max-Planck-Institut, Munich, Germany
Stewart, Walter W., B.A., National Institutes of Health, Bethesda, Maryland
Timpe, Leslie C., B.A., Harvard Medical School, Boston, Massachusetts
Tomosky-Sykes, Alisen J., M.A., Columbia University, New York, New York

SEMINARS

- Sawyer, R., University of California, Berkeley. *Biology of leeches.*
——— *Development of leeches.*
Nicholls, J.G., Stanford University Medical School. *Introduction.*
——— *Sensory and motor cells.*
——— *Afterpotentials.*
——— *Synaptic mechanisms and tissue culture.*
——— *Killing single cells.*
Muller, K., Carnegie Institution. *Sensory cell abnormalities.*
——— *Structure of synapses.*
——— *S cell regeneration.*
Wallace, B., Stanford University Medical School. *Heart cell synapses.*
——— *Regeneration.*
Stent, G., University of California, Berkeley. *Swimming.*
——— *Photoreceptors.*
——— *Heartbeat.*
Kristan, W., University of California, San Diego. *Swimming.*
Zipser, B., State University of New York Downstate Medical Center. *Neurons regulating sexual behavior.*
Cohen, L., Marine Biology Laboratories, Woods Hole. *Dyes for recording membrane potential.*
Salzberg, B., University of Pennsylvania. *Dyes for recording membrane potential.*

TECHNIQUES FOR STUDYING THE VERTEBRATE CENTRAL NERVOUS SYSTEM, June 11–July 1

INSTRUCTORS

- Kirkwood, Peter, Ph.D., Institute of Neurology, London, England
Shatz, Carla, Ph.D., Stanford University Medical School, California
Stryker, Michael, Ph.D., University of California, San Francisco

ASSISTANT

- Mates, Sharon, B.A., University of Washington Medical School, Seattle

One aim of the course was to provide students with the basic technical competence necessary for conducting independent experimental work. Another was to provide an introduction to the functional organization of several different areas in the vertebrate central nervous system. The course consisted of four different laboratory exercises. In all labs students learned the appropriate surgical techniques, the preparation of microelectrodes, and simple histological procedures for reconstructing electrode tracks. In one lab the reflex activity of motoneurons in the cat's spinal cord was studied using both intracellular and extracellular recording techniques. Two other labs focused on the visual system. In one, extracellular recordings were made from the cat's visual cortex, and the response properties of neurons to visual stimuli were examined. In the other, stereotaxic techniques were used to place electrodes within deeper structures (lateral geniculate nucleus) for recordings and microinjections. In this laboratory, the connections between the lateral geniculate nucleus and the visual cortex were studied. The retrograde transport of horseradish peroxidase injected into the visual cortex, and the anterograde transport of ^3H -proline injected into the lateral geniculate nucleus were used to identify these connections. In the fourth lab, the regions of the rat's cortex receiving somatosensory input from the whiskers were studied. Extracellular recordings were used together with the recently developed 2-deoxyglucose histochemical method for measuring local glucose utilization in neural tissue. The structure of this cortical region seen in cell-body stained sections was compared with findings from these physiological techniques.

PARTICIPANTS

Bach, Michael, M.S., Albert-Ludwigs Universität, Freiburg, Germany
Bowling, Doug B., Ph.D., Stanford University, California
Brecha, Nicholas, Ph.D., State University of New York, Stony Brook
Haber, Suzanne N., Ph.D., Stanford University, California
Kohlerman, Nicholas J., B.A., Johns Hopkins University, Baltimore, Maryland
Martin, John H., M.A., Columbia University, New York
Nixon, Bruce, B.S., McMaster University, Hamilton, Ontario
Sudarsky, Lewis R., M.D., Childrens Hospital, Boston, Massachusetts

SEMINARS

Burke, R.E., National Institutes of Health. *Anatomy and physiology of monosynaptic action in spinal motoneurons of the cat.*
Wiesel, T.N., Harvard Medical School. *The visual system: Adult organization, development, and the effects of deprivation.*
Moore, G.P., University of Southern California, Los Angeles. *Theory and use of cross-correlation technique in the mammalian nervous system.*
Kiang, N.Y.S., Massachusetts Eye and Ear Infirmary. *The mammalian auditory system.*
Bizzi, E., Massachusetts Institute of Technology. *The role of proprioceptive afferents in motor control.*
Karten, H.J., State University of New York Medical School, Stony Brook. *Displaced ganglion cells and the accessory optic system.*

MOLECULAR CYTOGENETICS, June 11—July 1

INSTRUCTORS

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

ASSISTANT

Erba, Harry, Yale University, New Haven, Connecticut

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

Students came from Chile, Denmark, Germany, Israel, Mexico, and various parts of the United States. Their wide range of previous research experience added a variety of expertise to the group.

PARTICIPANTS

Arancibia, Marco A., M.S., University of Chile, Santiago
Calzone, Frank J., B.A., University of Rochester, New York
Dale, Roderic M.K., Ph.D., Yale University, New Haven, Connecticut
Flores-Lopez, Margarita, B.S., Instituto Politécnico Nacional, Mexico City, Mexico
Grummt, Ingrid, Ph.D., Max-Planck-Institut, Munich, Germany
Leer, Johan, Ph.D., University of Aarhus, Denmark
Mertz, Janet E., Ph.D., University of Wisconsin, Madison
Sears, Barbara B., B.S., Duke University, Durham, North Carolina
Shilo, Ben, B.S., Sidney Farber Institute, Boston, Massachusetts
Strausbaugh, Linda, Ph.D., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Latt, S., Harvard Medical School. *Recent studies on chromosome fluorescence and sister chromatid exchange formation.*
Lindsley, D.L., University of California, San Diego. *Genetic control of spermatogenesis.*
Davis, R., Stanford Medical School. *Genes, transposons, and transformation of yeast.*
Gall, J., Yale University. *Gene amplification in tetrahymena.*
McClintock, B., Carnegie Institution of Washington. *How to make junk out of the genome.*
Hamkalo, B., University of California, Irvine. *Studies on the structure of metaphase chromosomes.*
Bonner, J.J., University of California Medical School. *Induction of puffing in isolated polytene nuclei from Drosophila.*
Pardue, M.L., Massachusetts Institute of Technology. *The heat shock response: A model system for the study of transcription in Drosophila.*

STRUCTURE AND FUNCTION OF THE SYNAPSE, June 11—July 1

INSTRUCTORS

Rahamimoff, Rami, M.D., Hebrew University Medical School, Jerusalem, Israel
Frank, Eric, Ph.D., Harvard Medical School, Boston, Massachusetts
Sanes, Joshua, Ph.D., University of California Medical School, San Francisco
Hudspeth, Albert James, M.D., California Institute of Technology, Pasadena

DEMONSTRATORS

Yaari, Yoel, Ph.D., Hebrew University Medical School, Jerusalem, Israel
Lev-tov, Aharon, M.Sc., Hebrew University Medical School, Jerusalem, Israel

This course was designed for graduate students and research workers interested in the structure and function of synapses. It consisted of lectures; reading of papers; group discussions; presentation of selected topics by participants; seminars by instructors, faculty, and participants; and demonstrations. After the course ended, eight participants who were uninitiated in electrophysiological techniques stayed on for several days to learn the usage of basic methods. Four additional participants stayed for four weeks learning in detail specific methods and starting their own research projects.

PARTICIPANTS

Augustine, George, J., B.S., University of Maryland, College Park
Ballivet, Marc, Ph.D., University of Geneva, Switzerland
Barish, Michael E., Ph.D., Stanford University, California
Green, Steven H., B.S., California Institute of Technology, Pasadena
Halegoua, Simon, B.S., State University of New York, Stony Brook
Jackson, Patrick C., B.S., McMaster University, Ontario, Canada
Jahnsen, Henrik, M.D., Institute of Neurophysiology, Copenhagen, Denmark
Kass, Leonard, M.S., Eye Research Institute, Boston, Massachusetts
Marchais, Dominique, B.S., Ecole Normale Supérieure, Paris, France
Marshak, David W., B.A., University of California, Los Angeles
Michler, Angelika, Ph.D., Max-Planck-Institut, Göttingen, Germany
Morrison-Graham, Kathleen, B.S., University of California, Los Angeles
North, Paula E., B.A., Vanderbilt University, Nashville, Tennessee
Pawson, Peter A., B.S., McGill University, Montreal, Canada
Pearse, Roger, B.A., McLean Hospital, Belmont, Massachusetts
Pickett, Jackson B.E., M.D., University of California, San Francisco
Rothman, Steven M., M.D., Washington University, St. Louis, Missouri
Sherman, Thomas G., B.S., University of Texas, Dallas
Stein, Cathy, B.A., University of Maryland, Baltimore
Zurn, Anne D., Ph. D., University of Geneva, Switzerland

SEMINARS

Hudspeth, A.J., California Institute of Technology. *Neurocytology.*

_____ *Structure of the synapse.*

_____ *Structure of the neuromuscular junction.*

_____ *Sensory transduction in hair cells.*

_____ *Recycling of synaptic vesicles.*

Frank, E., Harvard Medical School. *Passive electrical properties of nerve and muscle.*

_____ *Phenomenology of the action potential.*

_____ *Cable properties and the propagation of action potential.*

_____ *Excitatory synaptic transmission.*

_____ *The ionic basis of the EPSP.*

_____ *Inhibitory synaptic transmission.*

_____ *Quantal transmission in the central nervous system.*

_____ *Ionic selectivity and Eyring rate theory.*

_____ *Development of synapses in tissue culture.*

Rahamimoff, R., Hebrew University Medical School. *Ionic basis of membrane potential-forces.*

_____ *Fluxes through resting nerve membrane.*

_____ *Resting potential.*

_____ *Ionic basis of the action potential-currents.*

_____ *Ionic basis of the action potential-conductances.*

- _____ *Hodgkin-Huxley model.*
- _____ *Quantal release of transmitter.*
- _____ *Regulation of transmitter release by intracellular calcium.*
- _____ *Frequency modulation of transmitter release.*
- _____ *Leakage of transmitter.*
- _____ *Oscillations in transmitted release.*
- Raviola, E., Harvard Medical School. *Structure of the retina.*
- _____ *Workshop on freeze fracture.*
- Thomas, R., Bristol University. *The electrogenic sodium pump and ion-sensitive microelectrodes.*
- _____ *The regulation of intracellular H⁺ ion concentration.*
- Sanes, J., University of California Medical School, San Francisco. *Localization and structure of the acetylcholine receptor.*
- _____ *Localization and structure of acetylcholinesterase.*
- _____ *Development of the neuromuscular junction. I. Embryonic development.*
- _____ *Development of the neuromuscular junction. II. Denervation and reinnervation.*
- _____ *Development of the neuromuscular junction. III. Regulation and mechanisms.*
- Yaari, Y., Hebrew University Medical School. *Electrical synapses.*
- _____ *Presynaptic inhibition in CNS.*
- Goodenough, D., Harvard Medical School. *Gap junctions.*
- _____ *Introduction to X-ray diffraction and the structure of the gap junction.*
- Erlkar, S.D., University of Pennsylvania School of Medicine. *Neurotoxins.*
- _____ *Principles of synaptic pharmacology and false transmitters.*
- _____ *Chemical and electrical transmission in the spinal cord.*
- Albrecht-Buehler, G., Cold Spring Harbor Laboratory. *Geometry and cell movements.*
- Grinvald, A., Yale Medical School. *Voltage-sensitive fluorescent probes.*
- Yoshikami, D., Harvard Medical School. *Microphysiology of the postsynaptic membrane.*
- _____ *Postsynaptic potentiation interaction between quanta and the size of the quantum.*
- _____ *Transmission at automatic ganglia.*
- _____ *Slow synaptic potential.*
- Wallace, B., Stanford Medical School. *Octopamine synapses.*
- _____ *Leech demonstration.*
- Stevens, C., Yale Medical School. *Properties of the postsynaptic channel.*
- _____ *The voltage dependence of the postsynaptic channel.*
- _____ *Fluctuation analysis.*
- _____ *Applications of fluctuation analysis.*
- _____ *Ion permeation through channels.*
- Nicholls, J., Stanford Medical School. *Leech demonstration.*
- _____ *Long-term changes, single cell killing, regeneration in the nervous system of the leech.*
- Meuller, K., Carnegie Institution of Washington. *Leech demonstration.*
- _____ *Morphology and function of leech ganglia.*
- Fambrough, D., Carnegie Institution of Washington. *Biochemistry of the acetylcholine receptor.*
- _____ *Synthesis and degradation of the acetylcholine receptor.*
- Purves, D., Washington University, St. Louis. *Cell death and synapse elimination.*
- _____ *Nerve growth factor and the effects of axotomy.*
- _____ *Sprouting of axon terminals.*
- _____ *Specificity of neural connections.*
- Patterson, P., Harvard Medical School. *Migration of neural crest cells.*
- _____ *Influence of the environment on nerve cell differentiation.*

ADVANCED BACTERIAL GENETICS, July 4–July 24

INSTRUCTORS

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

ASSISTANTS

- Johnston, Mark, B.A., University of Utah, Salt Lake City
- Rose, Mark, B.A., Massachusetts Institute of Technology, Cambridge
- Stinchcomb, Dan T., B.A., Stanford University, California

Starting with a random pool of λ phages carrying cloned inserts of *Salmonella* DNA, students identified by plaque hybridization phages carrying the naturally occurring IS10 sequence and a Tn10 element present in the strain. From the same pool, students selected phages carrying portions of the histidine and tryptophan operon. Phages were characterized by restriction analysis, complementation tests, and electron microscopy. Inserts from phages were recloned in a plasmid vehicle.

Students also carried out a variety of genetic experiments involving use of the Tn10 insertion element in *Salmonella typhimurium*. These included isolation of insertional auxotrophs and fermentation mutants, selecting Tn10 insertions near particular genes and using Tn10 to direct insertion of a F_{TS}'Tn10 *lac*⁺ episome into the chromosome.

PARTICIPANTS

Armaleo, Daniele, M.S., Duke University, Durham, North Carolina
Bates, Marjorie A., B.S., University of California, Los Angeles
Cole, Patricia E., Ph.D., Columbia University, New York
Conklin, Mark A., M.S., National Institutes of Health, Bethesda, Maryland
Donnelly, Robert J., B.S., Johns Hopkins University, Baltimore, Maryland
Eccleshall, T. Ross, Ph.D., Albert Einstein College of Medicine, Bronx, New York
Esposito, Rochelle E., Ph.D., University of Chicago, Illinois
Farrell, Roberta L., M.S., University of Illinois, Urbana
Kwan, Sau-Ping, Ph.D., Albert Einstein College of Medicine, Bronx, New York
Lai, Elaine Y., Ph.D., Brandeis University, Waltham, Massachusetts
Lawther, Robert P., Ph.D., University of California, Irvine
Matteo, Martha R., Ph.D., Union Carbide Corp., Tarrytown, New York
McKenney, Keith H., B.S., National Institutes of Health, Bethesda, Maryland
Monahan, Thomas R., M.S., Schering Corp., Bloomfield, New Jersey
Neufeld, Berney R., Ph.D., Loma Linda University, California
Rice, Douglas A., B.A., University of Chicago, Illinois
Williamson, Patrick L., Ph.D., Amherst College, Massachusetts

SEMINARS

Abelson, J., University of California, San Diego. *T4 tRNAs*.
Beckwith, J., Harvard Medical School. *Genetic engineering in vivo*.
Kaiser, D., Stanford University. *Chemotaxis in myxobacteria*.
Federoff, N., Carnegie Institution of Washington. *Analysis of Xenopus 55S RNA genes in E. coli*.
Plashne, M., Harvard University. *Regulation of λ repressor*.
Low, B., Yale Medical School. *Potpourri of bacterial genetics*.
Parkinson, S., University of Utah. *Genetics of chemotaxis in E. coli*.
Roberts, R., Cold Spring Harbor Laboratory. *DNA sequencing (or Der Meistersänger)*.
Friedman, D., University of Michigan. *Nus and bolts of λ regulation*.
Gilbert, W., Harvard University. *Penninsulin*.
Franklin, N., Stanford University. *Regulation by anti-termination*.

ONCOGENIC VIRUSES, July 4–July 24

INSTRUCTORS

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

ASSISTANT

Camacho, Ann, Ph.D., University of Chicago, Illinois

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

of DNA were given. In addition to these activities, some students worked on the development of other procedures used in molecular virology.

PARTICIPANTS

Bowman, Benjamin F., M.S., Max-Planck-Institut, Munich, Germany
De La Vega, Humberto, B.S., Instituto Politécnico Nacional, Mexico City, Mexico
Dworkin, Mark B., Ph.D., Carnegie Institution of Washington, Baltimore, Maryland
Fiser, Ilja, Ph.D., University of Washington, Seattle
Freese, K.U., M.D., Institute of Virology, Freiburg, Germany
Gourlie, Brian B., Ph.D., Wesleyan University, Middletown, Connecticut
Hamelin, Richard, Ph.D., Hôpital Saint-Louis, Paris, France
Horowitz, Mia, Ph.D., Weizmann Institute of Science, Rehovot, Israel
Montenarh, Mathias, Ph.D., Universität Ulm, Germany
Neumann, Jeffrey R., Ph.D., Massachusetts Institute of Technology, Cambridge
Ohtsubo, Hisako, Ph.D., State University of New York, Stony Brook
Subramani, Suresh, M.S., University of California, Berkeley
Takeito, Makoto, Ph.D., Rockefeller University, New York, New York
Wrede, Paul, Ph.D., Massachusetts Institute of Technology, Cambridge

SEMINARS

Todaro, G., National Institutes of Health. *Study of viruses in vitro cell culture.*
Hynes, R., Massachusetts Institute of Technology. *Alterations of cells during transformation.*
Benjamin, T., Harvard Medical School. *Papovirus genetics and cell transformation.*
Weissman, S., Yale University. *SV40 genes and transcription.*
Nathans, D., Johns Hopkins University. *Mutants of SV40 constructed in vitro.*
Williams, J., Carnegie Mellon University. *Adenovirus genetics.*
Sharp, P., Massachusetts Institute of Technology. *Adenovirus transcription.*
Roberts, B., Brandeis University. *In vitro translation and gene mapping.*
Roizman, B., University of Chicago. *Herpes simplex virus-DNA replication and gene regulation.*
Spear, P., University of Chicago. *Herpes simplex virus-induced alterations of cellular membranes.*
_____. *Ana camacho-herpes simplex virus-transformation.*
Miller, G., Yale University. *Epstein-Barr virus—An overview.*
Berg, P., Stanford University. *SV40 DNA as a cloning vector.*
Shenk, T., University of Connecticut. *Isolation of adenovirus type 5 deletion mutants and analysis of the SV40 origin of DNA replication.*
Baltimore, D., Massachusetts Institute of Technology. *Molecular biology of retrovirus.*
Rowe, W., National Institutes of Health. *Murine leukosis virus.*
Temin, H., University of Wisconsin. *Avian retroviruses and provirus hypothesis.*
Robinson, H. H., Worcester Foundation. *Chicken genes which code for virus.*
Gilbert, W., Harvard University. *Structure of immunoglobulin genes of mouse.*

BASIC NEUROANATOMICAL METHODS, July 4—July 24

INSTRUCTORS

LaVail, Matthew M., Ph.D., University of California, San Francisco
LaVail, Jennifer H., Ph.D., University of California, San Francisco

ASSISTANTS

Ward, Patricia Ann, B.A., University of California, San Francisco
Sugino, Ilene, M.A., University of California, San Francisco

This course is designed for graduate students and research workers interested in learning basic neuroanatomical techniques. The course is primarily a series of laboratory exercises by the participants, with lectures and demonstrations by the instructors and visiting faculty.

The classical neuroanatomical methods that were covered in this laboratory course include: perfusion fixation; embedding in various media; cell staining methods that highlight neuronal and glial cell nucleic acids; silver salt impregnation methods in the intact nervous system and in selectively interrupted nerve fiber bundles; brightfield, darkfield, and fluorescence light microscopy; electron microscopy; and data gathering with camera lucida and photomicrography.

Among new methods covered were the following: various histochemical, radiochemical, and immunocytochemical techniques that demonstrate the structure and location of specific neurons or synaptic terminals based on their characteristic neurotransmitters or state of electrical activity; tracing axonal

pathways using the anterograde axoplasmic transport of radioactively labeled protein; determining the sources of neuronal pathways using the retrograde axonal transport of suitable markers; and computer-assisted techniques for the quantitative analysis of cellular features of the nervous system.

PARTICIPANTS

Fahle, W. Manfred, B.S., Max-Planck-Institut, Tübingen, Germany
Herschel, Michael B., M.D., Mailman Research Center, McLean Hospital, Belmont, Massachusetts
Innis, Robert B., B.S., Johns Hopkins School of Medicine, Baltimore, Maryland
Kelly, Amy S., Ph.D., Stanford Medical School, California
Marchand, Claudine, Ph.D., State University of New York, Stony Brook
Pinto, Lawrence, H., Ph.D., Purdue University, Lafayette, Indiana
Schein, Stanley Jay, Ph.D., Massachusetts Institute of Technology, Cambridge
Wu, Chun-Fang, Ph.D., California Institute of Technology, Pasadena

SEMINARS

Ralston, H.J., III, University of California, San Francisco. *Axonal degeneration methods.*
Moore, R.Y., University of California, San Diego. *Fluorescence techniques and aminergic systems.*
Hendrickson, A., University of Washington. *Light and electron microscope autoradiographic methods.*
Pfenninger, K., Columbia University College of Physicians and Surgeons. *Intramembranous structure of neural cells—Freeze fracture methods.*
Pickel, V., Cornell University Medical College. *Immunocytochemical methods.*
Hubel, D., Harvard Medical School. *The 2-deoxyglucose method and transneuronal autoradiography.*
Woolsey, T.A., Washington University School of Medicine. *Computer-assisted methods.*

NEURAL DEVELOPMENT, July 4—July 19

INSTRUCTORS

Purves, Dale, M.D., Washington University Medical School, St. Louis, Missouri
Patterson, Paul H., Ph.D., Harvard Medical School, Boston, Massachusetts

The aim of this intensive two-week course was to expose a group of approximately twenty students to the classical literature of neural development, with special emphasis on recent advances and controversies in this rapidly growing field. The selection of students was based primarily on recommendations and potential rather than background; thus this year's group of 21 students was composed of five postdoctoral fellows and sixteen graduate students, from the United States and Europe, involved in widely differing research programs. The common denominator was an interest in pursuing research related to neural development.

The format of the course was to have invited lecturers present a detailed review of particular fields in a three-hour morning session. The speaker then chose several important papers, generally ones not discussed in the morning, for the students to read during the course of the afternoon. In the late afternoon the speaker (and instructors) met with groups of six to seven students to discuss the reading and prepare one of the students in each group to present the papers at an evening meeting of the whole class. The evening then consisted of several students' presentations of aspects of the field not covered by the invited lecturer, as well as a general discussion of the issues raised during the day. Most evenings ended with one or two student research seminars in which an individual's work was described for discussion, with criticism by fellow students and instructors. In general, we attempted to have such presentations on an evening when the guest lecturer had a special interest in the student's field. An occasional variant of this format was to end the evening with a research seminar by an additional invited speaker whose material was deemed of interest, but not broad enough to devote an entire day to.

PARTICIPANTS

Anderson, Hilary, J., Ph.D., University of Oxford, England
Betz, Heinrich, M.D., Institut Pasteur, Paris, France
Caldwell, John H., Ph.D., University of Colorado, Denver
Feldman, Daniel H., B.A., University of California, San Francisco
Green, Steven H., B.S., California Institute of Technology, Pasadena
Hayes, Nancy L., B.A., University of North Carolina, Chapel Hill
Henke, Sigrid, M.D., Max-Planck-Institut, Tübingen, Germany
Holland, Robert L., B.A., University of Oxford, England
Hume, Richard I., Stanford University, California
Kato, Ann C., Ph.D., University of Geneva, Switzerland
Katz, David M., B.A., State University of New York, Stony Brook

Leber, Steven M., B.S., Albert Einstein College of Medicine, Bronx, New York
Lee, Matt T., B.S., University of California, San Diego
Lichtman, Jeff W., B.A., Washington University, St. Louis, Missouri
Lidov, Hart G.W., B.A., Johns Hopkins University, Baltimore, Maryland
Meiri, Hamutal, B.S., Hebrew University, Jerusalem, Israel
Pilgrim, Alison, J., B.A., University of Oxford, England
Shotwell, Sandra L., B.A., California Institute of Technology, Pasadena
Strong, Judith A., B.A., Yale Medical School, New Haven, Connecticut
Wallenfels, Barbara, Ph.D., Max-Planck-Institut, Tübingen, Germany
Wendon, Linda M.B., B.A., University College London, England

SEMINARS

Cowan, M.W., Washington University School of Medicine, St. Louis. *Overview of neural development.*
———. *Primary induction and cell proliferation.*
———. *Migration and selective aggregation.*
———. *Cytodifferentiation and cell death.*
LeDouarin, N., Centre National de la Recherche Scientifique. *Migration and differentiation of neural crest derivatives.*
Patterson, P.H., Harvard Medical School. *Control of transmitter biosynthesis in the autonomic nervous system.*
———. *Nerve growth factor.*
———. *Cell surface interactions as a basis for neuronal recognition.*
Purves, D., Washington University School of Medicine, St. Louis. *Nerve growth factor: Biological effects.*
———. *The elimination of synapses in postnatal development.*
———. *Sprouting and retrograde axotomy effects: Maintenance of synaptic connections in maturity.*
———. *The formation of specific connections in the peripheral autonomic nervous system.*
Nottenbohm, F., Rockefeller University. *Hormonal influences in neural development.*
Spitzer, N.W., University of California, San Diego. *Development of electrical-chemical excitability in nerve and muscle.*
Fambrough, D., Carnegie Institution. *The role and regulation of postsynaptic receptors in synaptogenesis.*
Dennis, M.J., University of California, San Francisco. *The electrophysiology of synaptic development.*
———. *The elimination of inappropriate synapses: Possible role in specific connectivity.*
Landmesser, L., Yale University. *The role of axon guidance in the formation of specific neural connection.*
Bentley, D., University of California, Berkeley. *Neural development in invertebrates.*
Macagno, E., Columbia University. *A mechanism for the formation of synaptic projections in the arthropod visual system.*
Hunt, R.K., Johns Hopkins Medical School. *The formation of specific retino-tectal connections.*
Black, I., Cornell Medical School. *The development of sympathetic and parasympathetic ganglia.*
Hubel, D.H., Harvard Medical School. *Effects of deprivation on the development of the visual system of cats and monkeys.*
Rakic, P., Yale University Medical School. *Genetic and epigenetic determinants of brain development.*

THE MOLECULAR BIOLOGY AND GENETICS OF YEAST, July 31–August 20

INSTRUCTORS

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

ASSISTANTS

Baim, Steve, B.S., University of Rochester, New York

This program emphasized the major laboratory techniques used in the genetic analysis of yeast: tetrad analysis, mitotic recombination, and fine-structure mapping. The isolation and characterization of both chromosomal and cytoplasmic mutants were undertaken. Biochemical studies were performed with chromosomal and mitochondrial mutants. Analysis of eukaryotic gene structure used digestion with restriction endonucleases and gel electrophoresis. Several aspects of the yeast transformation system were also investigated.

PARTICIPANTS

Barney, Michael C., M.S., Medical College of Wisconsin, Milwaukee
Bastia, Deepak, Ph.D., University of Alabama, Birmingham
Brake, Anthony J., M.A., University of California, Los Angeles
Carlson, Marian B., Ph.D., Stanford Medical Center, California
Colby, Diane S., Ph.D., University of California, San Francisco
Duncan, Craig H., Ph.D., Yale University, New Haven, Connecticut
Gallwitz, Dieter F.G., M.D., Universität Marburg, Germany
Guthrie, Christine, Ph.D., University of California, San Francisco

Hammond, Charlotte I., B.S., University of Connecticut, Farmington
Jaehning, Judith A., Ph.D., University of California, Berkeley
Kjellin-Straby, Kerstin, Ph.D., University of Umea, Sweden
Mery-Drugeon, Elena, Ph.D., Institut Pasteur, Paris, France
Savage, Margaret A., B.S., Cornell University Medical College, New York, New York
Scott, John F., B.S., Stanford University, California
Truffaut, Nicole, Ph.D., Institut Pasteur, Paris, France
Wernau, William C., Ph.D., Pfizer, Inc., Groton, Connecticut

SEMINARS

Byers, B., University of Washington, Seattle. *Cytology of the yeast life cycle.*
Petes, T.D., University of Chicago. *Structure and replication of yeast DNA.*
———. *Genetic analysis of replicating ribosomal DNA genes.*
Hicks, J., Cold Spring Harbor Laboratory. *Regulation of mating types in yeast.*
Warner, J., Albert Einstein College of Medicine. *Regulation of ribosomal proteins and RNA.*
McLaughlin, C.S., University of California, Irvine. *Protein and RNA synthesis in yeast.*
———. *Macromolecular synthesis through the cell cycle.*
Tzagoloff, A., Columbia University Medical School. *The mitochondrial genome.*
Mortimer, R.K., University of California, Berkeley. *Genetic mapping in yeast.*
———. *Gene conversion and post meiotic segregation.*
Lawrence, C., University of Rochester. *Radiation mutagenesis and repair in yeast.*
Esposito, M.S., University of Chicago. *Genetic control of recombination and sporulation.*
Fink, G.R., Cornell University. *Transformation of yeast with hybrid DNA.*
———. *Regulation of HIS4 in yeast.*
Sherman, F., University of Rochester. *Regulation of the iso-cytochromes c.*
———. *Nonsense suppression in yeast.*
Abelsen, J., University of California, San Diego. *Transcription and processing of intervening sequences in yeast tRNA genes.*
Guthrie, C., University of California, San Francisco. *Genetic and biochemical analysis of yeast suppressor tRNAs.*

ELECTROPHYSIOLOGICAL METHODS FOR CELLULAR NEUROBIOLOGY, August 1—August 21

INSTRUCTORS

Kehoe, JacSue, Ph.D., Ecole Normale Supérieure, Paris, France
Chiarandini, Dante, M.D., New York University, New York
Stefani, Enrique, M.D., Instituto Politécnico Nacional, Mexico City, Mexico

GUEST LECTURER

Kado, Ray, Ph.D., Centre National de la Recherche Scientifique, Gif-sur-Yvette, France

In this neurobiology course the neuromuscular junction of the frog and the central ganglia of the mollusc *Aplysia* were used as experimental preparations for training students in basic electrophysiological methods for cellular neurobiology. Examination of certain characteristics of the resting potential, action potential, and synaptic potentials of these two preparations served as a framework for introducing the following techniques: microdissection, fabrication of single and multibarrelled capillary microelectrodes, intracellular and extracellular application of ions and drugs (ionophoresis and pressure injection), and intracellular staining for light and electron microscopy.

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

PARTICIPANTS

Chillemi, Santi, Ph.D., Laboratorio di Cibernetica, Naples, Italy
Davis, Norman T., Ph.D., University of Connecticut, Storrs
Drager, Ursula C., M.D., Harvard Medical School, Boston, Massachusetts
Kass, Leonard, M.S., Eye Research Institute, Boston, Massachusetts
Nathanson, Neil M., Ph.D., University of California, San Francisco
Nanjundiah, Vidyanand, Ph.D., Indian Institute of Science, Bangalore
Nowycky, Martha C., Ph.D., Yale University, New Haven, Connecticut
Skinner, Kathleen J., B.A., University of California, Davis
Stein, Cathy, B.A., University of Maryland, Baltimore
Walz, Wolfgang W., M.S., Universität Konstanz, Germany

SEMINARS

- Rang, H.P., St. George's Hospital, London. *Basic electronics for neurobiologists. I.*
_____. *Basic electronics for neurobiologists. II.*
_____. *Basic electronics for neurobiologists. III.*

IMMUNOGENETICS AND TUMOR IMMUNOLOGY, August 1 – August 14

INSTRUCTORS

- Cantor, Harvey, M.D., Harvard Medical School, Boston, Massachusetts
Paul, William E., M.D., National Institutes of Health, Bethesda, Maryland

ASSISTANTS

- Bojanowski, Jo, B.S., Harvard University, Cambridge, Massachusetts

The course was intended for graduate students and research workers who are interested in studying the lymphocyte population of the mouse as material for analysis of the molecular biology of cell differentiation in higher organisms. Some of the general areas that were considered included (1) studies of lymphocyte development and the molecular basis of lymphocyte differentiation, (2) genetic and biochemical analysis of recognition structures expressed by different sets of lymphocytes, (3) the special requirements for activation of lymphocytes by antigen, (4) the role of interactions between different sets of lymphocytes in regulating the immune response to viruses and other antigens, (5) current information bearing on the molecular basis of viral leukemogenesis, and (6) the relationship of the immune system to leukemia resistance.

PARTICIPANTS

- Bear, Susan E., B.A., Tufts University, Medford, Massachusetts
Bleicher, Paul A., B.S., University of Rochester, New York
Bondada, Subbarao, Ph.D., National Institutes of Health, Bethesda, Maryland
Brodeur, Peter H., B.S., Tufts University, Medford, Massachusetts
Collins, Elizabeth T., B.S., University of Virginia, Charlottesville
Conlon, Paul J., M.S., University of Colorado, Denver
Conzelmann, Andreas, M.D., Swiss Institute for Cancer Research, Lausanne
Corley, Ronald B., Ph.D., Duke University, Durham, North Carolina
Fey, George H., Ph.D., Swiss Institute for Cancer Research, Lausanne
Fink, Pamela, B.S., Massachusetts Institute of Technology, Cambridge
Freeman, Gordon J., B.A., Harvard Medical School, Boston, Massachusetts
Geiger, Benjamin, Ph.D., University of California, San Diego
Gerassi, Esther E., Weizmann Institute, Rehovot, Israel
Greenstein, Julia, B.A., University of Rochester, New York
Guimezanes, Annick, Ph.D., INSERM, Paris, France
Harris, Nancy, Yale University, New Haven, Connecticut
Kraig, Ellen B., B.S., Brandeis University, Waltham, Massachusetts
Longacre, Shirley S., Ph.D., University of Geneva, Switzerland
Mely, Bernard, Ph.D., University of Paris, France
Marion, Tony, M.S., University of Alabama, Birmingham
Miozzari, Giuseppe F., Ph.D., Basel Institute for Immunology, Switzerland
Nakajima, Pamela, Tufts University, Medford, Massachusetts
Pretell, Judith O., Ph.D., Yale University, New Haven, Connecticut
Siekavitz, Miriam L., B.A., Massachusetts Institute of Technology, Cambridge
Singer, Dinah S., Ph.D., National Institutes of Health, Bethesda, Maryland

SEMINARS

- Boyse, E., Sloan-Kettering Cancer Center. *General introduction to immunogenetics.*
Weissman, I., Stanford University Medical School. *Development of T lymphocytes.*
Cantor, H., Harvard Medical School. *Genetic markers and functions of T lymphocytes.*
Gershon, R., Yale University Medical School. *Suppressor function of T lymphocytes.*
Shreffler, D., Washington University, St. Louis. *Genetics and organization of the MHC. I.*
_____. *Genetics and organization of the MHC. II.*
Paul, W., National Institutes of Health. *Specific Ir genes.*
Shevach, E., National Institutes of Health. *Genetic regulation of macrophage/T cell interaction.*
Marrack, P., University of Rochester. *Genetic regulation of T cell/IIb cell interaction.*
Hood, L., California Institute of Technology. *Chemistry of MHC gene products.*
Kapp, J., Washington University, St. Louis. *Genetic aspects of specific helper and suppressor factors.*
Zinkernagel, R., Scripps Clinic and Research Foundation. *Thymus processing of T lymphocytes.*
_____. *Virus/T cell interaction.*

- Janeway, C., Yale University Medical School. *Genetic aspects of alloreactivity.*
- Warner, N., University of New Mexico Medical School. *Genetic aspects of B cell development.*
- Mage, R., National Institutes of Health. *Ig classes, allotypes and idiotypes.*
- Eichmann, K., Heidelberg Institute of Immunology. *Idiotypes as markers for T and B cell receptors.*
- Leder, P., National Institutes of Health. *Molecular genetics of Ig.*
- _____ *Generation of diversity.*
- Geftter, M., Massachusetts Institute of Technology. *Genetics of Ig synthesis; hybridomas.*
- Lerner, R., Scripps Clinic and Research Foundation. *Genetics of immunopathologic states.*
- Rowe, W., National Institutes of Health. *Genetics of viral oncogenesis.*

BIOLOGICAL INFORMATION PROCESSING WORKSHOP, August 1—August 14

ORGANIZERS

- Marr, David, Ph.D., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge
- Zipser, David, Ph.D., Cold Spring Harbor Laboratory, New York

During the summer of 1978, the Sloan Foundation's program in cognitive sciences funded a Workshop here at Cold Spring Harbor on the topic of Biological Information Processing (BIP). BIP is concerned with analyzing the abilities of the nervous system in terms of information processing concepts. In order to do this the phenomena of interest must be characterized by biologically appropriate algorithms. "Algorithm" is used in a quite general sense of any computational procedure sufficiently precise to allow computer simulation. "Biologically appropriate" means that the algorithm has a plausible neurophysiological implementation. BIP is concerned with the relevant phenomena at several levels. Furthest from physiology is a characterization of the essential computational nature of a cognitive phenomena. Once this is available, attention can be focused on discovering the appropriate algorithms.

Because brains are so unlike computers, biologically appropriate algorithms are generally different from those developed for machines. Because of the physical complexity of brains, and their origin from random evolutionary processes, it is not usually possible to make explicit statements about neurophysiology or neuroanatomy on the basis of this kind of analysis alone. Rather, BIP can supply the neurosciences with a series of plausible algorithms, whose neuronal implementation would suffice to explain the observed behavioral phenomena. It then becomes an experimental and theoretical question for neurobiology to decide whether, in fact, the nervous system implements the algorithms proposed and if so, how? The important point here is that algorithms for complex, cognitive behavior are in general not directly discernible from physiological experiment alone; their discovery involves considerable analysis of the nature of the cognitive processes themselves.

The Workshop brought together about thirty people, from widely differing disciplines, chosen because their work is related to the biological information processing approach. Talks ranged in subject matter over a wide area. Although we had a talk on computer speech understanding and several on theories about how the brain as a whole might function, the main thrust of the Workshop was in the area of early cognition. This concept refers roughly to what is going on in that part of the nervous system between the transduction of external signals to internal form and their first reportable appearance as recognizable phenomena. The perception of shape, motion, depth, and textures are all good examples of the topics discussed in the realm of early cognition.

One of the purposes of the Workshop was to bring into contact a diverse group of people so that they could become familiar with each other and discuss their work. In general, the Workshop was characterized by a productive interchange of ideas among people whose fields are often disjointed but which have common themes. While this was exhilarating for all who attended, a situation was created in which many interesting issues could not be discussed fully or examined in detail. The Workshop was limited in its ability to develop any form of synthesis. Its main strength was in its educational value. In the future we plan to have workshops which are somewhat smaller and focus on more limited topics with the hope of encouraging more creative output.

PRESENTATIONS

- White, J.G., Medical Research Council, Cambridge, England: *Structural and functional aspects of the nematode nervous system.*
- Knight, T., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *Hardware.*

- Stent, G.S., University of California, Berkeley: *Neural circuits for rhythm generation.*
- Wehrhahn, C., Max-Planck-Institut für Biologische Kybernetik, Tübingen, Germany: *Signal processing in the fly's eye.*
- Von der Malsburg, D.C., Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany: *Ontogenesis of retinotopic mappings as a paradigm case of organization in the brain.*
- Zipser, D., Cold Spring Harbor Laboratory, New York: *Biological constraints.*
- Selfridge, O., Bolt Beranck & Newman, Inc., Cambridge, Massachusetts: *Problems in tracking and trailing.*
- Hoffmann, S., School of Medicine, State University of New York at Buffalo, Amherst, New York: *Computer simulation of specific, natural, neural networks.*
- Brady, M., University of Essex, Colchester, England: I. My experiences in building a program to read fortran coding sheets. II. Local Computation of structural descriptions.
- Selfridge, P., University of Rochester, New York: *Ongoing research in computer vision at the University of Rochester.*
- Kender, J., Carnegie-Mellon University, Pittsburg, Pennsylvania: *Texture gradients and inverse perspective.*
- Feldman, J., University of Rochester, New York: *Towards symbolic models of neural nets.*
- Julesz, B., Bell Telephone Laboratories, Inc., Murray Hill, New Jersey: *From a conjecture to a theory of texture perception.*
- Ullman, S., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *Interpretation of visual motion.*
- Stevens, K., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *Analysis and representation of visual surface motion.*
- Nishihara, H.K., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *Representation of the spatial organization of three-dimensional shapes for visual recognition.*
- Newell, A., Carnegie-Mellon University, Pittsburgh, Pennsylvania: *Production system architectures for human cognition.*
- Minsky, M., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *A brain theory.*
- Wilson, H., University of Chicago, Illinois: *Spatiotemporal information processing in the human visual system.*
- Braddick, O., Experimental Psychology Laboratory, Cambridge, England: *Low-level constraints on visual form and motion perception.*
- Marr, D., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *Human stereo vision.*
- Barlow, H.B., Physiological Laboratory, Cambridge, England: *Psychophysics of visual judgements on noisy figures.*
- Richards, W., Massachusetts Institute of Technology, Cambridge: *Why rods and cones?*
- Riesbeck, C.K., Yale University, New Haven, Connecticut: *Cognitive science in the Yale AI project: Theory and practice.*
- Kanade, T., Carnegie-Mellon University, Pittsburgh, Pennsylvania: *Theory of origami world and its application.*
- Marr, D., Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: *Closing remarks.*

THE CENTRAL NERVOUS SYSTEM WORKSHOP, August 1—August 31

ORGANIZER

Shatz, Carla J., Ph.D., Stanford University School of Medicine, California

This workshop brought together eight scientists with a strong interest in developmental neurobiology. Questions raised concerned the extent to which genetic programs, tissue interactions, and environmental influences control various aspects of development in animals.

Experiments were carried out in the two classical systems used for studying developmental questions, the frog spinal cord and the vertebrate visual system. M. Hollyday and V. Stirling examined the development of connections made in the spinal cord by afferent projections of dorsal root ganglion cells during metamorphosis. They discovered characteristic differences in the projection of lumbar and thoracic sensory fibers. M. Constantine-Paton and M. Law studied to what extent connectivities in the visual system of frogs are changed through embryonic microsurgery when either a third eye is implanted or the frogs are blinded. B. Harris and C. Shatz used the workshop to develop the visual system of the axolotl as a neurobiological preparation. The projection to visual nuclei was compared in normal and eyeless mutants. Interestingly in the eyeless mutant, the optic tectum was found to respond strongly to tactile stimulation. In other projects, D. Katz studied the sensory projections of cardiovascular afferents in the pigeon and E. Frank and M. Kennedy explored new anatomical methods to trace functional pathways.

The workshop led to a great deal of collaborative investigation. The participants acquainted each other with their different techniques. For example, the efficiency of horseradish peroxidase (HRP) and cobalt chloride nerve filling was compared and several variants of HRP histochemistry were explored. In addition, some workshop participants taught others electrophysiological techniques.

PARTICIPANTS

Development of sensory afferent projections in the frog spinal cord.

Hollyday, Margaret, Ph.D., University of Chicago, Illinois

Stirling, Victoria, Ph.D., National Institute for Medical Research, London, England

Anatomical methods for tracing functional pathways in the brain.

Frank, Eric, Ph.D., Harvard Medical School, Boston, Massachusetts

Kennedy, Mary, Ph.D., Yale University School of Medicine, New Haven, Connecticut

The effect of altered retinal input to the optic tectum of frogs.

Constantine-Paton, Martha, Ph.D., Princeton University, New Jersey

Law, Margaret, B.S., Princeton University, New Jersey

Anatomical and Physiological Studies of cardiovascular afferents in the pigeon.

Katz, David, B.S., State University of New York, Stony Brook

Investigations of the midbrain (tectum) in normal and eyeless mutant axolotls.

Harris, William A., Ph.D., Harvard Medical School, Boston, Massachusetts

Shatz, Carla J., Ph.D., Stanford University School of Medicine, California



COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

DNA: REPLICATION AND RECOMBINATION



Twenty-five years have now passed since I flew back from England to the Symposium on Viruses to talk about the double helix that Francis Crick and I had just found in Cambridge. We were very elated since almost certainly we knew in principle how DNA, that is, the gene, functioned as a template for its self-replication. If so, the long-elusive goal of the theoretical geneticist had been achieved, and with a marvelous simplicity that did not require years of training in high-powered chemistry or physics to understand. Yet on no occasion during those early June days do I remember any serious discussion of where enzymes would come in. But this was not surprising since the world of genetics and first-class enzymology were then more apart than connected and the thought that anyone might soon make functional DNA in a test tube was too far-out for serious minds.

We could not have been more wrong. Already by June 1956 Arthur Kornberg was reporting incorporation of radioactive thymidine into DNA and within several more years the first DNA polymerase had been characterized. The working out, however, of all the steps in DNA replication has proved far more complex than first imagined. Throughout this never-very-easy endeavor geneticists have learned that they could no longer think deeply about the gene without knowing more about enzymes than they ever wanted. Correspondingly, those biochemists who have wished for success with DNA needed to be conversant with the world of phages or risk producing data that would have no lasting significance. The net result, happily, has been a marvelous outpouring of new ways to look at the classical problems of gene replication and recombination. By now virtually no one has doubts that definitive statements on the enzymological level will soon be in hand.

This year thus seemed propitious for intellectual as well as sentimental reasons to hold our Symposium on DNA replication and recombination. Arranging it proved a major task and Tom Broker and Ahmad Bukhari did their job very well. Credit should also go to Arthur Kornberg, Bob Lehman, Charles Richardson, John Cairns, David Dressler, Bruce Alberts, Jun-Ichi Tomizawa, Frank Stahl, and Charles Radding for their thoughts as to possible speakers. They suggested more people than even a much overcrowded week could handle. So we knew we would make errors of omission that hopefully might be partially rectified by impromptu talks during the

various sessions. The resulting almost maddeningly full Symposium week was of such high intellectual quality that virtually everyone listened to most of the record number of 155 presentations that were given. The total attendance was 428, also a record.

A meeting of this size is possible only with much outside financial support and we wish to acknowledge the help of the National Institutes of Health, the National Science Foundation, and the United States Energy Research and Development Administration, which provided the key funds needed to bring visitors from abroad and to cover our speakers' expenses while at Cold Spring Harbor.

J.D. Watson

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

Opening Comments: A. KORNBERG, Stanford University, California

Session 1: General Recombination I—Models from Genetics

Chairperson: M. MESELSON, Harvard University, Cambridge, Massachusetts

- R. HOLLIDAY, Medical Research Council, London, England: Relationship between the correction of mismatched bases, gene conversion, and mutability.
- S. FOGEL,* R. MORTIMER,† K. LUSNAK,* and F. TAVARES,† *Department of Genetics and †Division of Medical Physics, University of California, Berkeley: Meiotic gene conversion—A signal of the basic recombination event in yeast.
- J.-L. ROSSIGNOL, N. PAQUETTE, and A. NICOLAS, Laboratoire de Génétique, Université Paris-Sud, Orsay, France: Aberrant 4:4, disparity in the direction of conversion and frequencies of conversion in *Ascomobolus immersus*.
- A. HINNEN,* J. B. HICKS,† and G. R. FINK,* *Department of Botany, Genetics, and Development, Cornell University, Ithaca, New York; †Cold Spring Harbor Laboratory, New York: Genetic transformation in yeast.
- R. P. ANDERSON and J. R. ROTH, Department of Biology, University of Utah, Salt Lake City: Legitimate and illegitimate recombination between sister chromosomes in *Salmonella typhimurium*.

Session 2: Unwinding

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

- H. M. SOBELL, Departments of Chemistry and Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, New York: How DNA unwinds.
- A. MCPHERSON,* F. JURNAK,* A. WANG,† F. KOLPAK,† A. RICH,† and I. MOLINEUX,† *Department of Biological Chemistry, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey; †Department of Biology, Massachusetts Institute of Technology, Cambridge; ‡Imperial Cancer Research Fund, Mill Hill Laboratories, London, England: The gene-V product of fd bacteriophage—Structure of a DNA-unwinding protein and its complexes with DNA.
- J. C. WANG, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Superhelical DNA, gyrase, and topoisomerase.
- M. GELLERT, K. MIZUUCHI, M. H. O'DEA, H. OHMORI, and J. TOMIZAWA, NIAMD, National Institutes of Health, Bethesda, Maryland: Studies on the mechanism of DNA gyrase.
- N. R. COZZARELLI,* P. O. BROWN,* N. P. HIGGINS,* K. N. KREUZER,* A. MORRISON,* C. L. PEEBLES,* and A. SUGINO,*† *University of Chicago, Illinois; †National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Structure, activities, and function of *Escherichia coli* DNA gyrase.
- M. ABDEL-MONEM, B. KUHN, and H. HOFFMANN-BERLING, Abteilung für Molekulare Biologie, Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany: DNA helicases.
- J. J. CHAMPOUX, L. S. YOUNG, and M. D. BEEN, Department of Microbiology and Immunology, School of Medicine, University of Washington, Seattle: Studies on the specificity and regulation of the DNA-unwinding enzyme.
- M. DUGUET, G. YARRANTON, and M. GEFTER, Department of Biology, Massachusetts Institute of Technology, Cambridge: Studies on the *rep* protein of *Escherichia coli*.

Session 3: Specialized Recombination I—The λ and Mu Paradigms

Chairperson: A. CAMPBELL, Stanford University, California

- A. LANDY, R. HOESS, K. BIDWELL, and W. ROSS, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Site-specific recombination in bacteriophage λ —Structural features of recombining sites.
- Y. KIKUCHI and H. A. NASHI, NIMH, National Institutes of Health, Bethesda, Maryland: Characterization of the proteins and DNA required for integrative recombination of phage λ .
- L. ENQUIST, A. KIKUCHI, and R. WEISBERG, NICHD, National Institutes of Health, Bethesda, Maryland: Differences between prophage insertion and excision.
- L. HEFFERNAN, M. BENEDIK, and A. CAMPBELL, Department of Biological Sciences, Stanford University, California: Regulation of genes encoding insertion and excision functions of bacteriophage λ .
- J. A. SHAPIRO and L. A. MACHATIE, Department of Microbiology, University of Chicago, Illinois: Integration and excision events involving bacteriophage λ and Tn9.
- A. I. BUKIARI,* M. S. DUBOW,* H. KHATOON,* L. AMBROSIO,* F. DEBRUIJN,* and †E. LJUNGCQUIST,* Cold Spring Harbor Laboratory, New York; *Karolinska Institute, Stockholm, Sweden: Integration of bacteriophage Mu DNA.
- D. KAMP, L. T. CHOW, T. R. BROKER, D. KWON, D. ZIPSER, and R. KAHMANN, Cold Spring Harbor Laboratory, New York: Site-specific recombination in phage Mu.
- M. FALEN,* A. RESBOIS,† and A. TOUSSAINT,* *Département de Biologie Moléculaire, Faculté des Sciences; †Laboratoire de Microscopie Electronique, Faculté de Médecine, Université Libre de Bruxelles, Belgium: Mini-Mu—Mu mutants with large internal deletions.
- P. VAN DE PUTTE and M. GIPHART-GASSLER, Department of Biochemistry, State University of Leiden, The Netherlands: Characteristics of a plasmid containing the early region of bacteriophage Mu.

Session 4: Specialized Recombination II—IS Elements and Transposons

Chairperson: W. SZYBALSKI, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison

- H. A. CHADWELL, H.-J. FRITZ, P. HABERMANN, R. KLAER, S. KÜHN, and P. STARLINGER, Institut für Genetik der Universität zu Köln, Germany: Studies with IS DNA.
- W. ARBER, Department of Microbiology, Biozentrum, University of Basel, Switzerland: The bacteriophage P1 plasmid can serve as a trap for transposing host insertion elements.
- H. SAEDLER, D. GHOSAL, and J. GROSS, Institut für Biologie III, Universität Freiburg, Germany: Multiplication and rearrangements of DNA sequences from both DNA strands of IS2 generate a turn-on signal for gene expression.
- G. WEINSTOCK and D. BOTSTEIN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Genetic and physical studies of Tn1 (*amp^R*) using bacteriophage P22.
- P. K. TOMICH, F. AN, and D. CREWELL, Department of Oral Biology, University of Michigan, Ann Arbor: Evidence for an erythromycin-resistance transposon (Tn917) in *Streptococcus faecalis*—Enhancement of transposition during induction of erythromycin resistance.
- M. CHANDLER, L. SILVER, D. LANE, and L. CARO, Department of Molecular Biology, University of Geneva, Switzerland: Properties of an autonomous r determinant from R100.1.
- D. ROSS,* J. SWAN,† D. STELE,† and N. KLECKNER,† *Department of Biology, Massachusetts Institute of Technology, Cambridge; †Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: *recA*-Independent recombination events promoted by Tn10.
- S. N. COHEN, J. CHOU, and M. CASADABAN, Department of Genetics and Medicine, Stanford University School of Medicine, California: Studies for the mechanism and specificity of Tn elements in bacteria.
- M. P. CALOS,* L. JOHNSRUD,* and J. H. MILLER,† *The Biological Laboratories, Harvard University, Cambridge, Massachusetts; †Department of Molecular Biology, University of Geneva, Switzerland: DNA sequences at insertion sites of IS1 and Tn9 in *Escherichia coli*.
- N. D. F. GRINDLEY, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Sequence analysis at IS1 insertion sites—Models for IS1 integration.
- E. OHNISHI, H. OHNISHI, and H. OHMORI, Department of Microbiology, State University of New York, Stony Brook: Nucleotide sequence analysis of a transposable element, Tn3 (TnA), and of an insertion element, IS1.

Session 5: Replication I—Priming and Okazaki Fragments

Chairperson: J. CAIRNS, Imperial Cancer Research Fund, London, England

- T. OKAZAKI, Y. KUROSAWA, T. OGAWA, T. SEKI, K. SHINOZAKI, S. HIROSE, A. FUJIYAMA, Y. KOHARA, Y. MACHIDA, F. TAMANOI, and T. HOZUMI, Institute of Molecular Biology, Faculty of Science, Nagoya University, Japan: Structure and metabolism of RNA primer in discontinuous replication of prokaryotic DNA.
- K. R. THOMAS, P. M. RAMOS, and B. M. OLIVERA, Department of Biology, University of Utah, Salt Lake City: Studies on the origin and structure of Okazaki pieces.
- R. WERNER, D. W. SIEGMANN, A. T. DIAZ, and H. JERING, Department of Biochemistry, School of Medicine, University of Miami, Florida: Characterization of 5' ends of nascent DNA chains.
- I. R. LEIMAN and B.-K. TYE, Department of Biochemistry, Stanford University, California: Excision-repair of uracil in DNA—Its role in the generation of Okazaki fragments.
- D. T. DENHARDT, C. HOURS, J. KOWALSKI, M. MATTHES, C. MIYAMOTO, and N. MIYAMOTO, Department of Biochemistry, McGill University, Montreal, Quebec, Canada: Some investigations into the nature of the 5' ends of cellular DNA molecules.
- M. GOULIAN, B. Y. TSENG, R. H. GRAFSTROM, and D. G. REVIE, Department of Medicine, University of California, San Diego: Studies on DNA replication in animal cells.
- B. BOGENHAGEN, A. M. GILLUM, P. A. MARIENS, and D. A. CLAYTON, Department of Pathology, Stanford University School of Medicine, California: Replication of mouse mitochondrial DNA—RNA priming and asynchronous synthesis of strands.
- P. RICHARD, Department of Biochemistry, Medical Nobel Institute, Karolinska Institute, Stockholm, Sweden: Priming of Okazaki pieces during polyoma DNA replication in isolated nuclei.

Session 6: Replication II—Small Phages and Plasmids as Replication Models

Chairperson: D. HELINSKI, University of California, San Diego

- R. R. MEYER, J. SHIMOMI, J. KOBORI, D. L. BATES, L. ROWEN, R. McMACKEN, K. UFIDA, and A. KORNBERG, Department of Biochemistry, Stanford University School of Medicine, California: Enzymatic conversion of single-stranded ϕ X174 and G4 circles to duplex forms—Discontinuous replication.
- S. EISENBERG, J. SCOTT, and A. KORNBERG, Department of Biochemistry, Stanford University School of Medicine, California: Enzymatic replication of ϕ X174 duplex circles (RF)—Continuous replication.
- S. WICKNER, NCI, National Institutes of Health, Bethesda, Maryland: DNA replication proteins of *Escherichia coli*.
- C. YASUMOTO, J. IKEDA, E. BENZ, R. VICUNA, K. MARIANS, and J. HURVITZ, Department of Developmental Biology, Albert Einstein College of Medicine, Bronx, New York: studies on in vitro synthesis of DNA.
- G. N. GODSON, Radiobiology Laboratories, Yale Medical School, New Haven, Connecticut: G4 DNA replication in vivo.
- J. SIMS, K. KOTHIS, and D. DRESSIER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Single-stranded phage DNA replication—Positive- and negative-strand DNA synthesis.
- D. S. RAY, S. V. SUGGS, T.-C. CHEN, J. KAGUNI, L. LAVERNE, and J. M. CIFARY, Molecular Biology Institute and Department of Biology, University of California, Los Angeles: Initiation and termination of rounds of replication in single-stranded phage DNA replication in vivo.
- K. HORIUCHI, J. V. RAVELCH, and N. D. ZINDER, Rockefeller University, New York, New York: DNA replication of bacteriophage ϕ 1 in vivo.
- H. SCHALLER,* R. SOMMER,* E. A. AUERSWALD,* E. BECK,* R. HERRMANN,* K. NEUGFBAUER,* T. MEYER,† and K. GEIDER,† *Mikrobiologie der Universität Heidelberg; †Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany: The origin of DNA replication of bacteriophage ϕ d.
- J. TOMIZAWA, T. ITOH, and H. OHMORI, NIAMD, National Institutes of Health, Bethesda, Maryland: Initiation of replication of plasmid ColE1 DNA.

Session 7: General Recombination II—Enzymological Considerations

Chairperson: C. RADDING, Yale University School of Medicine, New Haven, Connecticut

- G. MOSIG, A. LUDER, G. GARCIA, R. DANNENBERG, and S. BOCK, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: In vivo interactions of genes and proteins in DNA replication and recombination of phage T4.

- K. B. BURCK and R. C. MILLER, JR., Department of Microbiology, University of British Columbia, Vancouver, Canada: Marker rescue and partial replication of UV-irradiated T7⁻ bacteriophage DNA.
- E. WALDSTEIN, J. K. SELIGER, and L. SANIASER, Biology Department, Brookhaven National Laboratory, Upton, New York: A special type of UV-stimulated recombination of *Haemophilus influenzae*.
- R. PORIIR and B. LOW, Yale Medical School, New Haven, Connecticut: Transduction vs. "conjugation"—Evidence for multiple roles for Exo V in recombination in *Escherichia coli*.
- D. CHATTORAI, J. CRASEMANN, N. DOWER, D. FAULDS, P. FAULDS, R. MALONE, F. STAHL, and M. STAHL, Institute of Molecular Biology, University of Oregon, Eugene: Asymmetries in Chi activity.
- P. HOVARI-FLANDERS, E. CASSUTO, and P. ROSS, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Covalent circular *dx* DNA is cut in extracts of induced *Escherichia coli* *endA* (*arcC*⁻) when suitably damaged homologous DNA molecules are present.
- J. ROSANIGON, B. ENDLICH, and S. LINN, Department of Biochemistry, University of California, Berkeley: Translocation by the *LcoB* and *recBC* enzymes of *Escherichia coli*.

Session 8: Replication III—Replication Origins

Chairperson: C. RICHARDSON, Harvard Medical School, Boston, Massachusetts

- K. BACKMAN, M. BEILACH, H. W. BOYER, and S. YANOFSKY, Department of Biochemistry and Biophysics, University of California, San Francisco: Genetic and physical studies on the replication of ColE1.
- J. H. CROSA, L. K. LUTTROPP, and S. FALKOW, Department of Microbiology, University of Washington, Seattle: Molecular cloning of replication and incompatibility regions from the R plasmid R6K.
- D. HELINSKI, D. FIGURSKI, M. INUZUKA, M. KAHN, and R. KOLTER, Department of Biology, University of California, San Diego: Replication properties of low-molecular-weight derivatives of plasmids.
- G. ATTARDI, S. CREWS, J. NISHIGUCHI, D. OJALA, and J. POSAKONY, Division of Biology, California Institute of Technology, Pasadena: Sequence of the region of HeLa-cell mitochondrial DNA surrounding the origin of replication.
- K. KOIKE and M. KOBAYASHI, Cancer Institute (JFCR), Tokyo, Japan: Cloning and characterization of the replication origin from rat mitochondrial DNA.
- M.E. FURTH,* J.L. YATES,† and W.F. DOVE,* *McArdle Laboratory and †Enzyme Institute, University of Wisconsin, Madison: Positive and negative control of bacteriophage λ DNA replication.
- K. DENNISTON-THOMPSON, D. D. MOORE, K. E. KRUGER, B. G. WILLIAMS, M. E. FURTH, and F. R. BLATTNER, Department of Genetics, University of Wisconsin, Madison: Structure and function of the origin of replication of bacteriophage λ.
- G. HOBOM, M. LUSKY, R. GROSSCHEDL, and G. SCHERER, Institut für Biologie III der Universität Freiburg, Germany: Dissection of the origin of replication of bacteriophage λ.
- K. V. MFYENBURG, F. G. HANSEN, and E. RIISE, University Institute of Microbiology, Copenhagen, Denmark: Origin of replication, *oriC*, of the *Escherichia coli* chromosome on specialized transducing phages λ_{sm}.
- Y. HIROTA,* S. YASUDA,* A. NISHIMURA,* Y. TAKEDA,* M. YAMADA,* K. SUGIMOTO,† H. SUGISAKI,† A. OKA,† and M. TAKANAMI,† *National Institute of Genetics, Mishima; †Institute for Chemical Research, Kyoto University, Uji, Japan: Structural and functional properties of *Escherichia coli* DNA replication origin.

Session 9: Replication IV—Eukaryotic Systems I

Chairperson: P. REICHARD, Karolinska Institute, Stockholm, Sweden

- D.R.W. EDWARDS, J.B. TAYLOR, W.F. WAKELING, and I.R. JOHNSTON, Department of Biochemistry, University College London, England: Studies on the prereplicative phase of the cell cycle in *Saccharomyces cerevisiae*.
- L.M.S. CHANG, K. LURIE, and P. PLEVANI, Department of Biochemistry, Uniformed Services University of Health Sciences, Bethesda, Maryland: A stimulatory factor of yeast DNA polymerase.
- R.M. BENBOW, C.B. BREAU, M.R. KRAUSS, E.M. NELSON, N.S. WANG, and S.H. WHITE, Department of Biology, Johns Hopkins University, Baltimore, Maryland: Eukaryotic DNA replication: A multi-enzyme system from *Xenopus laevis* eggs.
- G.P. TOCCHINI-VALENTINI, M.I. BALDI, G. CARRARA, D. GANDINI-ATTARDI, and E. MATTOCCIA, Laboratory of Cell Biology, Consiglio Nazionale delle Ricerche, Rome, Italy: Cell-free systems constructed from *Xenopus laevis* oocytes: Formation of complex DNA and insertion of superhelical turns.

- P.A. FISHER, T.S.-F. WANG, and D. KORN, Department of Pathology, Stanford University, California: Structural and enzymological characterization of human DNA polymerases α and β .
- F. COBIANCIU,* G. MASTROIELI,† S. RIVA,* S. SPADARI,* G. PEDRINI-NOB,* and A. FALASCHI* †Laboratorio di Genetica Biochimica ed Evoluzionistica, C.N.R., Pavia; †Istituto di Genetica, Università di Pavia, Italy: A DNA-dependent ATPase from human cells.
- W.A. HASELTINE, Sidney Farber Cancer Institute; Department of Pathology, Harvard Medical School; Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts: In vitro studies of the mechanism of synthesis of DNA complementary to the genome of RNA tumor viruses.
- D. BALTIMORE, E. GILBOA, and E. ROTHENBERG, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Infective murine leukemia virus DNA made in vitro is mainly linear, entirely double-stranded molecules.
- H.F. VARMUS, P.R. SHANK, S. HUGHES, H.-J. KLING, D.H. SPECTOR, J.C. COHEN, G.M. RINGOLD, S. HLASLY, and J.M. BISHOP, Department of Microbiology, University of California, San Francisco: Synthesis and integration of the proviruses of RNA tumor viruses.
- D. STEFFEN, P. ANDERSON, M. GOLDFARB, and R.A. WEINBERG, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Integration of sarcoma and leukemia virus genomes into the chromosomes of mouse and rat cells.

Session 10: Replication V—Prokaryotic Systems

Chairperson: I.R. LEHMAN, Stanford University, California

- C.C. RICHARDSON, M.J. ENGLER, R. KOLODNER, J.F. LÉCLERC, D. RICHARDSON, I.J. ROMANO, and F. TAMANOL, Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts: Replication of bacteriophage T7 DNA by purified proteins.
- E. LANKA,* E. SCHERZINGER,* G. HILLENBRAND,* and W. STAUDENBAUER,* †Max-Planck-Institut für Molekulare Genetik, Berlin, Germany; †Max-Planck-Institut für Biochemie, Martinsried, Germany: T7 DNA primase: A multifunctional enzyme involved in DNA replication.
- C.C. LIU, R.L. BURKE, U. HIBNER, J. BARRY, and B. ALBERTS, Department of Biochemistry and Biophysics, University of California, San Francisco: Probing DNA replication mechanisms with the T4 bacteriophage in vitro system.
- J. ITO and N.E. HARDING, Department of Cellular Biology, Scripps Clinic and Research Foundation, La Jolla, California: *Bacillus* phage DNA-terminal proteins and their role in DNA replication.
- C. LARK and K.G. LARK, Department of Biology, University of Utah, Salt Lake City: Regulation of the stability of the DNA replication complex.
- H. SCHUSTER, E. LANKA, M. MIKOLAJCZYK, B. GIESCHKE, and M. SCHLICHT, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: Association of the prophage P1_{ban} protein with the DNA B protein of *Escherichia coli*.
- H. YOSHIKAWA,* K. YAMAGUCHI,† H. TOYODA,* M. SEKI,* and N. OGASAWARA,* †Cancer Research Institute, Kanazawa, Japan; †NIAMDD, National Institutes of Health, Bethesda, Maryland: Structure and function of DNA-protein complex containing replication origin markers of *Bacillus subtilis*.
- L.A. LOEB, Department of Pathology, University of Washington School of Medicine, Seattle: On the fidelity of DNA replication.
- M. ENGLER, R.C. PLESS, and M.J. BESSMAN, Department of Biology and McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland: Factors influencing the fidelity of DNA synthesis.

Session 11: Replication VI—Eukaryotic Systems II

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

- R. TJIAN, Cold Spring Harbor Laboratory, New York: Protein-DNA interactions at the origin of simian virus 40 (SV40) replication.
- D. SHORILF and D. NATHANS, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: SV40 mutants with base substitutions around the origin of viral DNA replication.
- Y. TSUBOTA, M.A. WAQAR, J.F. BURKE, B.I. MILAVETZ, M.J. EVANS, D. KOWALSKI, and J.A. HUBERMAN, Roswell Park Memorial Institute, Buffalo, New York: Association of enzymes with replicating and nonreplicating SV40 chromosomes.

- M.L. DIPAMPHILIS,* S. ANDERSON,* H. EDENBERG,* T. HERMAN,* B. KARAS,* G. KAUFMANN,[†] H. KROKAN,* E. SHULTON,* R. SU,* D. TAPPER,* and P. WASSARMAN,* *Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts; [†]Department of Biochemistry, Indiana University Medical School, Indianapolis; [‡]Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel: Replication and structure of SV40 chromosomes.
- M. BOICHAN, W. TOPP, and J. SAMBROOK, Cold Spring Harbor Laboratory, New York: Studies on SV40 excision from chromosomes.
- M.-C. YAO and J.G. GALL, Department of Biology, Yale University, New Haven, Connecticut: Amplification of rDNA in the protozoan, *Tetrahymena*.
- R. T. SCHIMKE, R. S. KAUFMAN, and J. NUNBERG, Department of Biological Sciences, Stanford University School of Medicine, California: Selective amplification of the dihydrofolate reductase genes in methotrexate-resistant cultured cells.
- C. WEISSMAN, H. WEBER, W. MÜLLER, and F. MEYER, Institut für Molekularbiologie I, Universität Zürich, Switzerland: Site-directed mutagenesis in the β -globin DNA insert of plasmid $\beta\beta$ G.

Session 12: Replication VII—Eukaryotic Systems III

Chairperson: D. NATHANS, Johns Hopkins School of Medicine, Baltimore, Maryland

- T.J. KELLY, JR. and R. LECHNER, Johns Hopkins University School of Medicine, Baltimore, Maryland: The structure of replicating adenovirus DNA molecules.
- B.W. STILLMAN and A.J.D. BELLETT, Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra: Replication of DNA in adenovirus type 5 (Ad5)-infected cells.
- M. GREFF, M. ARENS, and T. YAMASHITA, Institute for Molecular Virology, St. Louis University, Missouri: Adenovirus-2 (Ad2) DNA replication in vitro—A model for mammalian cell DNA replication.
- M.S. HORVITZ and L.M. KAPLAN, Albert Einstein College of Medicine, Bronx, New York: Adenovirus DNA synthesis in soluble extracts of cells infected with Ad5 or H5t125- -Characterization of proteins associated with replicating DNA.
- K.I. BERNIS and W.W. HAUSWIRTH, Department of Immunology and Medical Microbiology, University of Florida, Gainesville: Adeno-associated-virus (AAV) DNA replication.
- C. ASHII,* M. SMITH,* M. CHOW,[†] and D. WARD,[†] *Department of Biochemistry, University of British Columbia, Vancouver; [†]Departments of Human Genetics and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Nucleotide sequences near the origins of DNA replication for the parvoviruses minute virus of mice (MVM), H-1, H-3, Kilham rat virus (KRV), and Lull.
- M. ESTEBAN, C. CABRERA, M. SOŁOSKI, and J.A. HOŁOWCZAK, CMDNJ, Rutgers Medical School, Piscataway, New Jersey: Replication of vaccinia DNA.
- B. FRANCKE, Tumor Virology Laboratory, Salk Institute, San Diego, California: Cell-free DNA synthesis systems derived from herpes simplex virus type 1 (HSV1)-infected BHK cells.
- B. ROIZMAN, R.J. JACOB, L.S. MORSE, M.M. KNIPE, and W.T. RUYECHAN, Viral Oncology Laboratories, University of Chicago, Illinois: On the mechanism generating the inversions of the covalently linked L and S components in HSV DNA.
- N.M. WILLKIE, A. DAVISON, P. CHARTRAND, V. PRESTON, and N. STOW, Institute of Virology, Glasgow, Scotland: Recombination in HSV.

Session 13: General Recombination III—Structural Considerations

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

- R.C. WARNER, R.A. FISHEL, and F.C. WHEELER, Department of Molecular Biology and Biochemistry, University of California, Irvine: The figure-eight as an intermediate in recombination in small DNA phages—Characteristics of double-stranded branch migration.
- H. POTTER and D. DRESSLER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Genetic recombination: In vivo and in vitro studies.
- C.M. RADDING, R. CUNNINGHAM, C. DASGUPTA, and T. SHIBATA, School of Medicine, Yale University, New Haven, Connecticut: The role of superhelical DNA in general genetic recombination.
- M. FOX, C. DUDNEY, and E. SODERGREN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Heteroduplex regions in unduplicated bacteriophage λ recombinants.

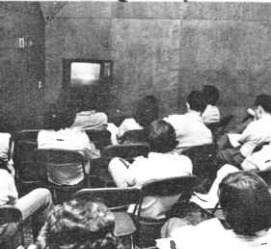
- H. IKEDA and I. KOBAYASHI, Institute of Medical Science, University of Tokyo, Japan: *recA*-Mediated recombination of phage λ : Structure of recombinant and intermediate DNA molecules and their packaging in vitro.
- K. SHIMADA, K. UMEF, T. NAKAMURA, and Y. TAKAGI, Department of Biochemistry, Kyushu University, School of Medicine, Fukuoka, Japan: Recombination in hybrid ColE1 DNAs as analyzed by λ -phage-mediated transduction.
- C.S. ROEDER and P.D. SAIDOWSKI, Department of Medical Genetics, University of Toronto, Canada: Pathways of bacteriophage T7 in vitro genetic recombination.
- H. ARAKI and H. OGAWA, Department of Pathology, Faculty of Science, Osaka University, Toyonaka, Japan: Genetic recombination of T7 phage in vitro.

Session 14: Repair and *recA* Function

Chairperson: E. WITKIN, Douglass College, Rutgers University, New Brunswick, New Jersey

- K. MCENTEE, Department of Biochemistry, Stanford University School of Medicine, California: Regulation of *recA* gene expression in vivo.
- J.W. ROBERTS, C.W. ROBERTS, and N.L. CRAIG, Department of Biochemistry, Cornell University, Ithaca, New York: The *Escherichia coli recA* gene product inactivates phage λ repressor.
- A.J. CLARK, A. GIRO, L.J. MARGOSSIAN, L.W. REAM, A. TEMPLIN, and M.R. VOLKERT, Department of Molecular Biology, University of California, Berkeley: Genetic studies of the regulation and function of *recA*.
- L. GROSSMAN,* S. RIAZUDDIN,* G. CHEN,* K. LINDEN,[†] and W. HASFLTINE,[†] *Department of Biochemistry, Johns Hopkins University; [†]Sidney Farber Cancer Research Institute, Harvard School of Medicine, Boston, Massachusetts: Nucleotide excision-repair of damaged DNA.
- M. OISHI, C.L. SMITH, and B. FRIEFLD, Public Health Research Institute of the City of New York, Inc., New York: The molecular events and molecules which lead to induction of prophage and SOS functions.
- M. RADMAN,* G. VILANI,* S. BOITEL,* B. CHICKMAN,[†] and S. SPADARI,[‡] *Département de Biologie Moléculaire, Université Libre de Bruxelles, Belgium; [†]Laboratory for Molecular Genetics, University of Leiden, The Netherlands; [‡]Laboratorio de Genetica Biochimica ed Evoluzionistica, Università di Pavia, Italy: Molecular mechanisms and genetic control of replicational fidelity—Involvement in spontaneous and induced mutagenesis.
- M. RYKOVSKI,* P. PUKKIA,* M. RADMAN,[†] R. WAGNER,* and M. MESFISON,* *The Biological Laboratories, Harvard University, Cambridge, Massachusetts; [†]Département de Biologie Moléculaire, Université Libre de Bruxelles, Belgium: Undermethylation and strand selection in DNA mismatch repair.

Summary: F. Stahl, University of Oregon, Eugene



SUMMER MEETINGS

PHORBOL ESTERS, May 11–May 14

arranged by

I. Bernard Weinstein, *Columbia University College of Physicians and Surgeons*

Walter Troll, *Institute of Environmental Medicine, New York University Medical Center*

68 participants

Opening Address: J.D. WATSON, Cold Spring Harbor Laboratory

Introduction: W. TROLL, New York University, New York, New York,
I.B. WEINSTEIN, Columbia University, New York, New York

Session 1: Studies on Two-stage Mouse Skin Carcinogenesis

Chairpersons: R. BOUTWELL, McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

B.L. VAN DUUREN, New York University, Medical Center, New York, New York

R. ALBERT, F. BURNS, M. PEREIRA, and B. ALTSHULER, Institute of Environmental Medicine, New York University Medical Center, New York: Pathways and kinetics of BaP and phorbol ester tumorigenesis in the mouse skin.

B.L. VAN DUUREN, Institute of Environmental Medicine, New York University Medical Center, New York: Mouse skin bioassays on analogs and stereoisomers of the tumor promoter phorbol myristate acetate (PMA) and on a series of cocarcinogenic agents.

R.K. BOUTWELL, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: The biochemical mechanism of skin tumor promotion.

A.K. VERMA and R.K. BOUTWELL, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: An organ culture of mouse skin—A simple in vitro model for studying the molecular mechanism of skin tumor promotion.

S. YUSPA, U. LICHTI, H. HENNING, M. POIRIER, and P. STEINERT, NCI, National Institutes of Health, Bethesda, Maryland: Tumor-promoter-induced alterations of epidermal maturation in cell culture.

U. LICHTI, S. YUSPA, and HENNINGS, NCI, National Institutes of Health, Bethesda, Maryland: Dissociation of tumor-promoter-stimulated ornithine decarboxylase activity and thymidine incorporation in primary mouse epidermal cell cultures.

Session 2: Cell Culture Effects—Growth and Transformation

Chairpersons: E. REICH, Rockefeller University, New York, New York

P. SHUBIK, Eppley Institute for Cancer Research, Omaha, Nebraska

- S. MONDAL, J.R. LILLEHAUG, C. BOREIKO, D.W. BRANKOW, and C. HEIDELBERGER, University of Southern California Cancer Center, Los Angeles: The effects of tumor-promoting phorbol esters on the morphology, growth, and 2-deoxy-D-glucose uptake of C3H/10T1/2 cells in culture.
- A.R. KENNEDY, H. NAGASAWA, and J.B. LITTLE, Harvard School of Public Health, Boston, Massachusetts: Effect of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and protease inhibitors on X-ray-induced oncogenic transformation and sister chromatid exchanges.
- V.E. STEELE, A.C. MARCHOK, and P. NETTESHEIM, Biology Division, Oak Ridge National Laboratory, Tennessee: Growth promotion and tumor promotion by TPA in cultured respiratory epithelium.
- V. KINZEL and R. SÜSS, Institute of Experimental Pathology, DKFZ, Heidelberg, Germany: Some early effects of phorbol esters in cell cultures.
- D.L. BERRY, T.L. SUTTON, A. VIAJE, S.M. FISCHER, and T.J. SLAGA, Biology Division, Oak Ridge National Laboratory, Tennessee: Uptake and metabolic conversion of TPA by newborn and adult epidermal cells in culture.
- R.D. ESTENSEN, D. SEEHAUSEN, C. COLE, and B. DRAZICH, Department of Laboratory Medicine and Pathology, University of Minneapolis, Minnesota: Binding of [³H]PMA to human peripheral blood lymphocytes.
- A. KINSELLA, S. MOUSSET, C. SZPIRER, and M. RADMAN, Département de Biologie Moléculaire, Université Libre de Bruxelles, Rhode-St.-Genèse, Belgium: The irreversible step in tumor promotion may be due to aberrant mitotic segregation.

Session 3: Membrane Effects and Prostaglandins

Chairpersons: A. SIVAK, Arthur D. Little, Inc., Cambridge, Massachusetts

T. SUGIMURA, National Cancer Center, Research Institute, Tokyo, Japan

- A. SIVAK and M. CHAREST, Arthur D. Little, Inc., Cambridge, Massachusetts: The plasma membrane as primary transducer of phorbol ester action in 3T3 mouse embryo fibroblasts.
- P.B. FISHER, D. SCHACHTER, and I.B. WEINSTEIN, Institute of Cancer Research and Division of Environmental Sciences, Columbia University College of Physicians and Surgeons, New York, New York: Tumor promoters alter membrane lipid fluidity of mammalian cells.
- S. BELMAN, W. TROLL, and S.J. GARTE, Institute of Environmental Medicine, New York University Medical Center, New York: Biological and biochemical antagonism between PMA and butyric acid in mouse epidermis—Correlation with their *in vitro* effects.
- L.S. LEE and I.B. WEINSTEIN, Institute of Cancer Research, and Division of Environmental Sciences, Columbia University College of Physicians and Surgeons, New York, New York: Interaction of phorbol esters with receptors of epidermal growth factor in normal and transformed cells.
- K. OHUCHI and L. LEVINE, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: Deacylation of cellular lipids and prostaglandin production in canine kidney cells by tumor-promoting phorbol esters.
- R.A. MUFSON,* P. KULKARNI,† J.D. LASKIN,* and I.B. WEINSTEIN,* *Cancer Center, Institute of Cancer Research; †Departments of Pharmacology and Ophthalmology, Columbia University College of Physicians and Surgeons, New York, New York: Phorbol-ester-induced aggregation of rabbit platelets occurs independently of synthesis of cyclooxygenase products.
- K. BRUNE,* H. ZUR HAUSEN,† and E. HECKER,† *Biozentrum der Universität Basel, Switzerland; †Institut für Virologie, Zentrum für Hygiene, Universität Freiburg, Germany; †Institut für Biochemie, Deutsches Krebsforschungszentrum, Heidelberg, Germany: Tumor promoters of the diterpene ester type stimulate prostaglandin release and induce persisting genomes of oncogenic viruses.

Session 4: Phenotypic Effects and Mimicry of Transformation

Chairpersons: G.C. MUELLER, McArdle Laboratory, University of Wisconsin, Madison
L. DIAMOND, The Wistar Institute, Philadelphia, Pennsylvania

- T.G. O'BRIEN and L. DIAMOND, The Wistar Institute, Philadelphia, Pennsylvania: The relationship between ornithine decarboxylase activity and DNA synthesis in cell cultures treated with tumor-promoting phorbol diesters.
- R.H. GOLDFARB and J.P. QUIGLEY, Downstate Medical Center, State University of New York, Brooklyn: Production of plasminogen activator by chick embryo fibroblasts—Synergistic effect of Rous virus transformation and treatment with the tumor promoter PMA.
- M.H. WIGLER, Cancer Center, Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York: Enhancement of transformation-specific traits of Rous-sarcoma-virus-infected chick embryo fibroblasts by tumor-promoting phorbol esters.
- P.M. BLUMBERG, P.E. DRIEDGER, and K.B. DELCLOS, Department of Pharmacology, Harvard Medical School, Boston, Massachusetts: The chick embryo fibroblast as an *in vitro* system for studying the activity of the phorbol esters.
- N.H. COLBURN, K. NELSON, and B. FORMER, NCI, National Institutes of Health, Bethesda, Maryland: Induction of anchorage-independent growth in epidermal cells by phorbol esters and its inhibition by tumor promotion inhibitors.
- L. KOPELOVICH and N. BIAS, Sloan-Kettering Institute for Cancer Research, New York, New York: TPA-treated human skin fibroblasts from individuals genetically predisposed to cancer growth without anchorage.
- C.E. WENNER and L.D. TOMEI, Roswell Park Memorial Institute, Buffalo, New York: The relationship of early membrane effects of TPA and $\text{PGF}_2\alpha$ to cell-cycle kinetics—An approach through G₁ mapping.

Session 5: Effects on Differentiation

Chairperson: H. HOLTZER, University of Pennsylvania, Philadelphia

- N.E. FUSENIG, German Cancer Research Center, Institute of Biochemistry, Heidelberg, Germany: Effects of the phorbol ester TPA on proliferation and differentiation of primary and permanent mouse epidermal cell cultures.
- H. HOLTZER, M. PACIFICI, C. WEST, and R. COHEN, Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia: Reversible and irreversible effects of PMA on cell differentiation.
- T. SUGIMURA,* T. KAWACHI,* N. NAKAYASU,* N. MATSUKURA,* and S. TAKAYAMA,† *National Cancer Center Research Institute; †Cancer Institute, Tokyo, Japan: Enhancement of differentiation of mouse leukemia cells by TPA and promotion of stomach carcinogenesis of rats by orally given croton oil.
- H. YAMASAKI, E. FIBACH, R.A. RIFKIND, P.A. MARKS, and I.B. WEINSTEIN, Cancer Center, Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York: Inhibition of Friend erythroleukemia cell differentiation by tumor promoters and the isolation and characterization of TPA-resistant clones.
- C.N. FRANTZ,* C.D. STILES,† H.N. ANTONIADES,‡ and C.D. SCHER,* *Departments of Pediatric Oncology and †Cell Biology, Sidney Farber Cancer Institute, Harvard Medical School; ‡Center for Blood Research, Harvard School of Public Health, Boston, Massachusetts: TPA induces DNA synthesis in density-inhibited Balb/c-3T3 cells in the presence of a variety of growth factors.
- G.C. MUELLER, T.W. KENSLER, P.W. WERTZ, and C.H. KWONG, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Retinoic acid inhibition of phorbol-ester-mediated comitogenesis, phospholipid metabolism, and capping in bovine lymphocytes.

Summation and Future Prospects: W. TROLL and I.B. WEINSTEIN

THE CYTOSKELETAL AND CONTRACTILE NETWORKS OF NONMUSCLE CELLS

May 17—May 21

arranged by

Robert D. Goldman, Mellon Institute, Carnegie-Mellon University

Joanna B. Olmsted, University of Rochester

188 participants

Session 1: Mitosis

Chairperson: L. I. REBHUN, University of Virginia, Charlottesville

- M. W. BERNS, S. BRENNER, J. B. RATTNER, and S. M. RICHARDSON, Department of Developmental and Cell Biology, University of California, Irvine: Laser microbeam studies on the mitotic apparatus.
- S. P. PETERSON and M. W. BERNS, Department of Developmental and Cell Biology, University of California, Irvine: Nucleic acid function in spindle formation in dividing PTK2 cells shown by laser induced psoralen photoreaction.
- A. FORER* and W. T. JACKSON,† *Biology Department, York University, Toronto, Canada; †Biology Department, Dartmouth College, Hanover, New Hampshire: Distribution of actin filaments in *Haemanthus* endosperm spindles.
- K. FUJIWARA,* M. E. PORTER,† and T. D. POLLARD,* *Department of Anatomy, Harvard Medical School, Boston, Massachusetts; †Department of Biology, University of Pennsylvania, Philadelphia: Comparative localization of myosin, α -actinin and tubulin in tissue-culture cells by double fluorescent antibody staining.
- W. Z. CANDE, Department of Botany, University of California, Berkeley: Studies of chromosome movement in lysed mitotic cells—Nucleotide dependence.
- D. P. KIEHART, Department of Biology, University of Pennsylvania, Philadelphia, and the Marine Biological Laboratory, Woods Hole, Massachusetts: Microinjection studies on spindle assembly and chromosome movement in vivo.
- T. KELLER and L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Studies of isolated spindles and spindle tubulin from sea urchin eggs.
- L. GRIFFITH and T. POLLARD, Department of Anatomy, Harvard Medical School, Boston, Massachusetts: Viscometric evidence for actin filament-microtubule interaction mediated by microtubule-associated proteins.
- M. J. WELSH, J. DEDMAN, S. COX, B. R. BRINKLEY and A. R. MEANS, Department of Cell Biology, Baylor College of Medicine, Houston, Texas: Calcium-dependent regulator protein—A dynamic component of the mitotic apparatus.
- J. M. MARCUS, J. R. DEDMAN, B. R. BRINKLEY, and A. R. MEANS, Department of Cell Biology, Baylor College of Medicine, Houston, Texas: Regulation of microtubule assembly-disassembly by calcium-dependent regulator protein.

Session 2: Microtubules I

Chairperson: J. B. OLMSTED, University of Rochester, New York

- M. OSBORN and K. WEBER, Max-Planck-Institut für Biophysische Chemie, Göttingen, Germany: Immunofluorescence microscopy of microtubules and other fibrous structures in cells in tissue culture.
- B. B. ASCH, D. MEDINA, and B. R. BRINKLEY, Department of Cell Biology, Baylor College of Medicine, Houston, Texas: Surface and cytoskeletal features of normal, preneoplastic, and neoplastic mouse mammary epithelial cells in vitro.
- R. W. TUCKER,* K. FUJIWARA,† C. D. STILES,* C. D. SCHER,* and A. B. PARDEE,* *Sidney Farber Cancer Institute; †Harvard Medical School, Boston, Massachusetts: Relationship of centriole cycle to DNA synthesis cycle in BALB/c-3T3 cells.
- M. DE BRABANDER,* J. DE MEY† M. JONIAU,‡ and G. GEUENS,* *Janssen Pharmaceutical Research Laboratories, Beerse, Belgium; †Medical Foundation Queen Elisabeth, V.U.B. Brussels, Belgium; ‡Catholic University of Leuven, Kortrijk, Belgium: The distribution of immunoreactive tubulin in cultured cells at the light- and electron-microscopic level studied with the unlabeled antibody enzyme method.
- B. S. ECKERT, Department of Anatomical Sciences, School of Medicine, State University of New York, Buffalo: Immunocytochemistry of tubulin and actin in glutaraldehyde-fixed cells by light and electron microscopy.

- B. A. PALEVITZ, Biology Department, State University of New York, Stony Brook: Microtubules, the plasmalemma and the control of cellulose orientation in plant cells.
- D. L. KIRK and G. I. VIAMONTES, Department of Biology, Washington University, St. Louis, Missouri: Cell shape changes as the basis for inversion in *Volvox* embryos.
- C. I. VIAMONTES and D. L. KIRK, Department of Biology, Washington University, St. Louis, Missouri: Cytological features underlying cell shape changes in inverting *Volvox* embryos.
- W. D. COHEN and I. NEMHAUSER, Department of Biological Sciences, Hunter College of City University of New York, New York: Phylogenetic distribution of the marginal band system.
- P. SATIR, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: Dynein arm structure and ciliary motion.
- L. BABISS,* R. B. LUFTIG,* J. A. WEATHERBEE,* R. R. WEIHING,* U. RAY,† and B. FIELDS,† *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; †Harvard Medical School, Boston, Massachusetts: Reovirus serotypes I and III differ in their in vitro association with microtubules.
- E. MANDELKOW and E. MANDELKOW, Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany: Image reconstruction of tubulin hoops.
- E. MANDELKOW and E. MANDELKOW, Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany: Aggregates formed by junctions between microtubule walls.

Session 3: Microtubules II

Chairperson: L. WILSON, University of California, Santa Barbara

- R. C. WEISENBERG, Department of Biology, Temple University, Philadelphia, Pennsylvania: Kinetic problems of microtubule and microfilament assembly.
- K. A. JOHNSON and G. B. BORISY, Laboratory of Molecular Biology, University of Wisconsin, Madison: The thermodynamics of microtubule self-assembly in vitro.
- R. V. ZACKROFF and R. C. WEISENBERG, Department of Biology, Temple University, Philadelphia, Pennsylvania: Assembly and stability of microtubules in glucose-D-phosphate.
- K. W. FARRELL, A. MORSE, and L. WILSON, Department of Biological Sciences, University of California, Santa Barbara: Characterization of outer doublet tubulin reassembly in vitro.
- A. H. LOCKWOOD, The Cancer Research Center and Department of Anatomy, Laboratories for Cell Biology, University of North Carolina Medical School, Chapel Hill: A protein essential for microtubule assembly—Biochemical and immunological characterization.
- M. E. STEARNS and D. L. BROWN, Department of Biology, University of Ottawa, Canada: Assembly of cytoplasmic tubulin of *Polytomella* on isolated basal body rootlet MTOCs.
- K. HINDS* and D. SOIFER,† *Cornell University Medical College, New York, New York; †Institute for Basic Research, Staten Island, New York: Absence of microtubule-associated proteins from hybrid microtubules assembled from chick brain and human fibroblast protein.
- L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Sulfhydryls and the assembly of microtubules.
- L. A. AMOS, Medical Research Council Laboratory of Molecular Biology, Cambridge, England: Arrangements of microtubule-associated proteins.
- W. S. ADAIR and U. W. GOODENOUGH, Department of Biology, Harvard University, Cambridge, Massachusetts: Identification of a membrane tubulin in *Chlamydomonas*.
- J. NATH and M. FLAVIN, NHLBI, National Institutes of Health, Bethesda, Maryland: State of tyrosylation of brain membrane tubulin.

Session 4: Microtubules and 10-nm Filaments

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

- L. VAN DE WATER III and J. B. OLMSTED, Department of Biology, University of Rochester, New York: Quantitation and characterization of antibody binding to tubulin.
- F. SOLOMON, M. MAGENDANTZ, and A. DUERR, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Analysis of cytoplasmic microtubules in cytoskeleton preparations of cultured cells.
- N. W. SEEDS and R. B. MACCIONI, Department of Biophysics and Genetics, University of Colorado Medical Center, Denver: Regulation of microtubule formation in nerve cells.

- J. A. WEATHERBEE, R. B. LUFTIG, and R. W. WEIHING, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Preparation and fractionation of microtubules from HeLa cells.
- K. HINDS,* B. S. DANES,* and D. SCIFER,† *Cornell University Medical College, New York, New York; †Institute for Basic Research, Staten Island, New York: Microtubules in Chediak-Higashi syndrome.
- P. LEFEBVRE, E. WIEBEN, and J. ROSENBAUM, Department of Biology, Yale University, New Haven, Connecticut: Control of flagellar protein synthesis in *Chlamydomonas*.
- D. P. WEEKS, P. S. COLLIS, and M. A. GEALT, Institute for Cancer Research, Philadelphia, Pennsylvania: Differential effects of inhibitors on tubulin induction in *Chlamydomonas reinhardtii*.
- G. R. CAMPBELL,* J. H. CHAMLEY-CAMPBELL,* U. GRÖSCHEL-STEWART,† J. V. SMALL‡, and P. ANDERSON,§ *Baker Medical Research Institute, Melbourne, Australia; †Institut für Zoologie, Darmstadt, Germany; ‡Institute of Molecular Biology, Salzburg, Austria; §University of Aarhus, Denmark: Immunofluorescent localization of 10-nm (100-Å) filaments in muscle and nonmuscle cells.
- E. WANG,* R. K. CROSS,* J. STARGER,† R. D. GOLDMAN,† and P. W. CHOPPIN,* *Rockefeller University, New York, New York; †Carnegie-Mellon University, Pittsburgh, Pennsylvania: Microtubules, 100-Å filaments, and intracellular movements.
- J. V. SMALL and J. E. CELIS, Institute of Molecular Biology, Salzburg, Austria, and Institute of Chemistry, University of Aarhus, Denmark: Ultrastructural studies of the cytoskeleton of cultured cells.
- G. P. TUSZYNSKI, E. FRANK, and C. H. DAMSKY, Wistar Institute, Philadelphia, Pennsylvania: Two-dimensional gel electrophoresis of cytoskeletal proteins.

Session 5: 10-nm Filaments and Microfilaments

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

- P. EAGLES, C. BELL, A. MAGGS, C. WAIS, and D. GILBERT, MRC Cell Biophysics Unit, Kings College, London: Neurofilament structure and its enzymatic modification.
- G. SHECKET and R. LASEK, Department of Anatomy, Case Western Reserve University School of Medicine, Cleveland, Ohio: Phosphorylation of 10-nm neurofilaments.
- P. F. DAVISON and B.-S. HONG, Fine Structure Department, Boston Biomedical Research Institute, Boston, Massachusetts: Classes of cytoplasmic filaments.
- G. SALOMON, R. LIEM, and M. SHELANSKI, Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts: Isolation of intermediate filaments from central and peripheral nerve.
- J. M. STARGER and R. D. GOLDMAN, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Properties of 10-nm filaments from various cell types.
- R. G. WHALEN, G. S. BUTLER-BROWNE, and F. GROS, Institut Pasteur, Paris, France: Multiplicity of the protein subunits of intermediate filaments.
- P. STEINERT and W. LIDFELT, NCI, National Institutes of Health, Bethesda, Maryland: Structural features of the mammalian epidermal keratin filament.
- I. K. BUCKLEY,* T. R. RAJU,* and M. STEWART,*† *Department of Experimental Pathology, John Curtin School of Medical Research, the Australian National University; †Division of Computing Research, CSIRO, Canberra, Australia: A reassessment of the distribution of F-actin in cultured fibroblasts.
- R. RUBIN and J. HOWARD, Department of Anatomy, University of Miami School of Medicine, Miami, Florida: A biochemical and ultrastructural comparison of Triton cytoskeletal models of normal and transformed cells.
- J. M. OLIVER and R. D. BERLIN, Department of Physiology, University of Connecticut Health Center, Farmington: Analogous ultrastructure and surface properties during Con-A capping and phagocytosis in leukocytes. I. Properties of the cytoskeleton.
- J. CONDEelis, Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York: The actin cytoskeleton and the mobility of cell-surface receptors for Con-A.
- R.W. MERRIAM, M. NOWINSKI, and K. HAAS, Department of Biology, State University of New York, Stony Brook: The demonstration of localized contractile programs in living oocytes and eggs of *Xenopus laevis*.

Session 6: Microfilaments I

Chairperson: E. TAYLOR, University of Chicago, Illinois

- S. S. BROWN, D. G. UYEMURA, and J. A. SPUDICH, Department of Structural Biology, Sherman Fairchild

- Center, Stanford University School of Medicine, California: Characterization of the polymerization properties of actin from *Dictyostelium discoideum*.
- T. CLARK and J. R. ROSENBAUM, Department of Biology, Yale University, New Haven, Connecticut: Characterization of nuclear actin from *Xenopus* oocytes.
- D. A. BEGG, R. C. MORELL, and L. I. REBHUN, Department of Biology, University of Virginia, Charlottesville: Studies of actin in the sea urchin egg cortex.
- K. T. EDDS, Marine Biological Laboratory, Woods Hole, Massachusetts: The ultrastructure and biochemical components of actin-containing filament bundles.
- J. A. SCHLOSS and R. D. GOLDMAN, Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Studies on the proteins and organization of cultured-cell native microfilaments.
- V. NACHMIAS, Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia: Effects of sulfhydryl inhibitors on AMP-induced filamentogenesis in *Physarum*.
- J. D'HAESE,* and H. HINSEN,† *Institut für Zoologie, Düsseldorf; †Institut für Cytologie, Bonn, Germany: Functional studies on the different organization forms of *Physarum* actomyosin.
- B. S. JACOBSON, Biochemistry Department, University of Massachusetts, Amherst: Plasma membrane isolation and inversion on cationic beads—Actin association with highly purified plasma membrane from *Dictyostelium discoideum*.
- C.-J. H. CHEN, D. J. LITMAN, and V. T. MARCHESI, Department of Pathology, Yale University School of Medicine, New Haven, Connecticut: Interactions between the cytoplasmic surface of the human erythrocyte membrane and potential cytoskeletal proteins.
- M. P. SHEETZ and D. SAWYER, Physiology Department, University of Connecticut Health Center, Farmington: Composition of the erythrocyte cytoskeleton and preliminary evidence for spectrin filaments.
- H. MARUTA, J. H. COLLINS, H. GADASI, and E. D. KORN, NHLBI, National Institutes of Health, Bethesda, Maryland: Catalytic site of *Acanthamoeba* myosin. I.
- J. H. COLLINS, H. GADASI, H. MARUTA, and E. D. KORN, NHLBI, National Institutes of Health, Bethesda, Maryland: Myosin-I heavy-chain kinase from *Acanthamoeba castellanii*.
- H. GADASI, J. H. COLLINS, H. MARUTA, and E. D. KORN, NHLBI, National Institutes of Health, Bethesda, Maryland: Tryptic digestion of *Acanthamoeba* myosin. II.

Session 7: Microfilaments II

Chairperson: D. HARTSHORNE, Carnegie-Mellon University, Pittsburgh, Pennsylvania

- C. MAHENDRAN and S. BERL, Mt. Sinai School of Medicine of City University of New York, New York: Resolution of troponin complex from bovine brain.
- D. M. WATTERSON, Department of Cell Biology, Rockefeller University, New York, New York: The role of calcium-modulated proteins in cell function.
- R. DABROWSKA* and D. J. HARTSHORNE,† *Nencki Institute of Experimental Biology, Warsaw, Poland; †Departments of Biological Sciences and Chemistry, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Ca²⁺-Dependent myosin light-chain kinase from nonmuscle tissues.
- M.-J. YERNA,* R. DABROWSKA,† D. J. HARTSHORNE,* and R. D. GOLDMAN,* *Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pennsylvania; †Department of Biochemistry of the Nervous System and Muscle, Nencki Institute of Experimental Biology, Warsaw, Poland: The regulation of BHK-21 myosin—Possible function of an endogenous modulator protein.
- D. R. HATHAWAY,* C. B. KLEE,† C. R. EATON,* and R. S. ADELSTEIN,* *NHLBI, National Institutes of Health; †NCI, National Institutes of Health, Bethesda, Maryland: Regulation of platelet and smooth-muscle myosin light-chain kinases.
- S. P. SCORDILIS and R. S. ADELSTEIN, NHLBI, National Institutes of Health Bethesda, Maryland: Developmental changes in myosin and myosin light-chain kinase during myogenesis.
- J. A. TROTTER and R. S. ADELSTEIN, NHLBI, National Institutes of Health, Bethesda, Maryland: Phosphorylation of macrophage myosin regulates actin activation.
- W. BLOOM, W. SCHOOK, C. ORES, and S. PUSZKIN, Department of Pathology, Mount Sinai School of Medicine of City University of New York, New York: Brain clathrin purification, assembly into basketlike structures and interaction with contractile proteins.
- R. R. WEIHING, Worcester Foundation, Shrewsbury, Massachusetts: Time course of heavy meromyosin (HMM) inhibition of actin-related gelation of HeLa cell extracts.

- E. A. BROTSCHI, J. H. HARTWIG, and T. P. STOSSEL, Medical Oncology Unit, Massachusetts General Hospital, Boston: Quantitative analysis of actin gelation by actin-binding protein, filamin, and myosin.
- P. DAVIES, D. WALLACH, M. WILLINGHAM, and I. PASTAN, Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland: Studies on the phosphorylation and function of the actin-binding protein, filamin.
- R. C. LUCAS, S. ROSENBERG, and A. STRACHER, Department of Biochemistry, Downstate Medical Center, State University of New York, Brooklyn, New York: Regulation of the cytoskeletal elements of platelets—Interaction of actin-binding protein and actin.
- U. LINDBERG, L. CARLSSON, and F. MARKEY, Cell Motility Group, Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden: Profilin: actin complex—Its role in cell motility.

Session 8: *New Concepts and Methods for Studying Cytoskeletal and Contractile Systems I*

Chairperson: K. PORTER, University of Colorado, Boulder

- G. ALBRECHT-BUEHLER, Cold Spring Harbor Laboratory, New York: The angular distribution of directional changes of guided 3T3 cells.
- E. BELL, L. MAREK, S. SHER, C. MERRILL, D. LEVINSTONE, and I. YOUNG, Massachusetts Institute of Technology, Cambridge: Relation of motility to cell states and previous history.
- S. TAMM, Laboratory of Molecular Biology, University of Wisconsin, Madison: Relations between membrane movements and cytoplasmic structures during rotational motility of a termite flagellate.
- R. A. BLOODGOOD, Albert Einstein College of Medicine, Bronx, New York: *Chlamydomonas* flagellar surface motility.
- J. L. HOFFMAN and U. GOODENOUGH, Department of Biology, Harvard University, Cambridge, Massachusetts: Flagellar surface motility in *Chlamydomonas*.
- K. R. STRAHS and M. W. BERNIS, Department of Developmental and Cell Biology, University of California, Irvine: Laser irradiation of stress fibers and bundles of intermediate filaments in cultured nonmuscle cells.
- M. C. WILLINGHAM, S. S. YAMADA, and I. PASTAN, NCI, National Institutes of Health, Bethesda, Maryland: Ultrastructural localization of intracellular antigens—A new procedure for cultured cells.
- H. R. BYERS* and K. R. PORTER,† *Department of Anatomy, Harvard Medical School, Boston, Massachusetts; †Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: A stereo high-voltage electron microscopic and cinematographic correlative study of pigment motion and the structure of the cytoplasmic matrix in erythrophores treated with colchicine.
- V. G. FONTE, K. L. ANDERSON, J. J. WOLOSEWICK, and K. R. PORTER, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: High-voltage electron microscope observations of the response of the cytoskeleton to dihydrocytochalasin-B treatment.
- M. H. ELLISMAN,* and K. R. PORTER,† *Department of Neurosciences, University of California, San Diego; †Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: The cytoskeleton of axoplasm examined by stereo high-voltage electron microscopy—A possible vehicle for axoplasmic transport.
- J. J. WOLOSEWICK and K. R. PORTER, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Response of the microtrabecular lattice (MTL) to low temperature and colchicine.
- C. ALLEN, Laboratory of Molecular Biology, University of Wisconsin, Madison: High-voltage electron microscope analysis of lamellar substructure in tissue cells.

Session 9: *New Concepts and Methods for Studying Cytoskeletal and Contractile Systems II*

Chairperson: V. NACHMIAS, University of Pennsylvania School of Medicine, Philadelphia

- M. CLARKE, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Isolation of motility mutants of *Dictyostelium discoideum*.
- J. M. ZENGEL, D. C. REIN, and H. F. EPSTEIN, Department of Pharmacology, Stanford University, Stanford, California: Genetic dissection of muscle development.
- D. N. JACOBSON, Department of Anatomy, Duke University, Durham, North Carolina: *Physarum* amoebal behavioral mutants have multiple effects on both regulation of cell movement and change of cell state.
- V. LING, A. CHASE, J. E. AUBIN, and F. SARANGI, Department of Medical Biophysics, University of Toronto, and Ontario Cancer Institute, Toronto, Canada: Chinese hamster ovary (CHO) cells with altered colcemid-binding activity.

- R. HYNES, I. ALI, and A. DESTREE, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Are there relationships between extracellular LETS protein fibers and intracellular filaments?
- S. PENMAN, A. BEN-ZE'EV, B. BENECKE, S. FARMER, R. LENK, and L. WEYMOUTH, Department of Biology, Massachusetts Institute of Technology, Cambridge: Skeletons and macromolecular function.
- W. E. GORDON III, A. BUSHNELL, and K. BURRIDGE, Cold Spring Harbor Laboratory, New York: Immunofluorescent and ultrastructural studies of actin, myosin, and α -actinin in the "sarcomeres" of stress fibers and in polygonal microfilament networks.
- D. L. GARD and E. LAZARIDES, Division of Biology, California Institute of Technology, Pasadena: Specific fluorescent labeling of chicken myofibril Z-line proteins catalyzed by guinea pig liver transglutaminase.
- J. W. SANGER and J. M. SANGER, Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia: The role of filaments in cell spreading.
- M. M. BLACK* and R. J. LASEK,[†] *Neuroscience Department, Children's Hospital, Boston, Massachusetts; [†]Anatomy Department, Case Western Reserve University, Cleveland, Ohio: Axonal transport of structural complexes within the axon—Possible identification of the polypeptides associated with the microtubecular system.
- W. S. LYNN and C. MUKHERJEE, Duke University Medical Center, Durham, North Carolina: Function of Na⁺ and extracellular protein in leukocyte motility and membrane ruffling.
- F. R. MAXFIELD* J. SCHLESSINGER,[†] M. C. WILLINGHAM,* Y. SHECHTER,[‡] and I. PASTAN,* *NCI, National Institutes of Health; [†]NCI, National Institutes of Health, Bethesda, Maryland, and Department of Applied Physics, Cornell University, Ithaca, New York; [‡]Burroughs Wellcome Company, Research Triangle Park, North Carolina: A common mechanism for the internalization of α_2 -macroglobulin, epidermal growth factor, and insulin by cultured fibroblasts.

RNA TUMOR VIRUSES, May 24—May 28

arranged by

J. Michael Bishop, University of California Medical School, San Francisco

Harold E. Varmus, University of California Medical School, San Francisco

387 participants

Session 1: The Genome—Structure and Genesis

Chairperson: P. VOGT, University of Southern California, Los Angeles

- R. SWANSTROM,* L. HALLICK,[†] J. E. HEARST,[‡] and J. M. BISHOP,* *Department of Microbiology, University of California, San Francisco; [†]Department of Microbiology and Immunology, University of Oregon, Portland; [‡]Department of Chemistry, University of California, Berkeley: Secondary structure in avian sarcoma virus RNA—Cross-linking as a molecular probe.
- M. M. C. LAI and S. S. F. HU, Department of Microbiology, University of Southern California, School of Medicine, Los Angeles: The presence of variable and constant sequences in the *env* gene of avian RNA tumor viruses.
- J. M. COFFIN and C. BARKER, Department of Molecular Biology and Microbiology and Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts: In vitro "evolution" of Rous sarcoma virus.
- A. SHIELDS, O. N. WITTE, E. ROTHENBERG,* and D. BALTIMORE, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Clonal isolates of Moloney murine leukemia virus aberrantly express viral genes at high frequency.
- J. ROMMELAERE, H. DONIS-KELLER, D. V. FALLER, J. SCHINDLER, and N. HOPKINS, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: The sequence of three T1 oligonucleotides associated with the N-, B-, or NB-tropism of certain murine leukemia viruses.
- D. V. FALLER, J. ROMMELAERE, and N. HOPKINS, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: T1 oligonucleotide analysis of Moloney and HIX genomic RNAs and of an intracellular, 21S, spliced Moloney virus RNA, the putative Moloney gp70 mRNA.

- D. J. DONOGHUE,* E. ROTHENBERG,† N. HOPKINS,* D. BALTIMORE,* and P. A. SHARP,* *Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; †Memorial Sloan-Kettering Cancer Center, New York, New York: Heteroduplex analysis of the nonhomology region between Moloney murine leukemia virus and the dual-host-range derivative HIX virus.
- P. ANDERSSON, M. GOLDFARB, and R. A. WEINBERG, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Assignment of the transforming function of Moloney sarcoma virus (Mo-MSV) to restriction-endonuclease-generated fragments of Mo-MSV cDNA.
- J. G. LEVIN and J. G. SEIDMAN, NICHHD, National Institutes of Health, Bethesda, Maryland: Assembly of host tRNAs into murine leukemia virus particles does not require genomic RNA.
- G. PETERS, C. GLOVER, and R. WEISS, Department of Viral Oncology, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, England: Absence of tRNA^{Trp} primer in polymerase-defective avian sarcoma viruses.
- M. LINAL,* E. MEDEIROS,* B. GALLIS,* and W. HAYWARD,† *Fred Hutchinson Cancer Research Center, Seattle, Washington; †Rockefeller University, New York, New York: A mutant of Rous sarcoma virus which packages cellular rather than genomic RNA.

Session 2: Viral DNA—Synthesis and Integration

Chairperson: R. ERIKSON, University of Colorado, Denver

- J. M. TAYLOR,* T. W. HSU,* G. E. MARK,* W. S. MASON,* J. L. SABRAN,† A. KAI,† M. M. C. LAI,‡ and R. R. GUNTAKA,§ *Institute for Cancer Research, Philadelphia, Pennsylvania; †University of Pennsylvania, Philadelphia; ‡University of Southern California Medical School, Los Angeles; §Columbia University, New York: Avian RNA tumor virus genome—Structure and replication.
- H. J. KUNG, P. R. SHANK, J. M. BISHOP, and H. E. VARMUS, Department of Microbiology, University of California, San Francisco: The structure of unintegrated viral DNA in cells infected with avian sarcoma virus—The size of the (+) strands in linear DNA and the identification of dimeric, closed, circular DNA.
- S. HUGHES,* P. R. SHANK,* D. SPECTOR,* H. J. KUNG,* H. L. ROBINSON,† E. STUBBLEFIELD,‡ P. K. VOGT,§ J. M. BISHOP,* and H. E. VARMUS,* *Department of Microbiology, University of California, San Francisco; †Worcester Foundation, Shrewsbury, Massachusetts; ‡M.D. Anderson Medical Center, Houston, Texas; §University of Southern California Medical School, Los Angeles: Mapping of virus-specific DNA in avian sarcoma virus-infected and uninfected cells.
- E. KESHET and H. M. TEMIN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Sites of integration of reticuloendotheliosis virus DNA in chicken cellular DNA.
- D. STEFFEN and R. A. WEINBERG, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Moloney murine leukemia virus integrates its DNA in multiple sites upon infection of cell cultures and induction of leukemia.
- P. NOBIS and R. JAENISCH, Heinrich-Pette-Institut, Hamburg, Germany: Virus-specific expression, integration and gene amplification in mice carrying Moloney leukemia virus in their germ lines.
- J. C. COHEN,* P. R. SHANK,* V. L. MORRIS,† R. CARDIFF,‡ and H. E. VARMUS,* *Department of Microbiology, University of California, San Francisco; †Department of Bacteriology, University of Western Ontario, London, Canada; ‡Department of Pathology, University of California, Davis: The endogenous and acquired proviruses of mouse mammary tumor virus.
- R. S. LEMONS, W. G. NASH, S. J. O'BRIEN, R. E. BENVENISTE, and C. J. SHERR, NCI, National Institutes of Health, Bethesda, Maryland: A gene (Bevi) on human chromosome six is a preferred integration site for baboon type-C DNA provirus in human cells.
- M. GOLDFARB and R. A. WEINBERG, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge: Physical mapping and transfection experiments with Harvey sarcoma virus DNA.
- G. M. COOPER and S. OKENQUIST, Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts: Mechanism of transfection of chicken embryo fibroblasts by Rous sarcoma virus DNA.
- A. ISHIMOTO, J. W. HARTLEY, and W. P. ROWE, NIAID, National Institutes of Health, Bethesda, Maryland: Rapid appearance of phenotypically mixed particles following superinfection of chronically infected cultures.

Session 3: Viral Gene Expression

Chairperson: N. HOPKINS, Massachusetts Institute of Technology, Cambridge

- M. GROUDINE,*† S. DAS,† P. NEIMAN,†‡ and H. WEINTRAUB,§ *Division of Radiation Oncology, University of Washington Hospital, Seattle; †Fred Hutchinson Cancer Research Center, Seattle, Washington; ‡Division of Oncology, University of Washington School of Medicine, Seattle; §Department of Biochemical Science, Princeton University, New Jersey: Subunit conformation and tissue-specific expression of the Rous associated virus-0 (RAV-0) genome.
- D. W. STACEY and H. HANAFUSA, Rockefeller University, New York: Envelope-glycoprotein mRNA and its nuclear precursor within Rous associated virus-2 (RAV-2) virus particles.
- S. R. WEISS, B. CORDELL, H. E. VARMUS, and J. M. BISHOP, Department of Microbiology, University of California, San Francisco: The 5' termini of avian sarcoma virus mRNAs contain transposed sequences.
- M. PERDUE, R. KRZYZEK, A. LAU, D. NOREEN, D. ANDERSON, and A. FARAS, Department of Microbiology, University of Minnesota Medical School, Minneapolis: Characterization of spliced nucleotide sequences present on Rous sarcoma virus-specific subgenomic RNAs from infected cells.
- M. GORECKI,* Y. ALONI,* S. BRATOSIN,* and A. PANET,† *Department of Organic Chemistry and Genetics, Weizmann Institute of Science, Rehovot, Israel; †Department of Virology, Hebrew University, Hadassah Medical School, Jerusalem, Israel: Moloney murine leukemia mRNAs are spliced.
- D. L. ROBERTSON and H. E. VARMUS, Department of Microbiology, University of California, San Francisco: Characterization of the murine mammary tumor virus-specific intracellular RNAs.
- R. D. PALMITER,* J. GAGNON,* V. M. VOGT,† S. RIPLEY,‡ and R. EISENMAN,‡ *Department of Biochemistry, University of Washington, Seattle; †Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York; ‡Fred Hutchinson Cancer Research Center, Seattle, Washington: Determination of the NH₂-terminal amino acid sequence of Pr76^{gag}.
- P. HACKETT, H. OPPERMANN, S. WEISS, A. ULLRICH, L. LEVINTOW, J. M. BISHOP, and R. GESTELAND,* Department of Microbiology, University of California, San Francisco; *Cold Spring Harbor Laboratory, New York: Translation in vitro of the mRNAs specifying the gag and pol proteins of avian sarcoma virus.
- H. OPPERMANN, L. S. CHEN, A. D. LEVINSON, H. E. VARMUS, J. M. BISHOP, and L. LEVINTOW, Department of Microbiology, University of California, San Francisco: Expression of avian sarcoma virus-specific proteins in permissive and nonpermissive cells.
- A. LAU,* R. KRZYZEK,* M. COLLETT,† J. BRUGGE,† R. ERIKSON,† and A. FARAS,* *Department of Microbiology, University of Minnesota Medical School, Minneapolis; †Department of Pathology, University of Colorado Medical Center, Denver: Src gene product is present in revertants of avian retrovirus-transformed mammalian cells.
- J. WYKE, J. BEAMAND, and K. QUADE, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, England: Phenotypic reversion of avian sarcoma virus-transformed rat cells—Variation in the expression of the virus genome.

Session 4: Viral Proteins

Chairperson: R. ARLINGHAUS, M.D. Anderson Hospital, University of Texas, Houston

- A. HIZI, M. A. MCCRAE, and W. K. JOKLIK, Duke University Medical Center, Durham, North Carolina: Studies on the amino acid sequences of polypeptides specified by the gag and pol genes of avian sarcoma virus B77.
- R. D. SCHIFF and D. P. GRANDGENETT, Institute for Molecular Virology, St. Louis University Medical Center, Missouri: Virus-coded origin of the 32,000-dalton endonuclease from avian retrovirus cores—Structural relatedness of p32 and the β polypeptide of avian retrovirus DNA polymerase.
- J. R. STEPHENSON,* A. S. KHAN,† F. H. REYNOLDS,† and T. L. SACKS,* *NCI, National Institutes of Health, Bethesda, Maryland; †Frederick Cancer Research Center, Frederick, Maryland: Mammalian sarcoma virus translational products—Identification and processing of precursors containing structural and nonstructural components.
- J.-S. TUNG, P. V. O'DONNELL, E. FLEISSNER, and E. A. BOYSE, Memorial Sloan-Kettering Cancer Center, New York, New York: Relationships of gp70 of murine leukemia virus envelopes to gp70 components of mouse lymphocyte plasma membranes.

- S. OROSZLAN, A. M. SCHULTZ, L. E. HENDERSON, and J. L. OLPIN, Viral Oncology Program, Frederick Cancer Research Center, Frederick, Maryland: Biochemical and primary structure studies of retrovirus gag polyproteins.
- P. TRAKTMAN, O. N. WITTE, and D. BALTIMORE, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Bypass by protease of temperature-sensitive lesions in the final morphogenetic event of retrovirus synthesis.
- A. PINTER, J. LIEMAN-HURWITZ, and E. FLEISSNER, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of murine leukemia virus structural subunits.
- J. HILKENS,* A. COLOMBATTI,* M. STRAND,† and J. HILGERS,* *Division of Genetics, The Netherlands Cancer Institute, Amsterdam; †Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University, Baltimore, Maryland: Identification of a mouse chromosome for type-C envelope glycoprotein membrane receptor by somatic cell genetics.
- D. ZARLING, R. DUKE, I. KESHET, A. WATSON, and F. BACH, University of Wisconsin, Madison: Physical association of H-2 and murine leukemia virus (MuLV) gp70 antigens on Rauscher MuLV transformed leukemia cells and their recognition by syngenic cytotoxic T lymphocytes.
- G. ROLOSON, C. CHAMBERS, D. HAAGENSEN, JR., R. MONTEJARO, and D. BOLOGNESI, Department of Surgery and Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: A single genetic locus determines the efficacy of serum therapy against murine adenocarcinoma 755a.

Session 5: Endogenous Viruses and Genetic Interactions

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

- L. M. SOUZA and M. A. BALUDA, Molecular Biology Institute, University of California, Los Angeles: The topology of endogenous proviral sequences in some chicken strains.
- E. HUMPHRIES, C. GLOVER, and J. ARRAND, Imperial Cancer Research Fund Laboratories, London, England: Restriction endonuclease analysis of Rous associated virus-0 (RAV-0)-related sequences in DNA of chicken embryo fibroblasts from different inbred and outbred chicken flocks.
- E. HUNTER,* A. S. BHOWN,* M. BARBACID,† and S. AARONSON,† *University of Alabama, Birmingham; †NCI, National Institutes of Health, Bethesda, Maryland: Evolutionary relationship of reticuloendotheliosis virus to avian and mammalian oncoviruses.
- R. CALLAHAN and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Four major endogenous retrovirus classes of the genus *Mus*.
- M. BARBACID and S. A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: In vitro generation of recombinants between mouse type-C RNA tumor viruses.
- J. W. GAUTSCH,* J. H. ELDER,* J. SCHINDLER,† F. C. JENSEN,* and R. A. LERNER,* *Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California; †Department of Biology, Massachusetts Institute of Technology, Cambridge: Structural markers of murine leukemia virus p30—Functional correlation with *Fv-1* tropism.
- L. BENADE,* J. IHLE,* and A. DECLIVE,† *NCI, Frederick Cancer Research Center, Frederick, Maryland; †Stanford University School of Medicine, California: C57BL B-tropic viruses—Evidence for origin by recombination with endogenous xenotropic virus.
- N. G. COPELAND and G. M. COOPER, Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts: Lack of infectivity of endogenous murine leukemia virus DNAs.
- D. R. LOWY, NCI, National Institutes of Health, Bethesda, Maryland: Infectious murine leukemia virus from normal mouse embryo cell DNA.
- H. L. NIMAN, M. B. GARDNER, J. W. PARKER, J. STEINER, and P. ROY-BURMAN, University of Southern California School of Medicine, Los Angeles: Endogenous RD-114 virus genome expression in immunological cells of the cat.
- C. MORONI,* R. P. MONCKTON,* J. F. DELAMARTER,* J. STOYE,* P. ERB,† G. SCHUMANN,‡ *Friedrich Miescher-Institut; †Institut für Mikrobiologie; ‡Research Department, Ciba-Geigy, Ltd., Basel, Switzerland: A putative role for endogenous C-type virus in the immune response.

Host Restriction

- R. SOEIRO, S. DATTAGUPTA, and N. BURNETTE, Albert Einstein College of Medicine, Bronx, New York: Host restriction of Friend leukemia virus—Structural studies of p30 and genome of N-, B-, and NB-tropic virions.
- J. SCHINDLER,* and J. GAUSCH† *Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; †Scripps Research Foundation, La Jolla, California: Further analysis of p30s of N-, B-, and B→NB-tropic murine leukemia viruses of BALB/c and of XLP-N recombinants between the N- and B-tropic viruses.
- G. DURAN-TROISE,* P. O. WEISLOGEL,† R. H. BASSIN,* and T. A. HURT,† *NCI, National Institutes of Health; †Litton Bionetics, Inc., Bethesda, Maryland: Use of amphotropic and heat-inactivated murine leukemia viruses in studies on the mechanism of Fv-1 restriction.
- B. M. BENJERS, R. H. BASSIN, A. REIN, and B. I. GERWIN, NCI, National Institutes of Health, Bethesda, Maryland: Abrogation of Fv-1 restriction in N-type cells.
- E. MEDEIROS and M. LINIAL, Fred Hutchinson Cancer Research Center, Seattle, Washington: Growth restriction of a Prague E avian sarcoma virus recombinant in chicken embryo fibroblasts.
- P. E. NEIMAN,* C. MCMILLIN-HELSEL,* and G. M. COOPER,† *Division of Oncology, University of Washington, and Fred Hutchinson Cancer Research Center, Seattle; †Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts: Specific restriction of avian sarcoma viruses by a line of transformed lymphoid cells.

Viral Genetics

- K. TOYOSHIMA, M. YUTSUDO, H. SUGIYAMA, S. TAHARA, and A. HAKURA, Research Institute for Microbial Diseases, Osaka University, Japan: Replication-defective mutants of avian sarcoma virus.
- A. REIN,* B. M. BENJERS,* R. H. BASSIN,* B. I. GERWIN,* and D. R. SLOCUM,† *NCI, National Institutes of Health; †Litton Bionetics, Inc., Bethesda, Maryland: Rescue and transmission of replication-defective variants of ecotropic murine leukemia virus.
- A. HORWICH,* C. DICKSON,* and B. OZANNE,† *Imperial Cancer Research Fund Laboratories, London, England, †University of Texas Health Science Center, Dallas: Isolation of absolute defective mutants of murine leukemia virus.
- G. S. MARTIN,* K. RADKE,* S. HUGHES,† N. QUINTRELL,† J. M. BISHOP,† and H. VARMUS,† *Zoology Department, University of California, Berkeley; †Department of Microbiology, University of California, San Francisco: Properties of quail cells transformed by replication-defective mutants of Prague Rous sarcoma virus.
- J. C. CHERMANN, F. SINOUSI, and R. LIDEREAU, Unité d'Oncologie Virale, Institut Pasteur, Paris, France: Evidence for a non-defective murine sarcoma virus.
- C. BERGHOLZ, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois: A hybrid of the simian sarcoma virus-related component of HL-23V with altered envelope properties.
- R. SHAIKH,** M. LINIAL,* and R. EISENMAN,* *Fred Hutchinson Cancer Research Center, Seattle, Washington; †Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts: Altered gag proteins of recombinants between PR-Rous sarcoma virus-C and Rous associated virus-0 (RAV-0).
- P. BALDUZZI, and J. R. CHRISTENSEN, Department of Microbiology, University of Rochester, New York: Marker-rescue experiments with temperature-sensitive mutants of Rous sarcoma virus.
- W. J. M. VAN DE VEN, Department of Biochemistry, University of Nijmegen, The Netherlands: Biological assay of in vitro synthesized subgenomic DNA fragments of murine leukemia virus by marker rescue.
- R. R. FRIIS,* and D. BECKER,† *Institut für Virologie, Giessen, Germany; †Rockefeller University, New York: Mapping transformation-defective temperature-sensitive mutants of Rous sarcoma virus into src.

Homo Sapiens

- F. WONG-STAAAL, S. JOSEPHS, and R. C. GALLO, NCI, National Institutes of Health, Bethesda, Maryland:

Hybridization of simian sarcoma virus RNA to endonuclease-restricted human DNA—Detection of endogenous and acquired viral-related fragments.

- P. C. JACQUEMIN, W. C. SAXINGER, and R. C. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Presence of IgG on the membrane of leukemic cells which specifically neutralizes reverse transcriptase from some mammalian type-C viruses.
- P. D. MARKHAM,* F. RUSCETTI,† S. Z. SALAHUDDIN,* R. E. GALLAGHER,† and R. C. GALLO,† *Litton Bionetics, Inc.; †NCI, National Institutes of Health, Bethesda, Maryland: Enhanced transformation of B lymphocytes from normal human blood by exposure to primate type-C retroviruses.
- P. HERBRINK, G. N. P. VAN MUYEN, and S. O. WARNAAR, Laboratory for Pathology, University of Leiden, The Netherlands: Search for C-type viral p30-related antigens and antibodies directed against C-type viral antigens in human tissues and sera.
- R. C. MELLORS and J. W. MELLORS, Hospital for Special Surgery, New York Hospital-Cornell University Medical College, New York: Evidence for type-C virus antibody in human systemic lupus erythematosus—Elution from renal lesions and demonstration by enzymeimmunoassay.
- R. KURTH, Friedrich-Miescher-Laboratory, Max-Planck-Institute, Tübingen, Germany: Antibodies reactive with purified primate type-C viral antigens.

Session 7: Poster Session

Viral Genome

- I. M. VERMA and M. A. MCKENNETT, Tumor Virology Laboratory, Salk Institute, San Diego, California: Physical maps of the in vitro synthesized murine leukemia and sarcoma viral DNA by restriction endonucleases.
- Y. H. CHIEN,* N. DAVIDSON,* I. VERMA,† P. H. DUESBERG,‡ T. Y. SHIH,[§] and E. M. SCOLNICK,[§] *Department of Chemistry, California Institute of Technology, Pasadena; †Salk Institute, San Diego, California; ‡Department of Molecular Biology, University of California, Berkeley; §NCI, National Institutes of Health, Bethesda, Maryland: Heteroduplex studies of murine sarcoma and focus-forming viruses.
- T. Y. SHIH, D. R. WILLIAMS, M. O. WEEKS, J. M. MARYAK, W. C. VASS, H. A. YOUNG, and E. M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Genomic organization of Kirsten and Harvey murine sarcoma viruses.
- D. SCHWARTZ and W. GILBERT, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Sequence analysis of the nucleotide region adjacent to the 3'-terminal poly(A) of Rous sarcoma virus 35S RNA.
- J. N. M. MOL and T. J. STOOF, Department of Experimental Pathology, Erasmus University, Rotterdam, Holland: The genomic complexity of the Rauscher leukemia virus.
- W. K. YANG,* D. L.-R. HWANG,† F. C. HARTMAN,* D. J. PRICE,* J. O. KIGGANS,* C. D. STRINGER,* and D. M. YANG,* *Biology Division, Oak Ridge National Laboratory; †Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee: Primer tRNA binding of cellular RNAs from retrovirus-infected, -transformed, and noninfected cells.
- G. G. LOVINGER, H. OKABE, and R. V. GILDEN, Viral Oncology Program, Frederick Cancer Research Center, Frederick Maryland: Nucleotide sequences close to the 5' terminus of Rauscher leukemia and baboon endogenous viruses.
- F. S. PEDERSEN, D. BUCHHAGEN, J. KOZLOV, Z. ROSENBERG, B. SAHAGAN, and W. A. HASELTINE, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: Structural studies of the genomes of RNA tumor viruses.

Reverse Transcriptase

- E. GILBOA, E. ROTHENBERG, and D. BALTIMORE, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Infective murine leukemia virus DNA made in vitro is mainly linear, entirely double-stranded molecules.
- M. GOLOMB, A. VORA, and D. P. GRANDGENETT, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Endonuclease activity of avian myeloblastosis virus RNA-directed DNA polymerase.
- J. C. OLSEN and K. F. WATSON, Department of Chemistry, University of Montana, Missoula: Priming properties of DNA complementary to sequences at the 5' terminus of the avian myeloblastosis virus genome.
- R. SOO, J. MATTERSBERGER, and P. H. HOFSCHEIDER, Max-Planck-Institut für Biochemie, Martinsried, Germany: Particles containing reverse transcriptase are released from primary quail embryo cultures.

- B. J. WEIMANN, Basel Institute for Immunology, Switzerland: Studies on the DNA polymerase of the sub-human primate simian sarcoma RNA tumor virus.
- R. FRIEDRICH, U. NOVAK, and K. MOELLING, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: Elongation of initial DNA of avian retroviruses synthesized in vitro.
- C. H. CLAYMAN and A. J. FARAS, Department of Microbiology, University of Minnesota, Minneapolis: In vitro synthesis of infectious, transforming DNA by Rous sarcoma virus.

Provirus

- B. VOGELSTEIN, D. GILLESPIE, B. NELKIN, and D. STRAYER, NCI, National Institutes of Health, Bethesda, Maryland: Preparative electrophoresis of integrated simian sarcoma associated virus sequences in human DNA.
- A. BERNIS and H. v. PUTTEN, Department of Biochemistry, University of Nijmegen, The Netherlands: Integration site of endogenous Moloney murine leukemia virus.
- R. KOSHY,* F. WONG-STAAI,* R. C. GALLO,* W. HARDY,† and M. ESSEX,‡ *NCI, National Institutes of Health, Bethesda, Maryland; †Memorial Sloan-Kettering Cancer Center, New York, New York; ‡Harvard University, Boston, Massachusetts: Distribution of feline leukemia virus DNA sequences in tissues of normal and leukemic domestic cats.
- J. G. ANDRE, D. R. LOWY, and G. L. HAGER, NCI, National Institutes of Health, Bethesda, Maryland: Separation of integrated mouse mammary tumor virus and murine leukemia virus DNA sequences from cell DNA.
- L. T. BACHELOR and H. FAN, Tumor Virology Laboratory, Salk Institute, San Diego, California: Integration sites for Moloney murine leukemia virus DNA in infected mouse cells.
- M. COHEN,* N. DAVIDSON,* R. M. MCALLISTER,† M. O. NICOLSON,‡ N. RICE,‡ and R. V. GILDEN,‡ *Department of Chemistry, California Institute of Technology, Pasadena; †Childrens Hospital of Los Angeles, California; ‡Frederick Cancer Research Center, Frederick, Maryland: Sequence organization of BAB8-K (BKD) provirus in the baboon and RD(BKD) genomes.
- W. MCCLEMENTS,* L. BOONE,* H. HANAFUSA,† and A. SKALKA,* *Roche Institute of Molecular Biology, Nutley, New Jersey; †Rockefeller University, New York, New York: Analyses of endogenous and infecting avian oncornavirus proviral DNAs.
- E. CANAANI and S. A. AARONSON, NCI, National Institutes of Health, Bethesda, Maryland: Restriction enzyme analysis of unintegrated and integrated mouse sarcoma virus.
- F. WONG-STAAI,* M. S. REITZ, JR.,† and R. C. GALLO,* *NCI, National Institutes of Health; †Litton Bionetics, Inc., Bethesda, Maryland: Integrated viral sequences in tissues from a leukemic gibbon ape and its "normal" contact—Evidence for partial provirus in the nonleukemic gibbon.
- J. J. O'REAR, E. KESHET, and H. M. TEMIN, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: Restriction enzyme maps of integrated and unintegrated spleen necrosis virus DNAs of differing specific infectivities.
- F. CATALA and P. VIGIER, Faculté des Sciences, Institut du Radium, Orsay, France: Transfection studies with DNA from nonpermissive hamster cells transformed by Rous sarcoma virus.

Endogenous Viruses

- R. KOMINAMI,* and M. HATANAKA,* *Viral Oncology Program, Frederick Cancer Research Center, Frederick, Maryland; †NCI, National Institutes of Health, Bethesda, Maryland: A commonly shared genetic region of mammalian retroviruses.
- V. L. MORRIS,* C. KOZAK,† W. FLINTOFF,* P. JOLICOEUR,‡ F. RUDDLE,§ H. VARMUS,** *University of Western Ontario, London, Canada; †NIAID, National Institutes of Health, Bethesda, Maryland; ‡Institut de Recherches, Clinique de Montreal, Canada; §Yale University, New Haven, Connecticut; **Department of Microbiology, University of California, San Francisco: The distribution of mouse mammary tumor virus DNA among mouse chromosomes as determined by somatic cell hybrids.
- D. STRAYER, D. GILLESPIE, R. C. GALLO, B. VOGELSTEIN, and D. WHEELER, NCI, National Institutes of Health, Bethesda, Maryland: Organization of RD114 sequences in domestic cat DNA.
- W. DROHAN and J. SCHLOM, NCI, National Institutes of Health, Bethesda, Maryland: Detection of novel mouse mammary tumor virus-related DNA sequences and virions in rodents.
- S. G. DEVARE and J. R. STEPHENSON, NCI, National Institutes of Health, Bethesda, Maryland: Endogenous type-C viruses of the RD114/baboon group and type-D viruses of the MPMV/SMRV group share common interspecies antigenic determinants in their envelope glycoproteins.

- A. TEREBA, St. Jude Children's Research Hospital, Memphis, Tennessee: Chemical activation and regulation of a C-type virus from ringnecked pheasant cells.
- M. L. BRYANT, A. SEN, C. J. SHERR, and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Biochemical and immunological characterization of five different endogenous primate retroviruses.
- T. I. BONNER and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Distantly related primate and certain nonprimate species contain DNA sequences related to primate viruses.
- M. E. FRAZIER,* M. J. HOOPER,* J. R. PRATT,[†] and R. N. USHIJIMA,[‡] *Battelle, Pacific Northwest Laboratory, Richland, Washington; [†]Schering Corporation, Kenilworth, New Jersey; [‡]Department of Microbiology, University of Montana, Missoula: Characterization of retrovirus isolated from miniature swine with radiation-induced leukemia.
- Y. IKAWA,* M. OBINATA,* Y. UCHIYAMA,* M. AIDA,* H. ICHIMURA,[†] and H. SATO,[†] *Department of Viral Oncology, Cancer Institute; [†]Department of Pathology, Sasaki Institute, Tokyo, Japan: Increased endogenous virus expression in morphologically undifferentiated forms of rat carcinoma lines.
- S. S. YANG and N. A. WIVEL, NCI, National Institutes of Health, Bethesda, Maryland: Integrated intracisternal A-particle-specific DNA sequences in mammary tumor cell hybrids and cybrids.
- D. JOSEPH and J. N. IHLE, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Genetic and restriction endonuclease analysis of integrated murine leukemia virus DNA genomes.
- H. L. ROBINSON,* J. M. COFFIN,[†] and R. N. EISENMAN,[‡] *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; [†]Department of Molecular Biology and Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts; [‡]Fred Hutchinson Cancer Research Center, Seattle, Washington: V-E7 and V-EC, two cellular genes for the expression of inducible subgroup E avian leukosis virus, code for distinct viral products.
- E. KUFF, K. LUEDERS, and E. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: A relationship between intracisternal type-A particles of *Mus musculus* and an endogenous retrovirus (M432) of *Mus cervicolor*.

Session 8: Transforming Genes I

Chairperson: T. GRAF, Max-Planck-Institut, Tübingen, Germany

- R. L. ERIKSON,* J. S. BRUGGE,* A. SIDDIQUI,* M. S. COLLETT,* F. DEINHARDT,[†] and B. MARCZYNSKA,[†] *Department of Pathology, University of Colorado, Denver; [†]Department of Microbiology, Rush Medical School, Chicago, Illinois: Antibody to virus-specific proteins in mammals bearing avian sarcoma virus-induced tumors.
- G. JAY, R. P. C. SHIU, F. T. JAY, B. M. PATERSON, A. S. LEVINE, and I. PASTAN, NCI AND NIAMDD, National Institutes of Health, Bethesda, Maryland: Identification of a transformation-specific protein induced by a Rous sarcoma virus.
- K. BEEMON, T. HUNTER, and B. SEFTON, Tumor Virology Laboratory, Salk Institute, San Diego, California: Cell-free translation of Rous sarcoma virus *src* RNA.
- A. LEVINSON, H. OPPERMAN, L. LEVINTOW, H. VARMUS, and J. M. BISHOP, Department of Microbiology, University of California, San Francisco: In vivo and in vitro phosphorylation of avian sarcoma transformation-specific proteins.
- C. C. HALPERN, W. S. HAYWARD, and H. HANAFUSA, Rockefeller University, New York, New York: Recovery of sarcoma-specific sequences from host cells by transformation-defective mutants.
- L.-H. WANG and H. HANAFUSA, Rockefeller University, New York, New York: Comparison of *src* sequences of Rous sarcoma virus (RSV) and recovered avian sarcoma viruses (rASVs) (recovered sarcoma viruses from chicken tumors induced by transformation-defective RSVs).
- R. VIGNE,* M. BREITMAN,* C. MOSCOVICI,[†] and P. K. VOGT,* *Department of Microbiology, University of Southern California, School of Medicine, Los Angeles; [†]Virus Research Laboratory, Veterans Administration Hospital, Gainesville, Florida: Isolation and characterization of culture-derived and animal-derived transforming avian oncoviruses.
- D. H. SPECTOR,* J. M. BISHOP,* H. E. VARMUS,* H. HANAFUSA,[†] P. K. VOGT,[‡] and C. MOSCOVICI,[§] *Department of Microbiology, University of California, San Francisco; [†]Rockefeller University, New York, New York; [‡]University of Southern California, Los Angeles; [§]Veterans Administration Hospital, Gainesville, Florida: Mapping of endogenous *src* sequences in uninfected avian cells.
- U. R. RAPP, J. R. STEPHENSON, and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Generation of new mouse sarcoma viruses in cell culture.

- S. RASHEED and M. B. GARDNER, Department of Pathology, University of Southern California, School of Medicine, Los Angeles: Isolation of rat sarcoma viruses.
- D. TROXLER, D. LINEMEYER, S. RUSCETTI, and E. M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Potential relationship of helper-independent mink cell focus-inducing strains of Friend murine type-C virus to the origin of spleen focus-forming virus.
- J. H. ELDER,* F. C. JENSEN,* J. W. GAUTSCH,* M. POTTER,† J. HARTLEY,‡ and R. A. LERNER,* *Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California; †NCI, National Institutes of Health; ‡NIAID, National Institutes of Health, Bethesda, Maryland: Envelope gene recombinants in murine tumor systems.

Session 9: Transforming Genes II

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

- S. SAULE, M. ROUSSEL, and D. STEHELIN, INSERM Virology Unit, Lille, France and Institut Pasteur de Lille, France: A cDNA probe specific for avian erythroblastosis virus.
- D. SHEINSS and J. M. BISHOP, Department of Microbiology, University of California, San Francisco: Characterization of nucleotide sequences which may encode the oncogenic capacity of avian retrovirus MC29.
- T. GRAF, N. ADE, and H. BEUG, Max-Planck-Institut für Virusforschung, Tübingen, Germany: A mutant of avian erythroblastosis virus (AEV) temperature sensitive for hemoglobin expression.
- P. MELLON and P. DUESBERG, Department of Molecular Biology, University of California, Berkeley: The genome of MC-29, an avian acute leukemia virus.
- S. HU, M. M.-C. LAI, and P. K. VOGT, Department of Microbiology, University of Southern California, School of Medicine, Los Angeles: Biochemical and genetic studies on a defective carcinoma-inducing avian oncovirus—MH2.
- O. WITTE,* N. ROSENBERG,† M. PASIND,* A. SHIELDS,* R. de BARA,‡ and D. BALTIMORE, *Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge; †Department of Pathology and Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts; ‡The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Identification of an Abelson murine leukemia virus-encoded protein correlated with maintenance of the transformed state.
- A. E. FRANKEL, J. GILBERT, P. J. FISCHINGER, and E. M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: The nature and distribution of feline sarcoma virus-specific nucleotide sequences in cat DNA and RNA.
- C. J. SHERR,* A. SEN,* G. J. TODARO,* A. SLISKI,† and M. ESSEX,† *NCI, National Institutes of Health, Bethesda, Maryland; †Harvard School of Public Health, Boston, Massachusetts: A feline sarcoma virus-specific protein in rescued pseudo-type particles contains FOCMA, p15, and p12 antigens.
- A. SEN, U. R. RAPP, C. J. SHERR, and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Mammalian sarcoma virus-specific phosphoproteins in "rescued" virions.
- B. K. PAL, S. RASHEED, and P. ROY-BURMAN, University of Southern California, School of Medicine, Los Angeles: Phosphorylated polypeptides of mammalian sarcoma viruses.

Session 10: Poster Session

Viral Gene Expression

- A. DOLEI,* M. R. CAPOBIANCHI,* L. CIOE,* P. MEO,* F. BELARDELLI,† E. AFFABRIS,† and G. B. ROSSI,† *Istituto di Virologia, University of Rome; †Section of Virology, Istituto Superiore di Sanità, Rome, Italy: Biphasic effect(s) of interferon on FLC erythroid differentiation.
- G. VECCHIO,* G. COLLETTA,* M. L. SANDOMENICO,* A. DOLE,† M. CAPOBIANCHI,† and G. B. ROSSI,† *Cattedra di Virologia Oncologica and Centro di Endocrinologia e Oncologia Sperimentale del CNR, University of Naples; †Istituto di Virologia, University of Rome; †Istituto Superiore di Sanità, Rome, Italy: Control of the viral genome expression in Friend cells.
- E. C. MURPHY, JR. and R. B. ARLINGHAUS, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston: Separate mRNA species for the Rauscher murine leukemia virus gag (*gag-pol*) and *env* gene products.
- J. J. KOPCHICK,* K. F. WATSON,† E. C. MURPHY, JR.,* and R. B. ARLINGHAUS,* *University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston; †Department of Chemistry,

- University of Montana, Missoula: Biosynthesis of reverse transcriptase from Rauscher murine leukemia virus by synthesis and cleavage of a gag-pol read-through viral precursor polyprotein.
- S. EDWARDS, and H. FAN, Tumor Virology Laboratory, Salk Institute, San Diego, California: Glycosylation of gag polyprotein in Moloney murine leukemia virus-infected fibroblasts.
- L. MONTAGNIER, F. SINOSSI, R. LIDEREAU, and J. C. CHERMANN, Viral Oncology Unit, Institut Pasteur, Paris, France: Enhancement of retrovirus production by anti-interferon serum.
- J. LEIS* and R. SMITH,† *Department of Surgery and †Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina: A mechanism for control of processing of oncornavirus RNA.
- J. S. LEE, N. QUINTRELL, H. E. VARMUS, and J. M. BISHOP, Department of Microbiology, University of California, San Francisco: Identification of virus-specific messenger RNA in permissive and nonpermissive cells infected with avian sarcoma virus.
- M. BONDURANT,* R. RAMABHADRAN, M. GREEN, and W. S. M. WOLD, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Sarc sequence transcription in Moloney sarcoma virus-transformed nonproducer cell lines.
- C. J. M. SARIS, H. C. M. VAN EENBERGEN, and H. P. J. BLOEMERS, Department of Biochemistry, University of Nijmegen, The Netherlands: Cell-free translation of RNA species included in virions of Moloney murine leukemia virus.

Viral Proteins

- S. J. ANDERSON and R. B. NASO, University of Texas Systems Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston: Characterization of mouse mammary tumor virus proteins and precursor proteins.
- K. MOELLING and K. DITTMAR, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: Proteolytic activity in RNA tumor viruses.
- T. G. WOOD, D. LYONS, E. C. MURPHY, JR., and R. B. ARLINGHAUS, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston: Characterization of viral proteins synthesized in murine sarcoma virus (MuSV)-transformed cells and in a mRNA-dependent cell-free protein synthesis system programmed with RNA from MuSV-excess preparations.
- R. NUSSE,* F. ASSELBERGS,† M. SALDEN,† and R. MICHALIDES,* *Division of Virology, Antoni van Leeuwenhoekhuis, Amsterdam; †Department of Biochemistry, University of Nijmegen, The Netherlands: Processing of primary translation products of mouse mammary tumor virus RNA.
- P. V. O'DONNELL, E. STÖCKERT, Y. OBATA, A. B. DELEO, H. W. SNYDER, JR., and L. J. OLD, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of ecotropic, xenotropic, and dual-tropic murine leukemia virus (MuLV) isolates by in vitro induction of MuLV gag and env gene-coded cell-surface antigens.
- A. P. BELL, and V. M. VOGT, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Avian gag precursor—Processing in vitro and lack of cleavage in mammalian cells in vivo.
- M. OSKARSSON,* G. VANDE WOUDE,* J. ELDER,† J. GAUTSCH,† and R. LERNER,† *NCI, National Institutes of Health, Bethesda, Maryland; †Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California: Chemical determination of the m1 Moloney sarcoma virus pP60 gag gene order—Evidence for unique peptides in the C terminus of the polyprotein.
- J. PAPAMATHEAKIS and D. MARCIANI, NCI, National Institutes of Health, Bethesda, Maryland: Isolation and physicochemical characterization of the major avian myeloblastosis virus glycoprotein.
- L. ROHRSCHEIDER, C. LEITCH, and G. COX, Fred Hutchinson Cancer Research Center, Seattle, Washington: Analysis of the viral glycoproteins of an amphotropic avian sarcoma virus.
- P. H. YUEN and P. K. Y. WONG, Department of Microbiology and School of Basic Medical Sciences, University of Illinois, Urbana: The ribonucleoprotein component of the Moloney strain of murine leukemia virus.
- A. H. LU, M. M. SOONG, and P. K. Y. WONG, Department of Microbiology, University of Illinois, Urbana: Maturation of Moloney murine leukemia virus.
- R. NOWINSKI, J. LEDBETTER, and R. EISENMAN, Fred Hutchinson Cancer Research Center, Seattle, Washington: Metabolism and biosynthesis of murine leukemia virus-coded cell-surface antigens.

- S. L. MARCUS, S. W. SMITH, J. RACEVSKIS, and N. H. SARKAR, Memorial Sloan-Kettering Cancer Center, New York, New York: Purification and characterization of the low-molecular-weight polypeptides of the murine mammary tumor virus—pp23 and p16 bind to oncornaviral RNA.
- Y. YOSHINAKA and R. B. LUTFIG, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts: Leucine aminopeptidase activity associated with purified murine leukemia viruses.
- R. B. LUTFIG,* Y. YOSHINAKA,* and S. OROSZLAN,† *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; †Frederick Cancer Center, Frederick, Maryland: TUFTSIN can stimulate murine leukemia virus production.
- R. KLEMENZ and H. DIGGELMANN, ISREC—Epalinges, Switzerland: A new intermediate in the processing of the glycoprotein precursor of Rous sarcoma virus.
- M. C. KEMP and R. W. COMPANS, University of Alabama, Birmingham: Glycopeptides of Rauscher murine leukemia virus.
- P. DEZÈLÈE and F. CATALA, Fondation Curie, Institut du Radium, Orsay, France: Differences in the phosphoproteins of avian sarcoma viruses related to their oncogenic capacity.
- G. SCHOCHEMAN, R. GILDEN, D. FINE and R. MASSEY, Frederick Cancer Research Center, Frederick, Maryland: Analysis of mouse mammary tumor virus and murine leukemia virus cell-surface antigens on mammary tumor cells and in vitro infected cells.

Session 11: Poster Session

Transformation

- R. R. FRIIS,* and M. J. WEBER,† *Institut für Virologie, Giessen, Germany; †Department of Microbiology, University of Illinois, Urbana: Indications for multiple functions encoded in the *src* genetic element.
- Y. C. CHEN,* F. J. JENKINS,* and L. B. ALLEN,† *Department of Biological Sciences and Genetics Center, North Texas State University, Denton; †Department of Microbiology, Texas College of Osteopathic Medicine, Fort Worth: Suppression of transformation by ribavirin (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide) in rat kidney cells infected with temperature-sensitive mutants of Rous sarcoma virus.
- J. H. CHEN,* M. G. MOSCOVICI,† and C. MOSCOVICI,† *Life Sciences Biomedical Research Institute, St. Petersburg; †Tumor Virology Laboratory, Veterans Administration Hospital, Gainesville, and Department of Pathology, University of Florida, College of Medicine, Gainesville, Florida: Defectiveness of avian myeloblastosis virus (AMV)—Analysis of viral gene products in AMV transformed myeloblasts.
- C. GURGO,* M. B. BONDURANT,* and S. H. BRIDGES,* *Institute for Molecular Virology, St. Louis University Medical Center; †Department of Pathology, Washington University School of Medicine, St. Louis, Missouri: Cytoplasmic particles and expression of leukemia-related RNA sequences in the thymus of AKR mice.
- P. R. ANDERSEN,* D. BOETTIGER,† and P. D. LUNGER,‡ *NCI, National Institutes of Health, Bethesda, Maryland; †Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia; ‡Department of Life and Health Sciences, University of Delaware, Newark: Transformation of reptilian cells in vitro by avian sarcoma viruses.
- G. R. ANDERSON, K. R. MAROTTI, and P. WHITAKER-DOWLING, Department of Microbiology, School of Medicine, University of Pittsburgh, Pennsylvania: Rat *src* genes induced in uninfected cells.
- K. F. MANLY, Roswell Park Memorial Institute, Buffalo, New York: Xenotropic viruses in thymuses of preleukemic mice.
- D. DINA, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: The "sarcoma-specific" region of Moloney murine sarcoma virus 124.
- M. YOSHIDA,* Y. HIRAYAMA,* A. NOMOTO,† and Y. IKAWA,* *Department of Viral Oncology, Cancer Institute; †Department of Public Health, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan: Partial *src*-gene sequence in transformation-defective mutants of Rous sarcoma virus and their transforming capacity.
- T. PAWSON,* P. DUESBERG,† and G. S. MARTIN,* *Department of Zoology and †Virus Laboratory, University of California, Berkeley: Cell-free translation of virion RNA from Rous sarcoma virus and MC29.
- T. HUNTER, K. BEEMON, and J. PAPKOFF, Tumor Virology Laboratory, Salk Institute, San Diego, California: Cell-free translation of Moloney murine sarcoma virus clone 124 RNA.
- J. KAMINE and J. M. BUCHANAN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Processing of the 60,000-dalton *src* gene protein synthesized in vitro.

- G. CALOTHY, F. POIRIER, and B. PESSAC, Fondation Curie, Institut du Radium, Orsay, France: Phenotypic properties of transformation-defective mutants of Rous sarcoma virus and of avian erythroblastosis virus inducing chick embryo neuroretinal cell proliferation.
- C. MOSCOVICI, L. GAZZOLO, and M. G. MOSCOVICI, Tumor Virus Laboratory, Veterans Administration Hospital, and Department of Pathology, University of Florida Medical School, Gainesville: Oncogenic and transforming expressions of avian acute leukemia viruses.
- T. W. MAK, M. E. MACDONALD, and A. BERNSTEIN, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Canada: Cellular and molecular mechanisms in Friend virus carcinogenesis.
- H. W. SNYDER, JR., E. E. ZUCKERMAN, W. D. HARDY, JR., K. J. PHILLIPS, M. FOX, and E. FLEISSNER, Memorial Sloan-Kettering Cancer Center, New York, New York: Characterization of molecular species carrying feline leukemia virus-induced cell-surface antigens and FOCMA.
- A. H. SLISKI and M. ESSEX, Department of Microbiology, Harvard University School of Public Health, Boston, Massachusetts: Cross-species induction of the feline oncornavirus-associated cell membrane antigen by feline sarcoma virus.
- L. J. VAN GRIENSVEN, Tumor Virology Laboratory, Salk Institute, San Diego, California: Properties of the RNA of a "mink cell focus-inducing" (MCF) virus derived from spontaneous thymomas of BALB/c mice carrying Moloney murine leukemia virus as an endogenous virus.
- S. GISELBRUCHT, P. DEBRE, C. BLAINEAU, M. A. HUROT, O. MULLER, and J. P. LEVY, Laboratoire d'Immunologie et Virologie des Tumeurs, INSERM, Hôpital Cochin, Paris, France: Genetic control of sensitivity to Moloney virus-induced leukemias in mice.
- L. P. TUREK and P. K. VOGT, Department of Microbiology, University of Southern California, School of Medicine, Los Angeles: Randomly derived avian sarcoma virus-infected normal rat kidney cell clones: Parameters of transformation and virus recovery.
- K. D. SOMERS, Eastern Virginia Medical School, Norfolk: A novel morphological revertant of mouse sarcoma virus-transformed rat cells.
- J. S. RHIM,* C. CHANG,† and R. J. HUEBNER,† *Microbiological Associates, Inc.; †NCI, National Institutes of Health, Bethesda, Maryland: Solubilization and characterization of cross-reacting rat tumor antigens.
- N. KOBAYASHI and A. KAJI, Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia: Differentiation and cellular transformation-expression of *src* gene in terminally differentiated myotubes.
- P. DEZÉLÉE, F. CATALO, and J. M. BIQUARD, Fondation Curie, Institut du Radium, Orsay, France: Differences in total proteins of normal and avian oncornavirus-infected chick embryo fibroblasts analyzed by high-resolution polyacrylamide gel electrophoresis and computerized quantification of protein bands.
- E. M. DURBAN and D. BOETTIGER, Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia: Effect of transformation by avian myeloblastosis virus on the progressive differentiation of avian yolk-sac cells *in vitro*.
- A. SCHINCARIOL and J. RIP, Cancer Research Laboratory, University of Western Ontario, London, Canada: Sequence complexity of polysomal RNA in murine sarcoma virus-transformed mouse cells.
- A. SCHINCARIOL, P. MAHER and J. RIP, Cancer Research Laboratory, University of Western Ontario, London, Canada: Alteration in chromatin of cells infected with RNA tumor viruses.
- M. S. MCGRATH and I. WEISSMAN, Department of Pathology, Stanford University Medical Center, California: Identification and characterization of murine leukemia virus receptors on thymic lymphoma cells.
- W. D. HARDY, JR. and E. E. ZUCKERMAN, Memorial Sloan-Kettering Cancer Center, New York, New York: Immune response of healthy cats to feline leukemia virus exposure.
- P. RICCIARDI-CASTAGNOLI,* M. LIEBERMAN,† O. FINN,† and H. S. KAPLAN,† *Istituto di Farmacologia, Università Statale di Milano, Italy; †Department of Radiology, Stanford University Medical Center, California: Lymphoma induction by radiation leukemia virus in athymic nude mice.
- K. ULRICH* and B. A. NEXØ,† *The Fibiger Laboratory, Copenhagen, Denmark; †Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine, Baltimore, Maryland: C-type virus activated during chemical leukemogenesis in mice.
- S. K. RUSCETTI, D. L. LINEMEYER, D. H. TROXIER, and E. M. SCOLNICK, NCI, National Institutes of Health, Bethesda, Maryland: Type-specific immunoassays for the gp70s of MCF murine leukemia viruses and the expression of cross-reacting antigens in spleen focus-forming virus-infected rat cells as well as certain normal mouse cell lines.

Session 12: Transformation

Chairperson: N. ROSENBERG, Tufts University, Boston, Massachusetts

- H. BEUG and T. GRAF, Max-Planck-Institut für Virusforschung, Tübingen, Germany: Role of the cell nucleus in the expression of Rous sarcoma virus-induced cell transformation.
- K. RADKE and G. S. MARTIN, Department of Zoology, University of California, Berkeley: Polypeptides of cells infected by Rous sarcoma virus.
- D. BOETTIGER, M. PACIFICI and H. HOLTZER, Departments of Microbiology and Anatomy, University of Pennsylvania, Philadelphia: The transition of mesenchymal cells to mature chondroblasts is irreversibly blocked by infection with Rous sarcoma virus.
- L. GAZZOLO,*† C. MOSCOVICI,† and M. G. MOSCOVICI,† *Unite de Virologie, INSERM, Lyon, France; †Veterans Administration Hospital and Department of Pathology, University of Florida School of Medicine, Gainesville: Expression of functional markers by leukemic cells.
- F. RUSCETTI, S. COLLINS, R. GALLAGHER, and R. GALLO, NCI, National Institutes of Health, Bethesda, Maryland: Inhibition of human myeloid differentiation in vitro by primate type-C retroviruses.
- I. WEISSMAN and M. S. MCGRATH, Department of Pathology, Stanford University Medical Center, California: The receptor-mediated model of leukemogenesis—Thymic maturation and AKR leukemogenesis.
- R. RISSE,* D. J. GRUNWALD,* E. STOCKERT,† P. JELEN,* C. SINAIKO,* and L. J. OLD,† *McArdle Laboratory for Cancer Research, University of Wisconsin, Madison; †Memorial Sloan-Kettering Cancer Center, New York, New York: The Abelson antigen—A viral tumor antigen that is related to a differentiation alloantigen.
- A. DECLÈVE,* M. LIEBERMAN,* J. N. IHLE,† and H. S. KAPLAN,* *Department of Radiology, Stanford University Medical Center, California; †NCI, Frederick Cancer Research Center, Frederick, Maryland: Characterization of the thymotropic and leukemogenic viruses isolated from the C57BL/Ka strain of mice.
- A. J. MAYER, M. L. DURAN-REYNALS, F. LILLY, and F. DURAN-STRUUCK, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: Association between suppression of endogenous ecotropic and xenotropic murine leukemia virus expression and suppression of spontaneous leukemia in crosses between AKR/J and RF/J mice.
- J. E. DE LARCO and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: Growth factors (SGFs) produced by murine sarcoma virus-transformed cells.

SV40, POLYOMA, AND ADENOVIRUSES, July 26–July 30

arranged by

Terri Grodzicker, Cold Spring Harbor Laboratory

Michael Botchan, Cold Spring Harbor Laboratory

295 participants

Session 1: SV40 and Polyoma—Genetics and Genome Structure

Chairperson: D. NATHANS, Johns Hopkins University, School of Medicine, Baltimore, Maryland

- D. SHORTLE, S. LAZAROWITZ, and D. NATHANS, Johns Hopkins University School of Medicine, Baltimore, Maryland: SV40 mutants with base substitutions around the origin of viral DNA replication.
- J. R. ARRAND, B. E. GRIFFIN, N. SMOLAR, and E. SOEDA, Imperial Cancer Research Fund Laboratories, London, England: The primary sequences of the late region of polyoma virus DNA.
- G. C. CARMICHAEL, J. HATTORI, and T. L. BENJAMIN, Department of Pathology, Harvard Medical School, Boston Massachusetts: Structural organization of the DNAs of nontransforming host-range mutants of polyoma virus.
- M. M. BENDIG and W. R. FOLK, Department of Biological Chemistry, University of Michigan, Ann Arbor: Polyoma mutants with alterations at the *Hind*III, *Hind*II, and *Hae*III cleavage sites.
- J. E. MERTZ and S.-D. YU, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison: A new complementation group of SV40.

- D. HAMER and P. LEDER, NICHD, National Institutes of Health, Bethesda, Maryland: SV40 recombinants carrying rabbit globin-gene sequences.
- W. SCHAFFNER, W. TOPP, and M. BOTCHAN, Cold Spring Harbor Laboratory, New York: SV40-sea urchin histone DNA recombinant integrated into mammalian chromosomes can be recovered in its original form.
- Y. GLUZMAN, Cold Spring Harbor Laboratory, New York: A new Ad5-SV40 hybrid which contains the entire SV40 genome.
- R. C. A. YANG and R. WU, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: BK virus DNA—Physical maps and coding sequences for T-antigens.

Session 2: SV40 and Polyoma—Early Proteins

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

- T. HUNTER, M. A. HUTCHINSON, and W. ECKHART, Tumor Virology Laboratory, Salk Institute, San Diego, California: Characterization of three polyoma T antigens.
- B. SCHAFFHAUSEN, J. SILVER, and T. L. BENJAMIN, Department of Pathology, Harvard Medical School, Boston, Massachusetts: T antigens of wild type and nontransforming mutants of polyoma virus.
- Y. ITO and J. E. SMART, Imperial Cancer Research Fund Laboratories, London, England: Three forms of polyoma virus early proteins share common tryptic peptides probably near the N termini of the proteins.
- E. PAUCHA, R. HARVEY, and A. E. SMITH, Imperial Cancer Research Fund Laboratories, London, England: Cell-free synthesis of SV40 T antigens.
- E. MAY, M. KRESS, and P. MAY, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Characterization of the two SV40 early mRNAs coding for small-t and large-T antigens—Evidence that nuclear RNA from infected mouse cells is translated very efficiently into small-t antigen.
- D. T. SIMMONS,* G. JAY,† P. T. MORA,‡ and M. A. MARTIN,* *NIAID and †NCI, National Institutes of Health, Bethesda, Maryland: Characterization of new species of tumor antigens from SV40- and polyoma-transformed cells.
- K. RUNDLELL, Department of Microbiology-Immunology, Northwestern University, Chicago, Illinois: Additional early proteins induced by SV40.
- J. A. MELERO, R. CARROLL, D. STIT,* and W. MANGEL,* Department of Pathology, New York University Medical Center, New York; *Department of Biochemistry, University of Illinois, Urbana: Multiple 48–55K antigenic species immuno-precipitable by antiserum raised to purified large-T antigen of SV40.
- A. GRAESSMANN, M. GRAESSMANN, and C. MUELLER, Institut für Molekularebiologie und Biochemie der Freien Universität Berlin, Germany: The biological activity of different early SV40 DNA fragments.

Session 3: Poster Session—SV40 and Polyoma

- C. BLACKBURN and J. D. HARE, Department of Microbiology, University of Rochester School of Medicine and Dentistry, New York: Quantification of DNA synthesis and template activity in SE3049 polyoma virus.
- J. N. BRADY, V. D. WINSTON, and R. A. CONSIGLI, Kansas State University, Manhattan: Characterization of a DNA-protein complex and capsomere subunits derived from polyoma virus by treatment with ethyleneglycol-bis-N,N'-tetraacetic acid (EGTA) and dithiothreitol (DTT).
- H. CHAN, M. ISRAEL, W. ROWE,* and M. MARTIN, Laboratory of Biology of Viruses and *NIAID, National Institutes of Health, Bethesda, Maryland: Construction of bacteriophage lambda-containing polyoma virus DNA.
- C. CHANG,* D. T. SIMMONS,† W. TOPP,‡ M. A. MARTIN,* and P. T. MORA,* *NCI and †NIAID, National Institutes of Health, Bethesda, Maryland; ‡Cold Spring Harbor Laboratory, New York: Identification of a new species of SV40-specific antigens.
- L. V. CRAWFORD,* D. C. PIM,* P. Z. O'FARRELL,† C. N. COLE,‡ H. VAN HEUVERSWEYN,§ and W. FIERS,§ *Department of Molecular Virology, Imperial Cancer Research Fund Laboratories, London, England; †Department of Biochemistry and Biophysics, University of California, San Francisco; ‡Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut; §Laboratory of Molecular Biology, University of Ghent, Belgium: The effect of alkylation on SV40 T antigens and related polypeptides.
- T. CREFFELD and W. KELLER, Department of Microbiology, University of Heidelberg, Germany: Transcription of SV40 in vivo and in vitro.

- W. DEPPERT, Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany: Distribution of SV40 T and U antigens in different nuclear subfractions of SV40-transformed cells.
- J. FAVALORO, B. KAMEN, J. PARKER, and R. TREISMAN, Imperial Cancer Research Fund Laboratories, London, England: Topography of polyoma virus transcripts.
- R. FEIGINY and M. CENTER, Division of Biology, Kansas State University, Manhattan: Polyoma DNA synthesis in isolated nucleoprotein complexes.
- B. FÖHRING, P. GRUSS, and G. SAUER, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Heidelberg, Germany: SV40 virions contain linear FOIII DNA molecules whose ends map close to the origin of DNA replication.
- J. P. FORD and M. T. HSU, Molecular Cell Biology Department, Rockefeller University, New York, New York: Late SV40 mRNA biogenesis.
- T. FRIEDMANN,* A. ESTY,* P. LAPORTE,* R. DOOLITTLE,† and G. WALTER,‡ Departments of *Pediatrics and †Chemistry, University of California, San Diego; ‡Salk Institute, San Diego, California: Nucleotide sequence studies of polyoma tumor antigens.
- E. GARBER, M. SEIDMAN, and A. J. LEVINE, Department of Biochemical Sciences, Princeton University, New Jersey: SV40 nucleoprotein complexes—Efficient virion formation in vivo and the lability of virions in vitro.
- S. GATTONI, D. ZOUZIAS, and C. BASILICO, Department of Pathology, New York University School of Medicine, New York: Control of the state of the viral DNA in rat cells transformed by polyoma virus.
- P. K. GHOSH,* V. B. REDDY,† J. SWINSOE,† P. LEBOWITZ* and S. M. WEISSMAN,† Departments of *Internal Medicine and †Human Genetics, Yale University, New Haven, Connecticut: Heterogeneity and 5'-terminal structure of late mRNAs of SV40.
- B. E. GRIFFIN, C. MADDOCK, N. SPURR, and Y. ITO, Imperial Cancer Research Fund Laboratories, London, England: Viable deletion mutants of polyoma virus.
- J. D. GRIFFIN and D. M. LIVINGSTON, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Intermediate size and very low molecular weight of SV40 T antigens.
- J. B. HISCOTT and V. DEFENDI, Department of Pathology, New York University Medical Center, New York: Characteristics of human fibroblasts transformed by subgenomic fragments of SV40 DNA.
- M. HOROWITZ, S. BRATOSIN, and Y. ALONI, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: Electron microscope evidence for splicing of polyoma late mRNAs.
- M.-T. HSU, M. COCA-PRADOS, and R. FERNANDEZ-MUNOZ, Rockefeller University, New York, New York: Characterization of intracellular SV40 nucleoprotein complexes.
- E. KINNEY-THOMAS and J. D. HARE, Department of Microbiology, University of Rochester School of Medicine and Dentistry, New York: Restriction analysis of a polyoma variant which produces an excess of virion-associated proteins.
- M. KRESS, E. MAY, and P. MAY, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France: Study of anti-T immunoprecipitable proteins present in addition to large-T and small-t antigens in SV40-transformed cell lines.
- R. E. LANFORD and J. S. BUTEL, Department of Virology, Baylor College of Medicine, Houston, Texas: Characteristics of a cytoplasmic form of SV40 tumor antigen.
- S. LAVI and Z. GROSSMAN, Department of Virology, Weizmann Institute of Science, Rehovot, Israel: The effect of chemical carcinogens on the expression of SV40 genes.
- M. F. LAW, K. K. TAKEMOTO, and P. M. HOWLEY, NCI, National Institutes of Health, Bethesda, Maryland: Characterization of the genome of K virus, a murine papovavirus.
- J. MARTIN, R. FRISQUE, B. PADGETT, and D. WALKER, Department of Medical Microbiology, University of Wisconsin Medical School, Madison: A physical map of the DNA of JC virus.
- M. P. MOYER and R. C. MOYER, Trinity University, San Antonio, Texas: SV40 rescue from "nonrescuable" cell lines.
- N. NEWELL and T. J. KELLY, JR., Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland: Base-sequence homologies among the genomes of the primate papovaviruses.
- M. PATER, A. PATER, and G. DI MAYORCA, Department of Microbiology, University of Illinois Medical Center, Chicago: Isolation and characterization of BK virus DNA from a human cell line from transitional cell carcinoma of the urethra.
- B. J. POMERANTZ and J. D. HARE, Department of Microbiology, University of Rochester School of Medicine and Dentistry, New York: A variant of polyoma virus that overproduces processed viral RNA.

- P. RIGBY,* J. COOK,* M. LOVETT,* P. GALLIMORE,† C. COLE,‡ and P. BERG,‡ *Department of Biochemistry, Imperial College of Science and Technology, London, England; †Department of Cancer Studies, University of Birmingham Medical School, England; ‡Department of Biochemistry, Stanford University Medical Center, California: Viral DNA integration patterns and the biological properties of SV40-transformed mouse cells.
- A. ROMAN, Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis: The growth of papovaviruses in primary isolates from different organs.
- A. SEN, C. M. PUCHALSKI, and G. J. TODARO, NCI, National Institutes of Health, Bethesda, Maryland: The replication of SV40 and the expression of gene A in human cells—Interactions between viral and cellular growth regulatory functions.
- C.-K. J. SHEN and J. E. HEARST, Department of Chemistry, University of California, Berkeley: Specific loop formation of denatured SV40 DNA—A possible mechanism for the splicing of early and late SV40 transcripts.
- A. E. SMITH, A. MELLOR, R. SMITH, E. PAUCHA, R. M. HEWICK, and M. D. WATERFIELD, Imperial Cancer Research Fund Laboratories, London, England: Sequence and fingerprint analysis of SV40 T antigens.
- E. SOEDA, J. ARRAND, N. SMOLAR, and B. E. GRIFFIN, Imperial Cancer Research Fund Laboratories, London, England: The early region of polyoma virus DNA.
- K. N. SUBRAMANIAN, Department of Microbiology, University of Illinois Medical Center, Chicago: Isolation and characterization of deletion mutants of SV40 mapping within the leader sequence of the major late viral mRNA.
- C. T. WAKE and J. H. WILSON, Department of Biochemistry, Baylor College of Medicine, Houston, Texas: Development of SV40 as a genetic system for investigating mammalian recombination mechanisms.
- F. O. WETTSTEIN and J. G. STEVENS, Department of Microbiology and Immunology, University of California School of Medicine, Los Angeles: Virus-specific DNA and RNA in Shope-papilloma virus-induced tumors.
- J. H. WILSON, Department of Biochemistry, Baylor College of Medicine, Houston, Texas: Interference in SV40 DNA infections—A possible basis for cellular competence.
- D. ZOULIAS,* K. K. JHA,† C. MULDER,‡ C. BASILICO,* and H. L. OZER,† *New York University School of Medicine, New York; †Hunter College, New York, New York; ‡University of Massachusetts Medical School, Worcester: Extrachromosomal SV40 DNA in transformed human fibroblasts.

Session 4: SV40 and Polyoma-Transformed Cells

Chairperson: P. TEGTMEYER, State University of New York, Medical School, Stony Brook

- G. JAY, F. T. JAY, C. CHANG, R. M. FRIEDMAN, and A. S. LEVINE, NCI and NIAMDD, National Institutes of Health, Bethesda, Maryland: Use of the Ad2*ND1 hybrid virus to identify the protein responsible for SV40 tumor rejection and its genetic origin.
- D. P. LANE and L. V. CRAWFORD, Department of Molecular Virology, Imperial Cancer Research Fund Laboratories, London, England: SV40 virus-coded proteins are displayed on the surface of SV40 transformed cells.
- A. M. LEWIS, JR. and J. L. COOK, NIAID, National Institutes of Health, Bethesda, Maryland: The association of tumor induction by Ad2-SV40 recombinants with a specific segment of SV40 DNA.
- D. G. ATTARDI, L. LANIA, M. GRIFFITHS, B. COOKE, and M. FRIED, Imperial Cancer Research Fund Laboratories, London, England: Polyoma virus sequences in transformed and untransformed rat cells.
- M. M. FLUCK and T. L. BENJAMIN, Department of Pathology, Harvard Medical School, Boston, Massachusetts: Properties of cells transformed by *ts-a/A* mutants of polyoma virus and SV40.
- M. RASSOULZADEGAN, P. GAUDRAY, B. PERBAL, N. CHENCINER, E. MOUGNEAU, and F. CUZIN, Centre de Biochimie, Université de Nice, France: Transformation of rat fibroblast cells by polyoma and SV40 viruses—Characterization of two classes of virus-induced transformants.
- R. SEIF, M. PERSICO-DILAURO, V. P. SETLOW, C. A. F. EDWARDS, and R. G. MARTIN, NIAMDD, National Institutes of Health, Bethesda, Maryland: Polyoma and SV40 early functions involved in transformation.
- C. C. ROBINSON and J. M. LEHMAN, Department of Pathology, University of Colorado Medical Center, Denver: DNA content changes in SV40 *tsA*-transformed Chinese hamster cells at the permissive and nonpermissive temperature.
- W. TOPP,* M. SLEIGH,† D. RIFKIN,‡ C. CHANG,§ and S. SHIN,** *Cold Spring Harbor Laboratory, New York;

[†]Molecular Biology Unit, CSIRO, Epping, Australia; [‡]New York University Medical School, New York; [§]NCI, National Institutes of Health, Bethesda, Maryland; ^{**}Department of Genetics, Albert Einstein College of Medicine, Bronx, New York: SV40 viral mutants defective in small t are unable to induce any of the phenotypes associated with maximally transformed cells.

Session 5: Adenoviruses—Transformed Cells and Early Transcription

- Chairperson: H. GINSBERG, Columbia University, College of Physicians and Surgeons, New York, New York
- N. JONES, W. COLBY, and T. SHENK, Department of Microbiology, University of Connecticut Health Center, Farmington: Isolation of Ad5 deletion mutants defective for transformation of rat embryo cells.
- K. DORSCH-HÄSLER, P. B. FISHER, and H. S. GINSBERG, Department of Microbiology and Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York: Integration patterns of viral DNA in cells transformed by WT5 adenovirus and H5ts125.
- J. GRONEBERG, D. SUTTER, H. IBEUGAUFIS, H. SOBOLL,* and W. DOERFLER, Institute of Genetics, University of Cologne; *Paul Ehrlich Institute, Frankfurt, Germany: Patterns of integration of Ad12 DNA in transformed hamster cells, in revertants, and in rat brain tumor cells.
- K. FUJINAGA, Y. SAWADA, Y. UEMIZU, S. OJIMA, K. SHIROKI,* and H. SHIMOJO,* Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College; *Institute of Medical Science, University of Tokyo, Japan: Analysis of the viral genome present in cells transformed by the Ad12 DNA fragment.
- H. JOCHEMSEN, J. H. LUPKER, A. DAVIES, and A. J. VAN DER EB, Sylvius Laboratories, University of Leiden, The Netherlands: Location of the transforming region and identification of the early gene products of Ad12 in vitro and in vivo.
- A. J. BERK and P. A. SHARP, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge: Sizing and mapping of pulse-labeled early Ad2 nuclear and cytoplasmic RNAs.
- L. T. CHOW, T. R. BROKER, and J. B. LEWIS, Cold Spring Harbor Laboratory, New York: Temporal differentiation in the splicing pattern of mRNA for Ad2 single-strand DNA-binding protein and characterization of new spliced early RNAs.
- T. H. CARTER and R. A. BLANTON, Pennsylvania State University College of Medicine, Hershey: Regulation of Ad5 early transcription by the 72,000 dalton DNA-binding protein.
- D. SPECTOR, D. HALBERT, M. MCGROGAN, and H. J. RASKAS, Department of Pathology, Washington University School of Medicine, St. Louis, Missouri: Gene products specified by early region 1 of the Ad2 genome.

Session 6: Poster Session—Adenoviruses

- A. R. BIATTI and J. WEBER, Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada: Core protein PVII-specific endoprotease of Ad2.
- S. BLANCHARD, E. DAVID, and W. WINTERS, University of Texas Health Science Center, San Antonio: DNA replication and capsid antigen synthesis in human cells infected with Ad5 and Ad31.
- B. DRAGON and H. S. GINSBERG, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, New York: Studies on the "100K" protein from Ad-5 infected cells.
- H. ESCHÉ, R. SCHILLING, and W. DOERFLER, Institute of Genetics, University of Cologne, Germany: In vitro translation of Ad12-specific mRNA prepared from infected and transformed cells.
- M. FEDOR, G. KIT, T. MULLENBACH, and E. DANIELL, Department of Molecular Biology, University of California, Berkeley: Adenovirus chromatin composition and production in infected cell nuclei.
- C. J. GOLDENBERG and H. J. RASKAS, Department of Pathology, Washington University School of Medicine, St. Louis, Missouri: Evidence for cleavage and ligation of a nuclear precursor during formation of mRNA from early region 2 of the Ad2 genome.
- S. HASHIMOTO and M. GREEN, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Evidence for at least six 5'-terminal structures for Ad2 early mRNA molecules.
- B. A. KILPATRICK and R. E. GELINAS, Cold Spring Harbor Laboratory, New York: Comparison of leader sequences and message locations among adenovirus serotypes.
- J. MAAT, H. VAN ORMONDT, and P. I. SCHRIER, Sylvius Laboratories, University of Leiden, The Netherlands: Nucleotide sequence studies in the transforming region of Ad5 DNA.
- R. RICCIARDI,* B. ROBERTS,* B. PATERSON,[†] and M. MATHEWS,[‡] *Department of Biology, Brandeis University, Waltham, Massachusetts; [†]NCI, National Institutes of Health, Bethesda, Maryland; [‡]Cold Spring Harbor Laboratory, New York: The organization of the Ad2 genome.

- P. I. SCHRIER and A. J. VAN DER EB, Sylvius Laboratories, University of Leiden, The Netherlands: Characterization of T antigens from cells transformed by fragments of Ad5 DNA.
- P. B. SEHGAL, N. W. FRASER, M. C. WILSON, and J. E. DARNELL, Rockefeller University, New York, New York: Mapping of early Ad2 promoters in HeLa cells using 5,6-dichloro- β -D-ribofuranosylbenzimidole (DRB) and UV irradiation.
- K. SHIROKI,* H. SHIMOJO,* Y. SAWADA,[†] and K. HUIJINAGA,[†] *Institute of Medical Science, University of Tokyo, Shirokanedai Minato-ku; [†]Cancer Research Institute, Sapporo Medical College, Japan: The transforming gene of Ad12 DNA.
- S. E. STRAUS, A. SERGEANT, M. A. TIGGES, and H. J. RASKAS, Department of Pathology, Washington University School of Medicine, St. Louis, Missouri: The infecting parental Ad2 genome is recovered from early or late nuclei as "circular" DNA-protein complexes.
- J. WEBER, D. PLAAT, and R. BHATTI, Département de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada: The processing of adenovirus core protein 50K to core protein V is linked to but not dependent on DNA packaging.
- C. S. H. YOUNG, Department of Microbiology Columbia University, College of Physicians and Surgeons, New York, New York: The biological characteristics of adenovirus recombination.

Session 7: Adenoviruses—Late Transcription

Chairperson: J. FLINT, Princeton University, Princeton, New Jersey

- A. R. DUNN, M. B. MATHEWS, L. T. CHOW, J. SAMBROOK, and W. KELLER,* Cold Spring Harbor Laboratory, New York; *Universität Heidelberg, Germany: Leader sequences associated with a hybrid mRNA which includes the start of the Ad2 fiber gene—Preliminary characterization of a clone (*E. coli* strain 776) containing a DNA copy of Ad2 fiber mRNA.
- L. T. CHOW, J. B. LEWIS, T. GRODZICKER, J. SAMBROOK, and T. R. BROKER, Cold Spring Harbor Laboratory, New York: Mapping of additional Ad2 late mRNAs and characterization of RNA processing intermediates for the late Ad2 and Ad2-SV40 hybrid viruses.
- K. K. KALGHATGI, B. A. ROLLER, and L. D. HODGE, Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut: Studies on adenovirus anti-mRNA.
- E. ZIFF and R. EVANS, Rockefeller University, New York, New York: The capped 5' terminus of Ad2 late nuclear transcripts.
- J. R. NEVINS and J. E. DARNELL, JR., Rockefeller University, New York, New York: Processing of late adenovirus nuclear RNA.
- S. BACHENHEIMER, Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill: Heterogeneity at the 5' end of adenovirus 35S nuclear RNA.
- R. M. EVANS and J. E. DARNELL, Rockefeller University, New York, New York: A new class of adenovirus small nuclear RNA.
- J. WEBER and J. M. BLANCHARD, Rockefeller University, New York, New York: In vitro processing of early adenovirus RNA in isolated nuclei.
- J. L. MANLEY, P. A. SHARP, and M. L. GEFTER, Department of Biology, Massachusetts Institute of Technology, Cambridge: Synthesis and processing of adenovirus RNA in isolated nuclei.

Session 8: SV40 and Polyoma—Replication

Chairperson: M. A. MARTIN, NIAID, National Institutes of Health, Bethesda, Maryland

- P. CLERTANT and F. CUZIN, Centre de Biochimie, Université de Nice, France: Induction of in vitro synthesis of polyoma virus DNA by partially purified T antigen.
- C. PRIVES, H. SHURE, Y. BECK, D. GIDONI, and M. OREN, Department of Virology, Weizmann Institute of Science, Rehovot, Israel: DNA binding and sedimentation properties of the proteins specified by the early region of SV40.
- D. M. LIVINGSTON, M. P. KRIEGLER, J. D. GRIFFIN, and J. HUDSON, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts: Complementation of the *tsA* mutant defect in viral DNA replication following microinjection of highly purified SV40 T antigen.
- E. FANNING, K.-H. KLEMPNAUER, B. OTTO, and I. BAUMGARTNER, Fachbereich Biologie, University of Konstanz, Germany: Structure of replicating and nonreplicating SV40 chromatin.
- T. M. HERMAN, M. L. DEPAMPHILIS, and P. M. WASSARMAN, Department of Biological Chemistry, Harvard

Medical School, Boston, Massachusetts: The structure of chromatin at the replication fork of SV40 chromosomes.

W. A. SCOTT and D. J. WIGMORE, Department of Biochemistry, University of Miami School of Medicine, Florida: Distribution of endonuclease-sensitive sites in SV40 chromatin.

B. I. MILAVETZ, J. A. BURKE, M. J. EVANS, J. PLUMMER, M. J. EDDY, M. A. WAGAR, Y. TSUBOTA, and J. A. HUBERMAN, Roswell Park Memorial Institute, Buffalo, New York: Proteins associated with SV40 chromosomes.

K. MANN and T. HUNTER,* Biology Department, University of Alaska, Anchorage; *Tumor Virology Laboratory, Salk Institute, San Diego, California: Association of SV40 T antigen with SV40 nucleoprotein complexes.

N. YAMAGUCHI and M. KUBOTA, Institute of Medical Science, University of Tokyo, Japan: Association of SV40 T antigen with chromatin in SV40-transformed cells.

Session 9: SV40 and Polyoma—Transcription

Chairperson: B. GRIFFIN, Imperial Cancer Research Fund Laboratories, London, England

A. COWIE, A. FLAVELL, B. KAMEN, and S. LEGON, Imperial Cancer Research Fund Laboratories, London, England: Heterogeneity at the 5' ends of polyoma virus late mRNAs.

Y. ALONI, S. BRATOSIN, M. HOROWITZ, and O. LAUB, Department of Genetics, Weizmann Institute of Science, Rehovot, Israel: Splicing of SV40 late mRNAs.

M. BINA-STEIN, M. THOREN, N. SALZMAN, and T. THOMPSON, NIAID, National Institutes of Health, Bethesda, Maryland: A new method for studying spliced regions in mRNA.

V. B. REDDY,* P. K. GHOSH,† P. LEBOWITZ,† and S. M. WEISSMAN,* Departments of *Human Genetics and †Internal Medicine, Yale University School of Medicine, New Haven, Connecticut: Primary structure of SV40 early mRNA.

G. HAEGEMAN, H. VAN HEUVERSWYN, A. VAN DE VOORDE, and W. FIERS, Laboratory of Molecular Biology, State University of Ghent, Belgium: Characterization of late SV40 mRNA.

H. MANOR, M. WU, and N. DAVIDSON, Department of Chemistry, California Institute of Technology, Pasadena: Electron microscopic analysis of the polyoma virus "late" RNA species.

N. H. ACHESON, Department of Virology, Swiss Institute for Experimental Cancer Research, Epalinges: Polyoma giant nuclear RNAs contain tandem repeats of the nucleotide sequence of the entire viral genome.

S. SEGAL and G. KHOURY, NCI, National Institutes of Health, Bethesda, Maryland: Expression of early and late SV40 genes in nonpermissive cells.

B. THIMMAPPAYA, M. FITZGERALD, and T. SHENK, Department of Microbiology, University of Connecticut Health Center, Farmington: Novel viral transcripts are synthesized in cells infected with a viable variant of SV40.

Session 10: Adenoviruses—Proteins

Chairperson: R. ROBERTS, Cold Spring Harbor Laboratory

O. BRISON, C. KEDINGER, and P. CHAMBON, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS et Faculté de Médecine, Unité 44 de l'INSERM, Strasbourg, France: Ad2 replication and transcription complexes.

T. YAMASHITA, M. ARENS, M. GREEN, K. BRACKMANN, and M. CARTAS, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: Ad2 DNA synthesized in vitro and in vivo in the absence of late protein synthesis contains a protein attached to both termini.

R. PADMANABHAN, M. SHINAGAWA, C. F. GARON, and J. A. ROSE, Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, and NIAID, National Institutes of Health, Bethesda, Maryland: Interaction of other virion proteins with a specific protein of 55,000 daltons at the termini of Ad2 DNA.

S. ROSS, S. J. FLINT, and A. J. LEVINE, Department of Biochemical Sciences, Princeton University, New Jersey: Adenovirus early proteins detected in infected and transformed cells.

T. G. STORCH and J. V. MAIZEL, JR., NICHD, National Institutes of Health, Bethesda, Maryland: The effects of glycosylation inhibitors on the synthesis of early Ad2 proteins.

- D. M. FOWLKES, S. T. LORD, T. LINNE, U. PETERSSON, and L. PHILIPSON, Department of Microbiology, Uppsala University Biomedical Center, Sweden: Interaction between the adenovirus DNA-binding protein and double-stranded DNA.
- D. KLESSIG and J. HASSELL, Cold Spring Harbor Laboratory, New York: Characterization of mutants of human adenovirus which multiply efficiently in simian cells.
- J. ARRAND, National Institute for Medical Research, London, England: Analysis of adenovirus gene function using DNA infection techniques.
- C. TIBBETTS, Department of Microbiology, University of Connecticut School of Medicine, Health Center, Farmington: Prospects for in vitro packaging of adenovirus DNA.

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BACTERIOPHAGE MEETING, August 15–August 19

arranged by

Ahmad I. Bukhari, Cold Spring Harbor Laboratory

Dietmar Kamp, Cold Spring Harbor Laboratory

152 participants

Session 1: Immunity—Repressor—Operator Interactions

- A. JOHNSON, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Interactions of the λ cro protein and λ repressor with operator DNA.
- R. T. SAUER, C. O. PABO, M. J. ROSS, and B. J. MEYER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Lambda repressor—Domain structure and function.
- N. L. CRAIG, E. M. PHIZICKY, C. W. ROBERTS, and J. W. ROBERTS, Department of Biochemistry, Cornell University, Ithaca, New York: Inactivation and cleavage of λ and P22 phage repressors by *E. coli* recA gene product.
- A. R. POTEETE, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Structure of the *immC* operators
- F. WINSTON and D. BOTSTEIN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Phenotypic distinctions among phage P22 *cly* mutants.
- B. MEYER, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: Lambda repressor regulation during lysogeny.
- R. PASTRANA, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Direct stimulation of repressor synthesis in λ imm434 lysogens by repressor.
- E. ROSEN, K. Z. MCCULLOUGH, and G. GUSSIN, Zoology Department, University of Iowa, Iowa City: Effects of mutations on binding of RNA polymerase to DNA containing the λ pr_m promoter.
- D. WULF, M. BEHER, S. IZUMI, M. LEWIS, J. BECK, M. MAHONEY, M. ROSENBERG,* and H. SHIMATAKE,* Department of Molecular Biology and Biochemistry, University of California, Irvine; *NCI, National Institutes of Health, Bethesda, Maryland: Genetic and sequence studies of λ c_y mutants.
- B. KNOLL, Department of Microbiology, Arizona Health Sciences Center, University of Arizona, Tucson: Single infection lysogenization by phage λ with dominant mutations of the *cIII* gene.
- C. EPP, M. L. PEARSON, M. O. JONES,* and I. HERSKOWITZ,* Department of Medical Genetics, University of Toronto, Ontario, Canada; *Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene: Control of the stability of the λ cII protein by the products of the phage gene *cIII* and the host gene *hfl*.
- R. FISCHER, M. JONES,* I. HERSKOWITZ,* and H. ECHOLS, Department of Molecular Biology, University of California, Berkeley; *Department of Biology and Institute of Molecular Biology, University of Oregon, Eugene: Location of the promoter for establishment of repression by bacteriophage λ .

Session 2: Regulation of Gene Expression I

- H. J. VOLLENWEIDER, M. FIANDT, and W. SZYBALSKI, McArdle Laboratory, University of Wisconsin, Madison: *E. coli* RNA-polymerase-binding sites lie preferentially within A+T-rich DNA regions.

- U. HANSEN and W. McCLURE, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: A noncycling activity assay for the sigma subunit of *E. coli* RNA polymerase.
- J. D. HARRIS, I. MARTINEZ, J. HEILIG, L. CSONKA, R. CALENDAR, and L. ISAKSSON,* Molecular Biology Department, University of California, Berkeley; *Molecular Biology Department, University of Uppsala, Sweden: Genetics of the sigma subunit of DNA-dependent RNA polymerase from *E. coli*.
- C. G. GOFF, Biology Department, Haverford College, Pennsylvania: Mutants affecting a 15,000 m.w. T4 protein tightly bound to *E. coli* RNA polymerase.
- D. H. HALL and R. D. SNYDER, School of Biology, Georgia Institute of Technology, Atlanta: *Sip* mutations affect the "modifier of transcription" (*mot*) gene of bacteriophage T4.
- B. SAUER, D. OW, and R. CALENDAR, Molecular Biology Department, University of California, Berkeley: Suppression of transcriptional polarity is nonessential for growth of phage P4.
- R. ZIVIN, W. ZEHRING, C. MALONE, L. HAYNES, and L. B. ROTHMAN-DENES, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois: In vivo and in vitro specificity of N4 transcription by N4-virion-associated RNA polymerase.
- G. LEE, A. KORMAN, N. HANNETT, and J. PERO, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Cloned fragments of SPO1 DNA fail to support transcription by phage-modified RNA polymerase.
- G. SELZER,* A. BOLLE, D. BELIN, G. VAN HOWE, and T. MATTSO, *National Institutes of Health, Bethesda, Maryland; Department of Molecular Biology, University of Geneva, Switzerland: Expression of a plasmid-carried T4 *rIIA* gene.
- S. STROME and E. T. YOUNG, Biochemistry Department, University of Washington, Seattle: Translational control of the expression of bacteriophage T7 gene 0.3.
- M. F. SHEMAKYIN, E. L. KAPITZA, L. I. PATRUSHEV, E. A. STUKACHEVA, and S. K. ZAVRIEV, M. M. Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow: The influence of *E. coli rho* factor and RNase III on the expression of ϕ X174 and T7 genomes in vitro.

Session 3: Regulation of Gene Expression II

- A. DAS, C. MERRILL, and S. ADHYA, NCI, National Institutes of Health, Bethesda, Maryland: Interaction between *rho* and RNA polymerase during transcription termination.
- A. DAS, D. COURT, S. ADHYA, and M. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: Transcription antitermination by bacteriophage λ *N*-gene product.
- S.-L. HU, J. S. SALSTROM,* and W. SZYBALSKI, McArdle Laboratory, University of Wisconsin, Madison; *The Biological Laboratories, Harvard University, Cambridge, Massachusetts: Stimulation of *Tof* function and $p_{R^{-}L_1}$ and $p_{R^{-}R_1}$ transcription by the *N* product of coliphage λ .
- N. C. FRANKLIN, M. L. PEARSON,* and G. N. BENNETT, Department of Biological Sciences, Stanford University, California; *Department of Medical Genetics, University of Toronto, Ontario, Canada: The *N* gene of λ codes only the 5' half of the 12S transcript.
- E. FLAMM, M. MOZOLA, and D. FRIEDMAN, Department of Microbiology, University of Michigan, Ann Arbor: Mutations affecting rightward transcription in bacteriophage λ .
- C. GAWRON and J. R. CHRISTENSEN, Microbiology Department, University of Rochester, New York: Lambda-*N*-mediated exclusion of T1—The escape of T1am23.
- S. HILLIKER, NCI, National Institutes of Health, Bethesda, Maryland: Comparison of *Salmonella* phages P22 and L by means of λ -P22 and λ -L hybrid phages.
- D. FORBES and I. HERSKOWITZ, Institute of Molecular Biology, University of Oregon, Eugene: The Q protein of bacteriophage λ —An antiterminator.
- M. G. SCHECHTMAN, J. D. SNEDEKER, and J. W. ROBERTS, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Activity of late gene regulators of λ and ϕ 82.
- K. R. LEASON, E. N. JACKSON, and T. F. WEIGHOUS, Department of Microbiology, University of Michigan, Ann Arbor: Products of P22 genes 12 and 18 are required for late gene transcription.

Session 4: Bacteriophage Mu

- M. M. HOWE, K. J. O'DAY, and D. W. SCHULTZ, Department of Bacteriology, University of Wisconsin, Madison: Identification of five new cistrons essential for development of bacteriophage Mu.
- C. J. THOMPSON and M. M. HOWE, Department of Bacteriology, University of Wisconsin, Madison: Tn5-induced mutations in bacteriophage Mu.

- J. ENGLER, Cold Spring Harbor Laboratory, New York: Mapping of Mu-specific RNA transcripts by electron microscopy.
- M. S. DUBOW and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Characterization of Mu proteins.
- C. CHASE and R. BENZINGER, Department of Biology, University of Virginia, Charlottesville: Transfection of *rec*⁺ *E. coli* spheroplasts with bacteriophage Mu DNA.
- R. KAHMANN and D. KAMP, Cold Spring Harbor Laboratory, New York: Structural analysis of the ends of bacteriophage Mu DNA.
- H. KHATOON and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Excision of Mu DNA.
- R. A. FITTS and A. L. TAYLOR, Department of Microbiology and Immunology, University of Colorado Medical Center, Denver: Replication of bacteriophage Mu in *dnaA*⁻ cells.
- M. L. PATO, N. TYLER, and B. T. WAGGONER, National Jewish Hospital and Research Center and University of Colorado Medical Center, Denver: Cellular location of Mu DNA replicas.
- L. AMBROSIO, A. I. BUKHARI, and E. LJUNGQUIST,* Cold Spring Harbor Laboratory, New York; *Karolinska Institutet, Stockholm, Sweden: Behavior of bacteriophage Mu DNA upon infection of *E. coli*.
- F. DE BRUIJN, R. KAHMANN, D. KAMP, H. KHATOON, and A. I. BUKHARI, Cold Spring Harbor Laboratory, New York: Manipulation of Mu ends.
- J. W. SCHUMM and M. M. HOWE, Department of Bacteriology, University of Wisconsin, Madison: Construction of λ phages containing both ends of Mu.
- N. SCHAUS, K. O'DAY, and A. WRIGHT, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: Properties of bacteriophage Mu carrying an *EcoRI*-generated internal deletion.
- D. KAMP and R. KAHMANN, Cold Spring Harbor Laboratory, New York: Properties of defective phage particles obtained from a Mu *gin*⁻G(-) lysogen.

Session 5: Recombination

- H. ECHOLS, Department of Molecular Biology, University of California, Berkeley: Some in vivo properties of integrative and general recombination inferred from interference (*int*) exchanges.
- M. KOTEWICZ, E. GRZESIUK, W. COURCHESNE, H. ECHOLS, R. W. DAVIES,* and P. H. SCHREIER,† Department of Molecular Biology, University of California, Berkeley; *Department of Biology, University of Essex, England; †Medical Research Council, Cambridge, England: Properties of the *int* protein of phage λ .
- T. POLLOCK and H. NASH, NIMH, National Institutes of Health, Bethesda, Maryland: Lambda *int*-dependent site-specific recombination in vitro.
- H. I. MILLER and D. I. FRIEDMAN, University of Michigan, Ann Arbor: Bacteriophage λ *int* gene mutants that bypass the need for the bacterial attachment site and integration host proteins.
- K. ABREMSKI and S. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: Excisive recombination of bacteriophage lambda.
- G. GUARNEROS and J. M. GALINDO, Department of Genetics and Molecular Biology, Centro de Investigación del IPN, Mexico City: Regulation of integrative recombination by the *cII* product and the *b2* region of bacteriophage λ .
- R. FISCHER, Y. TAKEEDA, and H. ECHOLS, Department of Molecular Biology, University of California, Berkeley: A new in vitro RNA-polymerase-binding site and transcription start generated by *int-c* point mutations.
- L. HEFFERNAN, M. BENEDIK, and A. CAMPBELL, Department of Biological Sciences, Stanford University, California: Regulation in the *int-xis* region of bacteriophage lambda.
- D. ROSS,* J. SWAN, and N. KLECKNER, *Department of Biology, Massachusetts Institute of Technology, Cambridge; Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts: "Nearly precise" excision of transposon Tn10 from bacteriophage λ .
- D. G. ROSS,* J. SWAN, and N. KLECKNER, *Department of Biology, Massachusetts Institute of Technology, Cambridge; Department of Molecular Biology, Harvard University, Cambridge, Massachusetts: Tn10-associated DNA rearrangements in a $\lambda::Tn10$ transducing phage.
- R. ZAGURSKY, C. WYMAN, and J. HAYS, Department of Chemistry, University of Maryland Baltimore County, Catonsville: Recombination of λ tandem duplication and triplication phages.
- B. KORBA* and J. HAYS,† Departments of *Biological Sciences and †Chemistry, University of Maryland Baltimore County, Catonsville: The DNA of recombinogenic λ phages grown on *E. coli arl* mutants contains sites susceptible to S1 endonuclease.

- J. HAYS, R. GOUGH, and S. BOEHMER, Department of Chemistry, University of Maryland Baltimore County, Catonsville: Repair-stimulated recombination of bacteriophage λ .
- N. HUTCHINSON and S. J. LEE, Department of Microbiology, University of Pennsylvania, Philadelphia: Frequencies of recombinants between primary and secondary phage in delayed superinfection.

Session 6: Small DNA Phages; Replication

- I. TESSMAN and B. B. STEFFEN, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Overlapping genes and multiple gene products of phages ϕ X174, S13, and G4.
- E. BECK, E. A. AUERSWALD, R. SOMMER, and H. SCHALLER, Department of Microbiology, Universität Heidelberg, Germany: Genome organization and DNA sequence of filamentous bacteriophages.
- A. RAZIN, K. ITAKURA, T. HIROSE, and A. D. RIGGS, Division of Biology, City of Hope National Medical Center, Duarte, California: Synthetic-DNA-directed change of a specific base in bacteriophage ϕ X174 DNA.
- J. SIMS and D. DRESSLER, The Biological Laboratories, Harvard University, Cambridge, Massachusetts: The negative-strand initiation site used by phages G4, St-1, ϕ K, and α 3.
- E. BENZ, R. VICUNA, and J. HURWITZ, Albert Einstein College of Medicine, Bronx, New York: DnaG-protein-mediated priming of bacteriophage DNA replication in vitro.
- M. LUSKY, R. GROSSCHEDL, and G. HOBOM, Institut für Biologie III, Freiburg, Germany: A dual system for initiation of λ replication.
- G. MOSIG and S. BOCK, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Is gene 41 of phage T4 analogous to the *dnaG* gene (primase) of *E. coli*?
- A. LUDER and G. MOSIG, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee: Specific interactions of mutations in gene 32 and DNA delay genes of phage T4D.
- R. C. MILLER, JR.,* E. T. YOUNG II,[†] R. H. EPSTEIN,[‡] H. M. KRISCH,[‡] T. MAITSON,[‡] and A. BOLLE,[‡] *Department of Microbiology, University of British Columbia, Vancouver, Canada; [†]Department of Molecular Biology, University of Geneva, Switzerland; [‡]Department of Biochemistry, University of Washington, Seattle: Regulation of the synthesis of the T4 DNA polymerase (gene 43).
- L. L. SILVER and N. G. NOSSAL, Laboratory of Biochemical Pharmacology, NIAMDD, National Institutes of Health, Bethesda, Maryland: The bacteriophage T4 DNA-delay gene 61 (58) product is required for a DNA chain initiating activity found in T4-infected cell extracts.
- L. S. RIPLEY, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: In vivo errors of replication in T4 DNA containing 2-aminopurine.
- A. C. RICE, T. A. FICHT, and R. W. MOYER, Department of Microbiology, Vanderbilt University, Nashville, Tennessee: Purification and properties of a bacteriophage-T5-encoded DNA-binding protein that exhibits multifunctional properties.
- H. R. WARNER, R. B. THOMPSON, and W. J. SWART, Department of Biochemistry, University of Minnesota, St. Paul: Isolation and properties of T5 mutants unable to induce dUTPase and thymidylate synthetase activities.

Session 7: Bacteriophage P1—Phage-Host Interaction

- N. STERNBERG, T. SOM, and S. AUSTIN, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Determinants for copy number, segregation fidelity, and incompatibility of the P1 plasmid prophage.
- N. STERNBERG, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland: Site-specific recombination in bacteriophage P1.
- J. B. RAZZA, C. A. WATKINS, and J. R. SCOTT, Department of Microbiology, Emory University, Atlanta, Georgia: Temperature-sensitive mutants of P1.
- J. A. COWAN,* J. R. SCOTT,* A. TOUSSAINT,[†] and N. LEFEBVRE,[†] *Department of Microbiology, Emory University, Atlanta, Georgia; [†]Laboratoires de Génétique et Microbiologie, Université Libre de Bruxelles, Rhode-Saint-Genèse, Belgium: Relationships of phages P1 and Mu.
- C. A. WATKINS, B. R. BAUMSTARK, and J. R. SCOTT, Department of Microbiology, Emory University, Atlanta, Georgia: Characterization of bacteriophage D6.
- S. GOTTESMAN, NCI, National Institutes of Health, Bethesda, Maryland: *Rap*—An *E. coli* mutation affecting λ growth.
- J. WAY, R. YOUNG, S. WAY, J. YIN, and M. SYVANEN, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: A new gene in λ affecting cell lysis.

- E. J. GRAYHACK, M. G. SCHECHTMAN, and J. W. ROBERTS, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York: Lambda genes *P* and *Q* under lactose operon control.
- M. MCEWEN, K. NAT, and P. SILVERMAN, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York: Chromosomal mutants of *E. coli* defective in bacteriophage $\text{Q}\beta$ replication.
- M. E. DRESSER, R. C. GREENE, and J. R. JOHNSON,* Duke University Medical Center, Durham, North Carolina; *Department of Biology, Texas A & M University, College Station: Isolation and characterization of specialized λ transducing phages carrying the *cytR* gene of *E. coli* K12.
- L. D. SIMON, K. TOMCZAK, and A. C. ST. JOHN,* Waksman Institute of Microbiology and *Department of Microbiology, Rutgers University, Piscataway, New Jersey: Inhibition of the degradation of abnormal proteins in *E. coli* by bacteriophages.
- K. DHARMALINGAM and E. B. GOLDBERG, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts: "SOS" prevents restriction of nonglucosylated phage T4 in *E. coli*.

Session 8: Morphogenesis

- D. W. BOWDEN and R. CALENDAR, Molecular Biology Department, University of California, Berkeley: In vitro P2 DNA maturation—A complex, site-specific system for cleavage.
- C. DIANA, J. GEISELSODER, and R. GOLDSTEIN, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Propagation and characterization of the diploid and triploid plaque-forming units of the capsid size determination mutant of satellite bacteriophage P4.
- J. GEISELSODER, M. CHIDAMBARAM, and R. GOLDSTEIN, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Transcriptional control of capsid size in the P2-P4 bacteriophage system.
- C. DIANA, G. DEHO, J. GEISELSODER, and R. GOLDSTEIN, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Viral interference at the level of capsid size determination by satellite phage P4.
- D. OW, M. KAHN,* B. SAUER, and R. CALENDAR, Molecular Biology Department, University of California, Berkeley; *Biology Department, University of California, San Diego: Marker-rescue study of bacteriophage P4 using ColE1-P4 hybrids.
- R. A. FISHER, M. FEISS, D. A. SIEGELE, D. A. NICHOLS, and J. DONELSON, Departments of Microbiology and Biochemistry, University of Iowa, Iowa City: Packaging of the bacteriophage λ chromosome—A role for sequences outside of *cos*.
- R. A. FISHER, K. KRIZANOVITCH-WILLIAMS, C. RUDOLPH, D. A. SIEGELE, J. YOCHER, and M. FEISS, Department of Microbiology, University of Iowa, Iowa City: Studies of bacteriophage λ assembly.
- P. YODERIAN, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts: Genetic control of the length of lambdaoid phage tails.
- R. J. DEANS and E. N. JACKSON, Department of Microbiology, University of Michigan, Ann Arbor: *Hind*III cleavage-site map of *Salmonella typhimurium* bacteriophage P22.
- E. JACKSON and F. LASKI, Department of Microbiology, University of Michigan, Ann Arbor: Specific cleavage of P22 DNA concatemers in absence of DNA packaging.
- S.-H. KAO and W. H. McCLAIN, Department of Bacteriology, University of Wisconsin, Madison: Involvement of bacteriophage T4 gene-5 protein in cell lysis.
- J. D. CHILDS, Biology and Health Physics Division, Atomic Energy of Canada Ltd., Chalk River, Ontario, Canada: Electrophoretic mutants of bacteriophage T4.
- N. L. INCARDONA, Microbiology Department, University of Tennessee Health Science Center, Memphis: Comparison of complex models for virus adsorption and eclipse by computer simulation of kinetic data.

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arranged by

John Abelson, University of California, San Diego

Paul Schimmel, Massachusetts Institute of Technology

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240 participants

Session 1a: Primary Structure of tRNA

Chairperson: U.L. RAJBHANDARY, Massachusetts Institute of Technology, Cambridge

- R.P. MARTIN,* J.M. SCHNELLER,[†] A.P. SIBLER,* A. STAHL,[†] and G. DIRHEIMER,* *Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, and [†]Laboratoire de Biochimie, Faculté de Pharmacie, Strasbourg, France: Yeast mitochondrial tRNAs—Isoacceptors, coding origin, and primary structure of tRNA^{Phe}.
- S. NOGUCHI,* Z. YAMAIZUMI,* Y. NISHIMURA,[†] Y. HIROTA,[†] T. OHGI,[‡] T. GOTO,[‡] and S. NISHIMURA,* *Biology Division, National Cancer Center Research Institute, Tokyo; [†]National Institute of Genetics, Mishima; [‡]Department of Agricultural Chemistry, Nagoya University, Japan: Isolation and properties of *E. coli* mutants that lack modified nucleoside Q in tRNA.
- N. OKADA,* N. SHINDO-OKADA,* S. SATO,[†] Y.H. ITOH,[‡] K. ODA,[§] and S. NISHIMURA,* *Biology and [†]Biochemistry Divisions, National Cancer Center Research Institute, Tokyo; [‡]Department of Molecular Biology, Keio University School of Medicine, Tokyo; [§]Institute of Medical Science, Tokyo University, Japan: Detection of unique tRNA species in tumor tissues by *E. coli* guanine insertion enzyme.
- Z. YAMAIZUMI,** Y. KUCHINO,* F. HARADA,* S. NISHIMURA,* and J.A. McCLOSKEY,[†] *Biology Division, National Cancer Center Research Institute, Tokyo, Japan; [†]College of Pharmacy, University of Utah, Salt Lake City: Primary structure of *E. coli* tRNA^{Arg}^{eu}—The presence of an unknown adenosine derivative in the anticodon that recognizes UU series of codon.
- R.C. GUPTA, B.A. ROE,* and K. RANDEPATH, Department of Pharmacology, Baylor College of Medicine, Houston, Texas; *Department of Chemistry, Kent State University, Ohio: Sequences of glycine tRNAs from human placenta.
- H.P. GHOSH,* K. GHOSH,* M. SIMSEK,[†] and U.L. RAJBHANDARY,[†] *Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada; [†]Department of Biology, Massachusetts Institute of Technology, Cambridge: Primary sequence of wheat germ initiator tRNA^{fMet}

Session 1b: Recognition of tRNA

Chairperson: P. SCHIMMEL, Massachusetts Institute of Technology, Cambridge

- L.H. SCHULMAN, H. PELKA, and R.M. SUNDARI, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York: Recognition of *E. coli* methionine tRNAs.
- J.P. EBEL, M. RENAUD, A. DIETRICH, M. BALTZINGER, R. EHRlich, F. FASIOLO, O. FAVOROVA, S. VASSILENKO, R. GIEGÉ, and P. REMY, Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Interaction between tRNA and aminoacyl-tRNA synthetase in the valine and phenylalanine systems from yeast.
- S.M. HECHT, B.L. ALFORD, Y. KURODA, and S. KITANO, Department of Chemistry, Massachusetts Institute of Technology, Cambridge: "Chemical aminoacylation" of tRNAs.

Session 2: Poster Session

- M. BALTZINGER, F. FASIOLO, P. REMY, and Y. BOULANGER, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Localization of the sites in the tetrameric yeast phenylalanyl-tRNA synthetase.
- C.J. BRUTON and L.A.-M. COX, Department of Biochemistry, Imperial College, London, England: CysteinyI-tRNA synthetase from *B. stearothermophilus* is both structurally and functionally monomeric.
- P. CHANG, P.A. SAFILLE, J. OMNAAS, and K.H. MUENCH, University of Miami School of Medicine, Florida: Location of the required cysteine residue in tryptophanyl-tRNA synthetase from a superproducing strain of *E. coli*.

- A. FAVRE* and E. HOLLER,† *Institut de Recherches en Biologie Moléculaire, Paris, France; †Institut für Biophysik und Physikalische Biochemie, Regensburg, Germany: Formation of the phenylalanyl-tRNA synthetase tRNA^{Phe} complex induces a conformational change of the tRNA.
- C. GÜNTNER and E. HOLLER, Institut für Biophysik und Physikalische Biochemie, Universität at Regensburg, Germany: The mechanism of phenylalanyl-tRNA synthetase of *E. coli* K10—The binding sites for free and aminoacylated tRNA are not identical.
- J. HUGHES and G. MELLOWS, Department of Biochemistry, Imperial College, London: Inhibition of isoleucyl-tRNA synthetase by the novel antibiotic pseudomonic acid A.
- D. KERN,* L. DUPLAIN,* S. POTIER,† Y. BOULANGER,† and J. LAPOINTE,* *Département de Biochimie, Université Laval, Quebec, Canada; †Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: The monomeric glutamyl-tRNA synthetases from *E. coli* and *B. subtilis*—Relations between their structural and catalytic properties, and their evolution.
- D. KERN, R. GIEGÉ, and J.P. EBEL, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Interaction of tRNAs with yeast valyl-tRNA synthetase and valylation mechanism in correct and incorrect systems.
- G. KRAUSS,* F. VON DER HAAR,† and G. MAASS,* *Medizinische Hochschule Hannover, Germany; †Max-Planck-Institut für Medizinische Chemie, Göttingen, Germany: Role of the CCA end of the tRNA in the recognition mechanism between tRNA^{Phe} (yeast) and phenylalanyl-tRNA synthetase (yeast).
- M. KRÖGER, H. STERNBACH, and F. CRAMER, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany: Reversible inactivation of tRNA nucleotidyltransferase from baker's yeast by tRNA^{Phe} containing iodoacetamide alkylated 2-thiocytidine in common and unusual positions.
- M.-R. KULA and H. TSAI, Gesellschaft für Biotechnologische Forschung mbH., Braunschweig-Sf ockheim, Germany: Structural studies on isoleucyl-tRNA synthetase.
- V. NIKODEM, R.C. JOHNSON, and J.R. FRESCO, Department of Biochemical Sciences, Princeton University, New Jersey: Purification, molecular weight, and asymmetry of leucyl-tRNA synthetase from baker's yeast.
- L. NILSSON, U. PACHMANN,* and R. RIGLER, Department of Medical Biophysics, Karolinska Institutet, Stockholm, Sweden; *Institut für Physiologische Chemie, Universität München, Germany: Conformational dynamics of yeast tRNA^{Phe} in the presence of Mg²⁺ spermine and phenylalanyl-tRNA synthetase.
- C. PLANTARD, B. LABOUESSE, P.V. GRAVES, and G. MÉRAULT, Département de Biochimie, Université de Bordeaux, France: Anticooperative binding of tryptophan and tRNA^{Trp} by tryptophanyl-tRNA synthetase from beef pancreas.
- S. ROBBE-SAUL, S. POTIER, F. FASIOLO, D. KERN, R. GIEGÉ, J. GANGLOFF, J. LAPOINTE,† and Y. BOULANGER, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France; †Faculté des Sciences et de Génie, Université Laval, Quebec, Canada: Structural studies on aminoacyl-tRNA synthetases—A tentative correlation between the subunit size and the occurrence of repeated sequences.
- R.P. SINGHAL and P.A. FALLIS, Chemistry Department, Wichita State University, Kansas: Study of tRNA structures for a primordial sequence—Modified base locations and synthetase recognition sites.
- R. THIEBE, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Universität München, Germany: Comparison of the aminoacylation mechanisms of tRNA^{Phe} and tRNA^{Arg} in the steady state.
- G. ZACCAÏ,* P. MORIN,* B. JACROT,* D. MORAS,† J.C. THIERRY,† R. GIEGÉ,† P. DESSEN,‡ and S. BLANQUET,† *Institut Max Von Laue-Paul Langevin, Grenoble; †Université Louis Pasteur and Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg; ‡Ecole Polytechnique, Palaiseau, France: tRNA interactions with aminoacyl-tRNA synthetases studied by neutron scattering.

Session 3: Structure and Function of Aminoacyl-tRNA Synthetases

Chairperson: B.S. HARTLEY, Imperial College, London, England

- J.R. RUBIN,* and D.M. BLOW,† *MRC Laboratory of Molecular Biology, Cambridge; †Blackett Laboratory, Imperial College, London, England: Tyrosyl-tRNA synthetase from *B. stearothermophilus*—Tertiary structure and ligand-binding studies.
- G.P. WINTER,* G.L.E. KOCH,* A. DELL,† and B.S. HARTLEY,† *MRC Laboratory of Molecular Biology, Cambridge; †Department of Biochemistry, Imperial College, London, England: The tryptophanyl- and tyrosyl-tRNA synthetases from *B. stearothermophilus*.
- A. FERSHT,* J. GANGLOFF,† and G. DIRHEIMER,† *Institut de Biologie Moléculaire et Cellulaire du CNRS,

Strasbourg, France; †MRC Laboratory of Molecular Biology, Cambridge, England: Reaction pathway and rate-determining step in the aminoacylation of tRNA^{Arg} catalyzed by the arginyl-tRNA synthetase from yeast.

- L. KISSELEV, G. KOVALEVA, and O. FAVOROVA, Institute of Molecular Biology, Moscow, USSR: Isolation, properties, and possible role of tryptophanyl-enzyme in the reaction of enzymatic aminoacylation of tRNA^{Trp}.
- G.L. IGLOI, F. VON DER HAAR, and F. CRAMER, Abteilung Chemie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany: Experimental proof for the misactivation of tyrosine by phenylalanyl-tRNA synthetase from yeast.
- H.G. FAULHAMMER, M. SPRINZL, and F. CRAMER, Abteilung Chemie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany: Interaction of tyrosyl-tRNA synthetase with fluoescamine-labeled tRNA^{Tyr} from yeast and participation of fluoescamine-modified *E. coli* tRNA^{Phe} in *in vitro* protein biosynthesis.

Session 4A: Crystal Structure of tRNA

Chairperson: S.-H. KIM, Duke University School of Medicine, Durham, North Carolina

- N.H. WOO,* B.A. ROE† and A. RICH,* *Department of Biology, Massachusetts Institute of Technology, Cambridge; †Department of Chemistry, Kent State University, Ohio: Three-dimensional structure of the *E. coli* initiator tRNA.
- G.J. QUIGLEY, M.M. TEETER, and A. RICH, Department of Biology, Massachusetts Institute of Technology, Cambridge: Role of spermine, magnesium, and water in yeast tRNA^{Phe}.
- A. DUCRUIX and A. RICH, Department of Biology, Massachusetts Institute of Technology, Cambridge: Conformation of peptidyl-tRNA^{Phe}.
- R.W. SCHEVITZ, A.D. PODJARNY, N. KRISHNAMACHARI, J. HUGHES, and P.B. SIGLER, Department of Biophysics and Theoretical Biology, University of Chicago, Illinois: A crystallographic analysis of yeast initiator tRNA.
- R. BOTT, C.D. STOUT,* and M. SUNDARALINGAM, Department of Biochemistry, University of Wisconsin College of Agricultural and Life Sciences, Madison; *Department of Crystallography, University of Pittsburgh, Pennsylvania: The structure of *E. coli* tRNA^{Arg} at 4Å resolution.
- H.T. WRIGHT, P.C. MANOR, K. BEURLING, R. KARPEL, and J.R. FRESCO, Department of Biochemical Sciences, Princeton University, New Jersey: Crystal structure of yeast tRNA^{Gly}—Molecular packing and comparison of the polynucleotide backbone conformation with that of yeast tRNA^{Phe}.

Session 4B: Physical Studies on tRNA in Solution

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

- B.R. REID and R.E. HURD, Biochemistry Department, University of California, Riverside: High-resolution nuclear magnetic resonance (NMR) studies on tRNA structure.
- P.D. JOHNSTON and A.G. REDFIELD, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: Pulsed FT-NMR double resonance studies of tRNA in H₂O.
- R. POTTS, D. FRITZINGER, N.C. FORD, and M.J. FOURNIER, University of Massachusetts, Amherst: Effect of aminoacylation and magnesium on the solution structure of yeast phenylalanine tRNA.

Session 5: Poster Session

- J. CANADAY, P. GUILLEMAUT, and J.H. WEIL, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Purification and nucleoside composition of chloroplastic and cytoplasmic initiator tRNAs from *Phaseolus vulgaris*—Preliminary sequence results.
- K. CHAKRABURTTY, Department of Biochemistry, Medical College of Wisconsin, Milwaukee: Effect of temperature and divalent cation on chemical modification of *E. coli* tRNA₁^{Arg}.
- S.H. CHANG,* F.K. LIN,* L.I. HECKER,† J.E. HECKMAN,‡ U.L. RAJBHANDARY,‡ and W.E. BARNETT,† *Department of Biochemistry, Louisiana State University, Baton Rouge; †Department of Biology, Massachusetts Institute of Technology, Cambridge; ‡Oak Ridge National Laboratory, Tennessee: Nucleotide sequence of blue-green algae phenylalanine tRNA.
- B. DUDOCK, J. LESIEWICZ, M.-Y. WANG, and R. GREENBERG, State University of New York, Stony Brook: A new

- class of tRNA modification reactions in *E. coli* and mammalian mitochondria—tRNA methyl esters.
- M. EHRENBURG, W. WINTERMEYER,* P. GRASSELLI, and R. RIGLER, Department of Medical Biophysics, Karolinska Institutet, Stockholm, Sweden; *Institut für Physiologische Chemie, Universität München, Germany: On the structure and conformational dynamics of tRNA_{yeast}^{Phe} in solution.
- R. EHRLICH, D. FRECHET, M. RENAUD, P. REMY, J.P. EBEL, C. REISS,* and J. GABAR,* Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg; *Institut du Radium, Orsay, France: High-resolution optical study of the thermal unfolding of RNAs—Evidence for fine transitions in tRNAs.
- A. FAVRE and G. THOMAS, Institut de Recherches en Biologie Moléculaire, Paris, France: The role of 4-thiouridine in *E. coli* tRNAs.
- R. GARBER* and S. ALTMAN,† Roche Institute of Molecular Biology, Nutley, New Jersey; Department of Biology, Yale University, New Haven, Connecticut: tRNA precursors and processing ribonucleases in *Bombyx mori*.
- I. GINZBURG, P. CORNELIS, and U.Z. LITTAUER, Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel: Assay of functionally impaired tRNA in injected *Xenopus* oocytes.
- J.P. GODDARD and M. LOWDON, Department of Biochemistry, University of Glasgow, Scotland: The thermal melting of *E. coli* tRNA^{Phe} studied by chemical reactivity.
- H. GROISJEAN,* S. DE HENAU,† and D. CROTHERS,‡ *University of Brussels, Belgium; †Medizinische Hochschule Hannover, Germany; ‡Yale University, New Haven, Connecticut: Studies on the complex between tRNAs with complementary anticodons—A direct approach to the “wobble” problem.
- C.L. HARRIS and F. MARASHI, Department of Biochemistry, West Virginia University, Morgantown: Purification and properties of tRNA^{Leu} from *E. coli* C6—A 4-thiouridine-containing species.
- N.K. HOWES and W.R. FARKAS, Memorial Research Center, University of Tennessee, Knoxville: Purification of and studies with homogeneous guanine insertase.
- R.E. HURD and B.R. REID, Biochemistry Department, University of California, Riverside: The structure and dynamics of regulatory and nonregulatory tRNAs in solution.
- H. ISHIKURA, K. MURAO, and Y. YAMADA, Laboratory of Chemistry, Jichi Medical School, Tochigi-ken, Japan: New uridine derivatives present in tRNAs from *B. subtilis*.
- R.L. KARPEL and A.C. BURCHARD, Department of Chemistry, University of Maryland Baltimore County, Catonsville: Physical studies of the interaction of a nucleic acid helix destabilizing protein with tRNA and synthetic polynucleotides.
- J. KATZE, Department of Microbiology, University of Tennessee Center for Health Science, Memphis: Serum factor required for nucleoside Q-containing tRNA in tissue culture.
- G. KEITH,* B. MENICHI,† T. HEYMAN,‡ H.H. ARNOLD,‡ R. RAETTIG,‡ H. KERSTEN,‡ and G. DIRHEIMER,* *Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France; †Institut du Radium, Orsay, France; ‡Institut für Physiologische Chemie der Universität Erlangen-Nürnberg, Germany: Comparison of the primary structures of tRNA^{Tyr} and tRNA^{Phe} of the gram-positive bacteria *B. subtilis* and *B. stearothermophilus*.
- T. LABONE and M. STEINMETZ KAYNE, Department of Biology, Trenton State College, New Jersey: A rapid assay for *E. coli* 4-thiouridine tRNA sulfurtransferase.
- J.L. LEROY and M. GUÉRON, Ecole Polytechnique, Palaiseau, France: Search for specific binding sites of divalent cations in tRNA.
- G.A. LUOMA and A.G. MARSHALL, Department of Chemistry, University of British Columbia, Vancouver, Canada: Laser Raman evidence for new cloverleaf secondary structures for 5S RNA and 5.8S RNA.
- T. NY, K. HJALMARSSON, and G.R. BJÖRK, Department of Microbiology, University of Umeå, Sweden: Noncoordinated regulation of tRNA biosynthetic enzymes.
- N. OKADA,* S. NOGUCHI,* H. KASAI,* N. SHINDO-OKADA,* T. OHGI,† T. GOTO,† and S. NISHIMURA,* *Biology Division, National Cancer Center Research Institute, Tokyo; †Department of Agricultural Chemistry, Nagoya University, Japan: Novel mechanism of posttranscriptional modification of tRNA—Insertion of base of Q precursor into tRNA by tRNA transglycosidase reaction.
- P.O. OLINS and D.S. JONES, Department of Biochemistry, University of Liverpool, England: The sequence of the major formylatable species of methionine tRNA from *S. obliquus*.
- A. PATKOWSKI* and B. CHU,† *Institute of Physics, A. Mickiewicz University, Poznań, Poland; †Department of Chemistry, State University of New York, Stony Brook: Intensity fluctuation spectroscopy and tRNA conformation.

- R. G. PERGOLIZI,* D. L. ENGELHARDT,[†] and D. GRUNBERGER,* Institute of Cancer Research, and Departments of *Biochemistry and [†]Microbiology, Columbia University, New York, New York: Methionine-dependent modification of a phenylalanine tRNA species lacking the hypermodified wye (Y) base in a monkey kidney cell line.
- B. A. ROE, E. Y. CHEN, H. L. RIZI, A. F. STANKIEWICZ, C. C. WEISZ, D. P. MA, C. Y. CHEN, and P. W. ARMSTRONG, Chemistry Department, Kent State University, Ohio: The role of modified nucleotides in mammalian tRNA—The Q nucleoside is replaced by guanosine in a tumor tRNA^{Asp}.
- J. C. THIERRY, R. GIEGÈ, J. FISCHER, R. WEISS, J. P. EBEL, and D. MORAS, Laboratoire de Cristallographie de l'ULP, and Laboratoire de Biochimie, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: Crystallographic studies on yeast tRNA^{Asp}.
- P. WREDE,* R. WURST,[†] J. VOURNAKIS,[‡] and A. RICH,* *Biology Department, Massachusetts Institute of Technology, Cambridge; [†]Biology Department, Syracuse University, New York: Localization of salt-induced conformational changes in 5'- and 3'-³²P-labeled tRNAs.
- R. M. WURST and J. N. VOURNAKIS, Department of Biology, Syracuse University, New York: Structure mapping of 5'-³²P-end-labeled yeast tRNA^{Phe} with S1 nuclease.

Session 6: tRNA Biosynthesis—RNA Processing

Chairperson: W. MCCLAIN, University of Wisconsin, Madison

- B. STARK, E. J. BOWMAN, R. KOLE, R. GARBER, R. KOSKI, and S. ALTMAN, Department of Biology, Yale University, New Haven, Connecticut: Ribonuclease P—An enzyme with an essential RNA component.
- Y. SHIMURA, H. SAKANO,* and F. NAGAWA, Department of Biophysics, Faculty of Science, Kyoto University, Japan; *Basel Institute for Immunology, Switzerland: Studies on precursors of *E. coli* tRNAs.
- H. SAKANO, Y. SHIMURA, T. IKEMURA, and H. OZEKI, Faculty of Science, University of Kyoto, Japan: tRNA precursors accumulated in RNase P mutants of *E. coli*.
- H. D. ROBERTSON, E. G. PELLE, and W. H. MCCLAIN,* Rockefeller University, New York, New York; *Department of Bacteriology, University of Wisconsin, Madison: RNA processing in an *E. coli* strain deficient in both RNase P and RNase III.
- V. DANIEL, M. ZEEVI, and A. GOLDFARB, Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel: In vitro tRNA synthesis.
- J. A. STEITZ, R. YOUNG, R. MACKLIS, and R. BRAM, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Sequences surrounding tRNA and rRNA processing sites in the rRNA operons of *E. coli*.
- E. LUND, J. E. DAHLBERG, and C. GUTHRIE,* Department of Physiological Chemistry, University of Wisconsin, Madison; *Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco: Processing of spacer and distal tRNAs from rRNA transcripts of *E. coli* in vitro.
- R. K. GHOSH and M. P. DEUTSCHER, Department of Biochemistry, University of Connecticut Health Center, Farmington: Isolation of tRNA-processing nucleases using synthetic tRNA precursors.
- N. R. PACE, B. MEYHACK, B. PACE, and D. STAHL, National Jewish Hospital and Research Center, and Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado: The in vitro metabolism of a 5S rRNA precursor from *B. subtilis*.
- A. K. HOPPER, University of Massachusetts Medical School, Worcester: The biosynthesis of yeast tRNAs.
- G. KNAPP, S. FUHRMAN, J. S. BECKMANN, P. F. JOHNSON, R. C. OGDEN, and J. ABELSON, Department of Chemistry, University of California, San Diego: Transcription and processing of intervening sequences in the tRNA genes of yeast.
- C. GUTHRIE, R. ATCHISON, D. COLBY, M. C. ETCHEVERRY, and M. SALVATO, University of California, San Francisco: Processing of tRNA in *E. coli* and *S. cerevisiae*.
- P. VALENZUELA, P. Z. O'FARRELL, B. CORDELL, T. MAYNARD, H. GOODMAN, and W. J. RUTTER, Department of Biochemistry and Biophysics, University of California, San Francisco: Structure and in vitro processing of yeast precursor tRNAs containing intervening sequences.

Session 7a: Coding and Suppression

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

- F. YAMAO, H. INOKUCHI, and H. OZEKI, Department of Biophysics, Faculty of Science, Kyoto University, Japan: Recognition mutants in *E. coli*.
- J. KOHLI, F. ALTRUDA, T. KWONG, G. WAHL,* A. RAFALSKI, R. WETZEL, and D. SÖLL, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut; *Department of Biology, University of Utah, Salt Lake City: UGA-suppressor tRNAs in *S. pombe*.
- J.F. ATKINS and R.F. GESTELAND, Cold Spring Harbor Laboratory, New York: Effect of tRNA balance on gene expression.
- R.H. BUCKINGHAM, J. VACHER, and C.G. KURLAND, Institut de Biologie Physico-Chimique, Paris, France, and Wallenberg Laboratory, Uppsala, Sweden: Suppressor activity of tRNA^{Trp}.

Session 7b: Involvement of tRNA in Regulation

Chairperson: H.E. UMBARGER, Purdue University, West Lafayette, Indiana

- H.E. UMBARGER, Department of Biological Sciences, Purdue University, West Lafayette, Indiana: Some observations on the involvement of aminoacyl tRNAs—The current status of tRNA involvement in regulation of amino acid biosynthesis.
- L. BOSSI and J.R. ROTH, Biology Department, University of Utah, Salt Lake City: Importance of anticodon-region pseudouridine for in vivo tRNA function.
- S.C. QUAY* and D.L. OXENDER,† *Massachusetts General Hospital, Harvard Medical School, Boston; †Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor: Role of tRNA^{Leu} in branched-chain amino acid transport regulation.

Session 8: Poster Session

- P.F. AGRIS and S.A.H. KOVACS, Division of Biological Sciences, University of Missouri, Columbia: A possible regulatory role for tRNA in melanin synthesis.
- M. ALBANI,* A. HOBURG,* H. KERSTEN,* P. WURMBACH,† and K.H. NIERHAUS,† *Institut für Physiologische Chemie und Biochemie, Erlangen; †Max-Planck-Institut für Molekulare Genetik, Berlin, Germany: Interaction of specific prokaryotic ribothymidine-containing and ribothymidine-lacking tRNAs with the programmed ribosome.
- A. ARAYA, J. LABOUESSE, and S. LITVAK, Département de Biochimie, Université de Bordeaux II, France: Studies on the specific interactions between reverse transcriptase and primer tRNA.
- A. BHUTA, K. QUIGGLE, G. BUTKE, R. GOLDBERG,* and S. CHLADEK, Michigan Cancer Foundation, and *Department of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan: Interaction of the AA-tRNA acceptor terminus with *E. coli* ribosomes—Stereochemical control of the peptidyl-transferase reaction.
- R.M. BOCK, H. LATEN, and J. GORMAN, Laboratory of Molecular Biology, University of Wisconsin, Madison: Isopentenyladenosine-deficient tRNA from an antisuppressor mutant of *S. cerevisiae*.
- M.M. COMER, Department of Biology, Clark University, Worcester, Massachusetts: Genes for threonine and lysine tRNAs—Approximate map location in *E. coli*.
- M.R. CULBERTSON,* C. CUMMINS,* and G.R. FINK,† *University of Wisconsin, Madison; †Cornell University, Ithaca, New York: Frameshift suppressors in *S. cerevisiae*.
- S.M. DESAI, C. HUNT, and S.B. WEISS, Franklin McLean Memorial Research Institute, and Department of Biochemistry, University of Chicago, Illinois: Organization of tRNA genes in the T5 bacteriophage chromosome.
- H.J. DRABKIN and L.N. LUKENS, Department of Biology, Wesleyan University, Middletown, Connecticut: Discovery of a new glycine isoaccepting tRNA in chick embryo tissues and its preferential utilization in collagen synthesis.
- M.Y. FELDMAN, Veterinary Institute, Beit Dagan, Israel: The "covalent cross-linkage" model for interactions of tRNA, mRNA, and ribosome.
- E. GRIFFITHS, National Institute for Medical Research, Mill Hill, London, England: Alterations in *E. coli* tRNAs induced by iron-binding proteins—A possible role in pathogenicity.

- H. GROSJEAN,* D. SANKOFF,[†] W. MIN-JOU,[‡] W. FIERS,[‡] and R. CEDERGEN,[†] *University of Brussels, Belgium; [‡]University of Ghent, Belgium; [†]University of Montreal, Quebec, Canada: MS₂ RNA—A correlation between the stability of the codon-anticodon interaction and the choice of code words.
- H. GUILLEY, Laboratoire de Virologie, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France: In vitro aminoacylation of viral RNA—A kinetic study of the reaction and structure of the amino acid-accepting 3'-OH extremity of turnip-yellow mosaic virus and tobacco mosaic virus RNA.
- H.A. HOSBACH, M. SILBERKLANG, and B.J. MCCARTHY, Department of Biochemistry and Biophysics, University of California, San Francisco: Structure of *D. melanogaster* tRNA genes.
- T. IKEMURA, Faculty of Science, Kyoto University, Japan: Relative amount of individual tRNAs and 5S RNA of *E. coli*.
- K.B. JACOBSON, Biology Division, Oak Ridge National Laboratory, Tennessee: Correlation between the absence of a tRNA^{Trp} isoacceptor and the activity of a mutant form of tryptophan oxygenase in *Drosophila*.

Session 9: Cloning of tRNA Genes

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

- K. FUKADA, J. VELTEN, and J. ABELSON, Department of Chemistry, University of California, San Diego: Cloning and sequencing of the T4 tRNA gene cluster.
- J. ROSSI, J. EGAN, M. BERMAN, and A. LANDY, Division of Biology and Medicine, Brown University, Providence, Rhode Island: Structure, organization, and regulation of the two tRNA^{Trp} gene clusters in *E. coli*.
- M.J. RYAN,* E.L. BROWN,* R. BELAGAJE,* H.-J. FRITZ,[†] and H.G. KHORANA,* *Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge; [†]Institut für Genetik der Universität Köln, Germany: Cloning of two chemically synthesized genes for a precursor to the tyrosine suppressor tRNA.
- P. PHILIPPSEN, J.R. CAMERON, and R.W. DAVIS, Department of Biochemistry, Stanford University School of Medicine, California: Analysis of tyrosine tRNA genes in yeast.
- G. PAGE, M. OLSON, and B.D. HALL, Department of Genetics, University of Washington, Seattle: Molecular characterization of seryl- and tyrosyl-tRNA genes of *S. cerevisiae*.
- R. CORTESE, D. MELTON, T. TRANQUILLA, and J.D. SMITH, MRC Laboratory of Molecular Biology, Cambridge, England: Cloning of nematode tRNA genes and their expression in frog oocytes.
- O. SCHMIDT, B. HOVEMANN, J. MAO, S. SILVERMAN, and D. SÖLL, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut: Specific transcription of eukaryotic tRNA genes in *Xenopus* germinal vesicle extracts.

Session 10a: tRNA Gene Arrangement

Chairperson: G. TENER, University of British Columbia, Vancouver, Canada

- G.M. TENER,* S. HAYASHI,* R. DUNN,* A. DELANEY,* I.C. GILLAM,* T. GRIGLIATTI,[†] T. KAUFMAN,[†] and D.T. SUZUKI,[†] Departments of *Biochemistry and [†]Zoology, University of British Columbia, Vancouver, Canada: tRNA genes of *D. melanogaster*.
- N.D. HERSHEY, P. YEN, R. ROBINSON, and N. DAVIDSON, Department of Chemistry, California Institute of Technology, Pasadena: Sequence organization of *Drosophila* tRNA genes.
- R. ELDER, P. SZABO, and O. UHLENBECK, Department of Biochemistry, University of Illinois, Urbana: tRNA gene organization in *D. melanogaster*.
- E. KUBLI, A.H. EGG, and T. SCHMIDT, Zoological Institute, University of Zürich, Switzerland: Localization of tRNA^{Asp} genes from *D. melanogaster* by in situ hybridization.
- E.A. MORGAN, T. IKEMURA, and M. NOMURA, Institute for Enzyme Research, University of Wisconsin, Madison: Identification of tRNA genes in rRNA operons of *E. coli*.
- A. STEINMETZ,* A.J. DRIESE,[†] M. MUBUMBILA,* E.J. CROUSE,[†] K. GORDON,[†] H.J. BOHNERT,[†] M. KELLER,* G. BURKARD,* R.G. HERRMANN,[†] and J.H. WEIL,* *Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France; [†]Botanisches Institut der Universität Dusseldorf, Germany: Mapping of the tRNA genes on the circular DNA molecule of *S. oleracea* chloroplast.

- H. INOKUCHI, M. KODAIRA, F. YAMAO, and H. OZeki, Faculty of Science, Kyoto University, Japan: tRNA gene clusters including suppressors in *E. coli*.
- F. MÜLLER, V. KÜRER, and S.G. CLARKSON, Institut für Molekularbiologie II, Universität Zürich, Switzerland: Structure and transcription of a cloned tRNA gene fragment of *X. laevis*.
- J. HECKMAN, B. ALZNER-DEWEERD, and U.L. RAJBHANDARY, Department of Biology, Massachusetts Institute of Technology, Cambridge: Mitochondrial tRNAs of *Neurospora crassa*—Sequence studies, gene mapping, and cloning.

Session 10b: Other Functions of tRNA

Chairperson: J.E. DAHLBERG, University of Wisconsin, Madison

- A.L. HAENNI,* S. JOSHI,* E. HUBERT,† G. HUEZ,† and G. MARBAIX,† *Université Paris VII, France; †Université Bruxelles, Rhode-St.-Genèse, Belgium: In vivo aminoacylation and "processing" of tumip-yellow mosaic virus RNA injected into *X. laevis* oocytes.
- W.K. YANG* and D.L. HWANG,† *Biology Division, Oak Ridge National Laboratory; †University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences: Binding activities of selective tRNA species to cellular retrovirus-specific tRNAs.
- S.P. EISENBERG, L. SOLL, and M. YARUS, Department of Molecular, Cell, and Developmental Biology, University of Colorado, Boulder: Undermodification of specific tRNAs affects regulation of the *trp* operon.
- J.R. MENNINGER, Zoology Department, University of Iowa, Iowa City: Macrolide antibiotics enhance the dissociation of peptidyl-tRNA from ribosomes of *E. coli*.
- R.L. SOFFER, Department of Biochemistry, Cornell University Medical College, New York, New York: Aminoacyl-tRNA-protein transferases.

Session 11: Poster Session

- H. KERSTEN, T. DINGERMAN, and M. MACH, Institut für Physiologische Chemie und Biochemie, Erlangen, Germany: Importance of ribothymidine-lacking tRNAs in developing *Dictyostelium discoideum*.
- M. KRAUSKOPF and J. VILLANUEVA, Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia: On the nature of the *N*-acetyl-phenylalanyl-tRNA hydrolase recognition site.
- T.A. KRUSE and B.F.C. CLARK, Institute of Chemistry, Aarhus University, Denmark: Possible influence of elongation factor Tu on the tRNA anticodon loop conformation.
- Y. KUCHINO, T. SEKIYA, and S. NISHIMURA, National Cancer Center Research Institute, Tokyo, Japan: Multiple copies of mammalian DNA fragment that specifically hybridizes with tRNA^{Asp}.
- I. KUDO, J.D. SMITH,* and U.L. RAJBHANDARY, Department of Biology, Massachusetts Institute of Technology, Cambridge: *MRC Laboratory of Molecular Biology, Cambridge, England: Cloning of *E. coli* tyrosine suppressor tRNA gene and expression of the cloned tRNA gene in *E. coli* minicells.
- J.A. LAKE, Molecular Biology Institute and Department of Biology, University of California, Los Angeles: Ribosome structure and tRNA-binding sites.
- M. LITT and K. WEISER, Department of Biochemistry and Division of Medical Genetics, University of Oregon School of Medicine, Portland: Studies in the control of specific tRNA levels in Friend leukemia cells.
- N. MARTIN,* D. MILLER,† and J.E. DONELSON,† *University of Minnesota, Minneapolis; †University of Iowa, Iowa City: Cloning of yeast mitochondrial tRNA genes in *E. coli*.
- E.J. MURGOLA, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston: Novel missense suppressors in *E. coli*.
- G. NASS and J. THOMALE, Abteilung Molekulare Biologie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany: Change of concentration of isoaccepting tRNAs caused by amino acid deficiency and not by uncharged tRNA.
- M.P. OESCHGER, N.S. OESCHGER, S.L. WOODS, G.T. WIPRUD, and P.M. CUCCARO, Department of Microbiology, Georgetown University, Washington, D.C.: Suppressor-enhancing mutations in *E. coli* K12.
- E. OHTSUKA, S. NISHIKAWA, R. FUKUMOTE, H. UEMURA, A.F. MARKHAM, S. TANAKA, T. MIYAKE, T. TANAKA, E. NAKAGAWA, and M. IKEYAMA, Faculty of Pharmaceutical Sciences, Osaka University, Japan: Joining and reconstitution of chemically synthesized formyl-methionine tRNA fragments.
- B.J. ORTWERTH, C. CONLON-HOLLINGSHEAD, and O.M.Y. CHU-DER, University of Missouri, Columbia: Correlation between tRNA₄^{Lys} and cell division in tissue-culture cells.
- J.B. PRINCE,* S.S. HIXSON,† and R.A. ZIMMERMANN,† Departments of *Biochemistry and †Chemistry, Univer-

sity of Massachusetts, Amherst: Photochemical cross-linking of tRNA^{Lys} and tRNA₂^{Glu} to 16S RNA at the ribosomal P site.

- D. RICHTER, Institut für Physiologische Chemie, Universität Hamburg, Germany: Discrimination between purine and pyrimidine base at the 3' terminus of the tRNA molecule by the stringent factor system from *E. coli*.
- J.M. ROBERTSON, H. WEIDNER, and W. WINTERMEYER, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Universität München, Germany: Fluorescence studies on tRNA-ribosome interactions.
- R.J. ROTHSTEIN, Department of Biochemistry, Cornell University, Ithaca, New York: Spontaneous deletion of the tyrosine tRNA locus *SUP4* of *S. cerevisiae*.
- S.J. SPENGLER, Space Sciences Laboratory, University of California, Berkeley: The biosynthesis of pseudouridine in tRNA—Isolation and characterization of the *hisT* gene product.
- R. WIRTH, D. ELHARDT, and A. BÖCK, Lehrstuhl für Mikrobiologie, Universität Regensburg, Germany: In vitro synthesis of ribosomal protein S20 and isoleucyl-, phenylalanyl-, and threonyl-tRNA synthetases.

Session 12: Interaction of tRNA with Ribosomes

Chairperson: C.R. CANTOR, Columbia University, New York, New York

- E. KUECHLER, A. BARTA, and A.J.M. MATZKE, Institute of Biochemistry, University of Vienna, Austria: Translocation of photo cross-linked complexes between phenylalanyl-tRNA from yeast and poly(U) on *E. coli* ribosomes.
- J. OFENGAND,* M. BOUBLIK,* S. GATES,† L. HSU,* M. KEREN-ZUR,* F.-L. LIN,* R. LIU,* I. SCHWARTZ,† and R. ZIMMERMANN,† *Roche Institute of Molecular Biology, Nutley, New Jersey; †University of Massachusetts, Amherst: Topography of tRNA-binding sites on ribosomes.
- W. WINTERMEYER, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Universität München, Germany: tRNA-tRNA interactions in solution.
- W. HILLEN, E. EGERT, H.-J. LINDNER, and H.G. GASSEN, Institut für Organische Chemie und Biochemie, Darmstadt, Germany: Function of "wobble nucleosides" in tRNA—Possible helix terminators in the first position of some anticodons.
- A.E. JOHNSON,* D.L. MILLER,† and C.R. CANTOR,‡ *Department of Chemistry, University of Oklahoma, Norman; †Department of Biochemistry, Roche Institute of Molecular Biology, Nutley, New Jersey; ‡Department of Chemistry, Columbia University, New York, New York: Affinity labeling of elongation factor Tu and of ribosomes using a functional analog of lysyl-tRNA.
- M. SPRINZL and K. WATANABE, Abteilung Chemie, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Germany: Interaction of the TΨC region of tRNA with the ribosomal A site.

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Russell Ross, University of Washington

Gordon Sato, University of California, San Diego

139 participants

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

Session I

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

- S. COHEN,* H. HAIGLER,* G. CARPENTER,** L. KING,†‡, Departments of *Biochemistry and †Medicine, Vanderbilt University, and ‡Veterans Administration Hospital, Nashville, Tennessee: Epidermal growth factor (EGF). I. Visualization of the binding and internalization of EGF in cultured cells. II. Enhancement of phosphorylation by EGF in membrane preparations in vitro.

- C. F. FOX, University of California, Los Angeles: Specific labeling and dynamics of the receptor for EGF.
- I. PASTAN, F. MAXFIELD, J. KEEN, Y. SHECHTER,* J. SCHLISSINGER, and M. WILLINGHAM, National Institutes of Health, Bethesda, Maryland; *Burroughs Wellcome Company, Research Triangle Park, North Carolina: Direct visualization of hormone binding and internalization in living cells.
- R. W. HOLLEY, Salk Institute, San Diego, California: Control of growth of kidney epithelial cells in culture.
- M. L. SMITH and J. M. BUCHANAN, Department of Biology, Massachusetts Institute of Technology, Cambridge: Activation of de novo purine synthesis after mitogenic stimulation of resting fibroblasts.

Session 2

Chairperson: R. BRADSHAW, Washington University, St. Louis, Missouri

- R. ROSS, A. VOGEL, P. F. DAVIES, E. RAINES, B. KARIYA, M. J. RIVEST, C. GUSTAFSON, and J. GLOMSET,* Departments of Pathology and *Medicine, University of Washington School of Medicine, Seattle: The platelet-derived growth factor.
- C.-H. HELDIN, B. WESTERMARK,* and Å. WASTESON, Institute of Medical and Physiological Chemistry, and *Wallenberg Laboratory, Uppsala, Sweden: Purification and characterization of human growth factors.
- C. D. STILES, W. J. PLEDGER, J. J. VAN WYK, H. ANTONIADES, and C. D. SCHER, Sidney Farber Cancer Institute and Center for Blood Research, Boston, Massachusetts, and University of North Carolina Medical School, Chapel Hill: Serum components which regulate the commitment of BALB/c-3T3 cells to DNA synthesis.
- D. GOSPODAROWICZ, I. VLODAVSKY, G. GREENBURG, and L. K. JOHNSON, Cancer Research Institute, University of California Medical Center, San Francisco: Cellular shape is determined by the extracellular matrix and is correlated with the control of cellular growth and physiological function.
- S. SCHWARTZ, P. BOWMAN, and S. SELDEN, Department of Pathology, University of Washington, Seattle: Growth control of aortic endothelium at wound edges.
- W. H. BEERS, Rockefeller University, New York, New York: The transmission of hormonal stimulation by a cell-contact-dependent mechanism—In vitro studies and implications for hormone active in vivo.

Session 3

Chairperson: M. STOKER, Imperial Cancer Research Fund Laboratories, London, England

- E. ROZENGURT and J. B. SMITH, Imperial Cancer Research Fund Laboratories, London, England: Monovalent ion fluxes in quiescent cultures of 3T3 cells—Mechanism and significance in the regulation of cell growth.
- I. S. EDELMAN, Department of Biochemistry, Columbia University, New York, New York: Thyroid thermogenesis in hepatocytes in primary cultures.
- A. H. RUBIN, Department of Molecular Biology and Virus Laboratory, University of California, Berkeley: The coordinate response of cells to hormones and its mediation by the intracellular availability of magnesium.
- L. JIMENEZ DE ASUA, K. M. V. RICHMOND, M. K. O'FARRELL,* A. M. OTTO, A. M. KUBLER, and P. S. RUDLAND,* Friedrich Miescher-Institut, Basel, Switzerland; *Imperial Cancer Research Fund Laboratories, London, England: Growth factors and hormones interact in a series of temporal steps to regulate the rate of initiation of DNA synthesis in mouse fibroblasts.
- A. B. PARDEE and A. YEN, Sidney Farber Cancer Institute, and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts: Variability of the cell cycle.
- R. BASERGA, M. ROSSINI, J. FLOROS, and R. WEINMANN,* Fels Research Institute, Temple University Medical School, and *Wistar Institute, Philadelphia, Pennsylvania: Induction of cellular DNA synthesis in resting cells by serum, polyoma, and Ad2.

Session 4

Chairperson: R. HOLLEY, Salk Institute, San Diego, California

- H. A. ARMELIN, M. C. S. ARMELIN, S. E. FARIAS, A. G. GAMBARINI, and E. KIMURA, Instituto de Quimica, Universidade de São Paulo, Brazil: Effects of hydrocortisone on the growth control of a mutant derived from Swiss mouse 3T3 fibroblasts.
- V. J. CRISTOFALO, J. M. WALLACE, and B. A. ROSNER, Wistar Institute, Philadelphia, Pennsylvania: Glucocorticoid enhancement of proliferative activity in WI-38 cells.
- W. H. DAUGHADAY, Washington University, St. Louis, Missouri: Somatomedins as mediators of growth hormone action in vitro.

- L. FRYKLUND, A. SKOTTNER, K. HALL,* A. FORSMAN, and S. CASTENSSON, Recip Polypeptide Laboratory, Aktiebolaget KABI, and *Karolinska Hospital, Stockholm, Sweden: Somatomedins A and B—Chemical nature and *in vivo* activity.
- P. S. RUDLAND, M. J. WARBURTON, and D. C. BENNETT, Imperial Cancer Research Fund Laboratories, London, England: Growth and differentiation of cultured rat mammary cells.
- T. KANO-SUEOKA, J. E. ERRICK, and D. M. COHEN, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder: Effects of hormones and a novel mammary growth factor on a rat mammary carcinoma MCCLX in culture.

Session 5

Chairperson: R. IGLESIAS, Estado 57, Department 706, Santiago, Chile

- D. D. CUNNINGHAM, D. H. CARNEY, and K. C. GLENN, Department of Medical Microbiology, University of California, Irvine: Role of the cell surface in initiation of cell division by thrombin.
- J. P. QUIGLEY, B. M. MARTIN, R. H. GOLDFARB, C. J. SCHEINER, and W. D. MULLER, Downstate Medical Center, State University of New York, Brooklyn: The involvement of serine proteases in growth control and malignant transformation.
- E. REICH, Rockefeller University, New York, New York: Plasminogen activator—Modulation of enzyme production and application in studying hormonal regulation of tissue function.
- M. LACROIX, F. SMITH, and I. B. FRITZ, Banting and Best Department of Medical Research, University of Toronto, Canada: The control of plasminogen activator secretion by Sertoli cells in culture, and its possible role in spermatogenesis.
- T. D. GELEHRTER, S. A. CARLSON, and B. L. FREDIN, Departments of Internal Medicine and Human Genetics; University of Michigan Medical School, Ann Arbor: Glucocorticoid regulation of plasminogen activator in rat hepatoma cells.
- L. OSSOWSKI, Rockefeller University, New York, New York: Mammary plasminogen activator—Hormonal control and life-cycle correlations in normal and malignant tissue.

Session 6

Chairperson: G. SATO, University of California, San Diego

- E. R. FROESCH, J. ZAPP, E. RINDERKNECHT,* and R. E. HUMBEL, Departments of Medicine and *Biochemistry, University of Zürich, Switzerland: Insulinlike growth factors (NSILA)—Structural and functional similarities with insulin.
- J. LARNER, J. C. LAWRENCE, P. J. ROACH, A. A. DEPAOLI-ROACH, R. J. WALKENBACH, J. GUINOVAR, and R. J. HAZEN, Department of Pharmacology, University of Virginia School of Medicine, Charlottesville: About insulin and glycogen.
- J. ROTH, Diabetes Branch, National Institutes of Health, Bethesda, Maryland: Insulin receptors *in vivo* and *in vitro*.
- A. TIXIER-VIDAL, N. BRUNET, and D. GOURDJI, Groupe de Neuroendocrinologie Cellulaire, Collège de France, Paris: Plasma membrane modifications related to the action of thyrotropin-releasing hormone (TRH) on rat prolactin cell lines.
- G. N. GILL, P. J. HORNSBY, and M. H. SIMONIAN, Department of Medicine, University of California, San Diego: Regulation of growth and differentiated function of cultured bovine adrenocortical cells.
- A. H. TASHJIAN, Jr., A. SCHONBRUNN, and T. F. J. MARTIN, Harvard Medical School and Harvard School of Public Health, Boston, Massachusetts: Interactions of TRH and somatostatin with pituitary cells in culture—Modulation of peptide receptors and hormone release and synthesis.
- B. P. SCHIMMER, P. A. RAE, N. S. GUTMANN, and V. M. WATT, Banting and Best Department of Medical Research, University of Toronto, Canada: Genetic dissection of ACTH action in adrenal tumor cells.

Session 7

Chairperson: H. EAGLE, Albert Einstein College of Medicine, Bronx, New York

- S. P. NISSLEY, M. M. RECHLER, A. C. MOSES, H. J. EISEN, O. Z. HIGA, I. FENNOY, C. B. BRUNI, R. M. WHITE, E. E. SCHILLING, J. PODSKALNY, and P. A. SHORT, National Institutes of Health, Bethesda, Maryland: Evidence that multiplication-stimulating activity (MSA) purified from the BRL-3A rat-liver cell line is found in rat serum and fetal liver organ culture.

- A. LIPTON, M. F. SCHULER, E. MUSSELMAN, R. C. FELDHOFF, and L. S. JEFFERSON, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey: Liver as a source of transformed-cell growth factor.
- G. J. TODARO and J. E. DE LARCO, NCI, National Institutes of Health, Bethesda, Maryland: Growth factors produced by sarcoma-virus-transformed cells.
- W. TOPP,* D. RIFKIN,¹ A. GRAESSMAN,² and M. SLEIGH,³ *Cold Spring Harbor Laboratory, New York; ¹New York University Medical School, New York; ²Institut für Molekularebiologie und Biochemie, Freie Universität Berlin, Germany; ³CSIRO, Epping, Australia: The role of early SV40 gene products in the maintenance of the transformed state.
- L. LEVINE, Department of Biochemistry, Brandeis University, Waltham, Massachusetts: Deacylation of cellular lipids and archidonic acid metabolism in cultured cells.
- K. NISHIKAWA, C. OKITSU, and G. H. SATO,* Department of Chemistry, Kanazawa Medical University, Ishikawa, Japan; *Department of Biology, University of California, San Diego: Control of BALB/3T3 growth by factors present in tumor extract.
- R. IGLESIAS, Estado 57, Department 706, Santiago, Chile: Tumors produced by hormones, hormones produced by tumors, and tumors produced by tumors.

Session 8

Chairperson: J. ROTH, National Institutes of Health, Bethesda, Maryland

- R. GOODMAN, C. CHANDLER, and H. R. HERSCHMAN, University of California School of Medicine, Los Angeles: Pheochromocytoma cell lines as models of growth-factor and hormone-induced neuronal differentiation.
- S. OHNO, Department of Biology, City of Hope National Medical Center, Duarte, California: Testis-organizing H-Y antigen as a short-range hormone in gonadal development.
- H. ISHIKAWA, M. SHIINO, and E. G. RENNELS, Department of Anatomy, University of Texas Health Science Center, San Antonio: Normal functional clones of pituitary cells derived from Rathke's pouch epithelium of fetal rats.
- A. G. GILMAN, P. C. STERNWEIS, A. C. HOWLETT, and E. M. ROSS, Department of Pharmacology, University of Virginia School of Medicine, Charlottesville: Resolution and reconstitution of some components of catecholamine-stimulated adenylate cyclase.
- P. COFFINO, Departments of Medicine and Microbiology, University of California, San Francisco: Regulation of mouse lymphoma cell growth by AMP.
- K. KRATOCHWIL, R. DÜRNBERGER, B. HEUBERGER, and G. WASNER, Institut für Molekularbiologie, Österreichische Akademie der Wissenschaften, Salzburg, Austria: Hormone-induced cell and tissue interaction in the embryonic mammary gland.
- F. T. KENNEY, Oak Ridge National Laboratory, Tennessee: Control of enzyme synthesis in hepatoma cells by steroids and other hormones.

Session 9

Chairperson: A. B. PARDEE, Sidney Farber Cancer Institute, Boston, Massachusetts

- M. R. STALLCUP, J. RING, and K. R. YAMAMOTO, Department of Biochemistry and Biophysics, University of California, San Francisco: Glucocorticoids regulate mammary tumor virus gene expression at the level of initiation of transcription.
- J. D. BAXTER, P. H. SEEBURG, J. A. MARTIAL, J. SHINE, L. K. JOHNSON, R. D. IVARIE, B. SCHACTER, and H. M. GOODMAN, Departments of Biochemistry and Medicine, University of California, San Francisco: Structure, expression in bacteria, and regulation in cultured cells of the growth hormone gene.
- D. GRANNER, M. DIESTERHAFT, T. NOGUCHI, P. OLSON, J. HARGROVE, and G. VOLENTINE, Departments of Medicine and Biochemistry, University of Iowa, and Veterans Administration Hospital, Iowa City: Regulation of liver and HTC cell tyrosine aminotransferase mRNA by glucocorticoids and dibutyl AMP.
- H. H. SAMUELS, F. STANLEY, L. E. SHAPIRO, J. CASANOVA, Z. D. HOROWITZ, and D. KLEIN, Department of Medicine, New York University Medical Center, New York: Multihormonal control of the growth hormone genome in cultured GH₄ cells by thyroid and glucocorticoid hormones.
- E. B. THOMPSON,* D. K. GRANNER,[†] T. D. GELEHRTER,[‡] S. S. SIMONS,[§] and G. HAGER,* *NCI, National Institutes of Health, Bethesda, Maryland; [†]Department of Medicine, University of Iowa Medical School, Iowa City;

*Department of Human Genetics, University of Michigan Medical School, Ann Arbor; §NIAMDD, National Institutes of Health, Bethesda, Maryland: Unlinked control of multiple glucocorticoid-sensitive processes in spontaneous HTC cell variants.

- J. L. ROBERTS,* M. BUDARF,† R. G. ALLEN,† J. D. BAXTER,* and E. HERBERT,† *University of California Medical School, San Francisco; †Chemistry Department, University of Oregon, Eugene: Effect of glucocorticoids on the synthesis and processing of the common precursor to adrenocorticotropin and endorphin in mouse pituitary tumor cells.
- S. L. SABOL, A. LING, and M. P. DANIELS, NHLBI, National Institutes of Health, Bethesda, Maryland: Endorphin production and its regulation by glucocorticoids in clonal pituitary tumor cells.

Session 10

Chairperson: R. ROSS, University of Washington, Seattle

- A. B. LERNER, Department of Dermatology, Yale University, New Haven, Connecticut: Action of MSH on pigment cells.
- D. A. SIRBASKU and R. H. BENSON, Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston: Estrogen-inducible growth factors which may act as mediators (estromedins) of estrogen-promoted tumor cell growth.
- M. E. LIPPMAN, J. C. ALLEGRA, J. S. STROBL, and L. W. ENGEL, NCI, National Institutes of Health, Bethesda, Maryland: Growth requirements of a human breast cancer cell line in serum-free medium.
- M. L. WALSH, B. DAMIANI, D. WONG, E. J. WOLINSKY, and L. B. CHEN, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts: A possible endogenous growth factor—*Src* gene product.
- J. HOCHSTADT,** D. C. QUINLAN,* A. J. OWEN,† and K. O. COOPER,† *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts; †Department of Biochemistry and Biophysics, University of Rhode Island, Kingston; †Department of Microbiology, New York Medical College, Valhalla: Regulation of transport upon interaction of fibroblast growth factor (FGF) with plasma membrane vesicles isolated from quiescent (G₀) BALB/3T3 cells.
- J. BOTTENSTEIN, J. MATHER,* and G. SATO, Department of Biology, University of California, San Diego; *The Population Council, Rockefeller University, New York, New York: Growth of neuroepithelial derived cell lines in serum-free hormone-supplemented media.
- G. R. SERRERO, D. B. MCCLURE,* and G. H. SATO, Centre de Biochimie, Faculté SPCNI, Nice, France; *Department of Biology, University of California, San Diego: Growth of mouse 3T3 fibroblasts in serum-free, hormone-supplemented media.
- S. STRICKLAND, Rockefeller University, New York, New York: Differentiation in teratocarcinoma stem cells by retinoic acid.

This meeting was supported in part by funds received from the National Cancer Institute, National Institute on Aging, National Institute of Child Health and Human Development, National Institute of General Medical Science, Fogarty International Center, and the National Science Foundation.

IN-HOUSE SEMINARS



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

J. Broach giving seminar in Blackford Hall

1977-1978

October

Olke Uhlenbeck, University of Illinois, Chicago: *RNA ligase and its application.*

Virginia Walbot, Washington University, St. Louis, Missouri: *Clonal development in corn and cytoplasmic organelles.*

November

Herbert Heyneker, University of California, San Francisco: *Expression in E. coli of a cloned somatostatin gene.*

Nicholas Cozzarelli, University of Chicago, Illinois: *DNA gyrase—The enzymatic alteration of super helix density and the inhibition of DNA replication by nalidixic acid.*

Neil Wilkie, Glasgow University, Scotland: *Genetic and physical maps of herpesvirus genomes.*

Alexander J. Varshavsky, Massachusetts General Hospital, Boston: *On the structure of eukaryotic, prokaryotic, and viral chromatin.*

December

Frederick Alt, Massachusetts Institute of Technology, Cambridge: *Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant cell lines.*

Robert Sauer, Harvard University, Cambridge, Massachusetts: *The lambda repressor.*

James Stringer, University of California, Irvine: *Mapping HSV transcripts by electron microscopy.*

February

Kurt Drickamer, Harvard University, Cambridge, Massachusetts: *Structure of band 3 in red blood cell membrane.*

Richard Axel, Columbia University, New York, New York: *Isolation, transfer, and integration of eukaryotic genes into mutant mouse cells.*

Shirley Tilgman, National Institutes of Health, Bethesda, Maryland: *Insertion in the mouse hemoglobin gene.*

Gary Strobel, United States Department of Agriculture, Bethesda, Maryland: *Biochemical basis of disease resistance in plants.*

March

Peter Lengyel, Yale University, New Haven, Connecticut: *Studies on the interferon system.*

S. S. Tevethia, Tufts University Medical Center, Boston, Massachusetts: *Immunobiology of SV40 TSTA.*

Brooks Low, Yale University, New Haven, Connecticut: *Different modes of recombination during conjugation and transduction in E. coli.*

Eugene Bell, Massachusetts Institute of Technology, Cambridge: *Semiautomated analysis of cell lineages in vitro.*

Jim Feramisco, University of Washington, Seattle: *Synthetic peptides as model substrates and inhibitors for the cAMP-dependent protein kinase.*

Mark Furth, University of Wisconsin, Madison: *Control of lambda DNA replication.*

April

- Aaron Shatkin, Roche Institute of Molecular Biology, Nutley, New Jersey: *5' cap of eukaryotic messenger RNA.*
- Pamela Stanley, Albert Einstein College of Medicine, Bronx, New York: *Alterations at the cell surface in lectin-resistant CHO cell mutants.*
- Lucia Rothman-Denes, University of Chicago, Illinois: *Control of development in the bacteriophage N4.*
- Bruce Zetter, University of California, San Francisco: *Effects of growth factors and carcinogens on vascular endothelial cells.*
- Jerry Rubin, Sidney Farber Cancer Center, Boston, Massachusetts: *Cloned drosophila genes.*
- Helen Donis-Keller, Harvard University, Cambridge, Massachusetts: *RNA sequencing.*
- Stephen Blose, University of Pennsylvania, Philadelphia: *10 nm filaments in endothelial cells—Structure and possible function.*
- Tom Schenk, University of Connecticut Medical Center, Storrs: *Deletion mutants of SV40.*
- Demetrius Spandidos, University of Toronto, Canada: *Transfer of genes which cause malignancy in mammalian cells.*

May

- John Fiddes, University of California School of Medicine, San Francisco: *Bacteriophage G4—A comparison of its sequence and overlapping genes with ϕ X174.*
- Michael Wigler, Columbia University, New York, New York: *Gene transfer in tissue culture.*
- Kevin Struhl, Stanford University, California: *Transformation in yeast.*
- Anton Jetten, Roche Institute of Molecular Biology, Nutley, New Jersey: *Action of retinoids on fibroblasts and embryonic carcinoma cells.*
- John Smart, Imperial Cancer Research Fund Laboratories, London, England: *The biological implications of a comparative tryptic peptide map analysis of the tumor antigens of polyoma virus.*
- Richard Frisque, University of Wisconsin, Madison: *Biological and biochemical properties of JC virus DNA.*
- Janice Pero, Harvard University, Cambridge, Massachusetts: *RNA polymerase from SPO1-infected B. subtilis.*

UNDERGRADUATE SUMMER RESEARCH PROGRAM

Another aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 195 students have completed the course, and many have gone on to creative careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology, (2) an increased awareness of major problem areas under investigation, (3) better understanding of the physical and intellectual tools for modern research and the pertinence of this information to future training, and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from a large number of applicants, took part in the program, which is now mainly supported by Laboratory funds. They are listed below, with their laboratory sponsors and topics of research. We gratefully acknowledge the generosity of Central General Hospital, Plainview, New York, which provided additional support.

Ezekiel J. Emanuel, Amherst College <i>Supervisor:</i> J.B. Hicks	Isolation of mutations in the mating type locus of <i>S. cerevisiae</i>
Debra Sue Erdmann, University of Wisconsin <i>Supervisor:</i> D.Y. Kwok	Genetic recombination and complementation between bacteriophage Mu and cloned fragments of Mu DNA
Scott Finley, State University of New York, Stony Brook <i>Supervisor:</i> N. Harter	Immunological identification of Ad2 early proteins
Judith R. Krieger, Harvard University <i>Supervisor:</i> K. Burridge	The use of monoclonal antibodies to study cell surface antigens
James Lupski, New York University <i>Supervisor:</i> A.I. Bukhari	Construction of plasmids containing ends of phage Mu DNA
Kenneth B. McElwain, Wesleyan University <i>Supervisor:</i> T.R. Broker	Identification of recombinant plasmids with Tn5 (the Kanamycin transposon) inserted in cloned phage Mu DNA
Jeremy H. Nathans, Massachusetts Institute of Technology <i>Supervisor:</i> R.J. Roberts	Modifications of Sanger's chain termination DNA sequencing method as applied to adenovirus 2 DNA

Susan Rolseth, University of Connecticut
Supervisor: Y.-S. E. Cheng

Adam Leon Schulman, University of Chicago
Supervisor: G. Albrecht-Buehler

Michael J. Stern, Stanford University
Supervisor: J.R. Broach

Olney Fellowship

Steven Robinow, University of California, Berkeley
Supervisor: R. Tjian

Characterization of *E. coli* K12 mutants defective in
protease III

The effect of cold shocks on the mirror-symmetrical
migration of sister 3T3 cells

Cloning *SUP61*, a yeast serine-inserting,
recessive-lethal, nonsense-suppressor gene

Enzymatic and DNA binding properties of the
SV40 A-gene product

NATURE STUDY PROGRAM

The Nature Study Program is designed for elementary and high school students who wish to achieve a greater understanding of their environment. During the summer and fall a total of 324 students participated in these activities. When weather permitted, most of the courses were held outdoors on Laboratory grounds or at Uplands Farm Nature Preserve of The Nature Conservancy, where the Laboratory has equipped and maintains classroom/laboratories for the study of field specimens collected by the students.

This summer the series of one day "Marine Biology Workshops" was extended to two weeks, including two cruises for adults. The studies, conducted on Long Island Sound, were performed aboard the R/V Tradewind, chartered from Schooner Inc., New Haven, Connecticut. The vessel is equipped with a variety of instrumentation and staffed by a Captain, mate, and Marine Biologist. Students participated in the biological studies and in the actual sailing of the vessel.

This past summer "Adventure Education" was added, which included an 18-mile bicycle hike, a 12-mile canoe trip, and sailing on Long Island Sound.

In the Fall, "Observational Astronomy" was again offered as an introduction to all facets of astronomy. Photography as related to Astronomy was included, with students utilizing our fully equipped darkroom. Telescopes for the observational sessions were provided by Ehrenreich Photo-Optical, and technical assistance by The Astronomical Society of Long Island and the Long Island Observers Association.

Program director: Sanford Kaufman, M.S., M.P.A., biology teacher, Hewlett High School

INSTRUCTORS

Don Dunn, M.S., art and photography teacher, Hewlett High School
Phylis Hechtlinger, M.S., science teacher, East Rockaway High School
Carolyn Hess, M.A. candidate, SUNY at Stony Brook
Lois Joseph, M.A., biology teacher, Hewlett High School
Fred Maasch, M.Ed., biology teacher, Islip High School
James Romansky, M.S., biology teacher, Bay Shore High School
Edward Tronolone, M.S., science teacher, Lynbrook North Middle School

COURSES

General Nature Study
Advanced Nature Study
Elementary Geology
Geology
Bird Study
Seashore Life
Animals with Backbones
Reptiles and Amphibians
Aquatic Biology
Marine Biology
Nature Photography I and II
Fresh Water Life
Observational Astronomy
Adventure Education

BANBURY CENTER



The Banbury program of small meetings on environmental health risks began the hard way. It poured with rain on the Sunday in May when most of the scientists attending our first meeting were to arrive. Thus, there were few to eat the first of a series of excellent meals prepared by our local caterer. The details of the meeting, including the contract for it, were arranged in just a few weeks during which it seemed that a new chemical carcinogen was cropping up almost daily. And the subject of the first meeting was inherently difficult. Leading students of animal and human genetics spent three days arguing about the risk to future generations from mankind's rapidly increasing commitment to chemical technology. The conclusions seemed to be that there is a risk, albeit hard to measure, and that geneticists should warn governments of the urgent need to gather data rigorously on the occurrence of mutations in newborns. This would provide a background against which the effects of new pollutants could be measured.

We taped it all, deciphered almost all of it, let the authors see our transcript, and participated in the difficult task of writing a summary of the conference. In November, a final draft report was produced for the meeting's sponsor, the Office of Toxic Substances of the Environmental Protection Agency, using the same IBM Office System 6 equipment that is producing the proceedings volume that the Laboratory is publishing April 1.

While learning hard lessons about rapid production of proceedings volumes from tape recordings, the Banbury staff was planning a series of 1979 meetings on six topics: short-term mutagenicity tests using mammalian cells, risk assessment with the Mormon data bank, the consequences of finding that ethylene dichloride causes cancer in animals, the possibilities of a safe cigarette, the quantification of industrial cancer, and prolactin inducers.

The planning involved many steps. The Banbury Director, Victor McElhenny, attended several conferences on environmental health problems, including the massive sessions in June arranged for the New York Academy of Sciences by Dr. Irving Selikoff. There were visits to the libraries of Dr. Selikoff at Mt. Sinai Hospital and Dr. Norton Nelson at Sterling Forest, New York. A select library on biological risk assessment was established at Banbury, and the Director's 20-year file of news clipping and releases on toxic substances was reworked. Advice was sought widely. We held a two-hour session with the participants of the Laboratory's phorbol ester meeting last May, and a smaller session with participants in the Symposium on DNA replication and recombination.

There were frequent contacts with John Cairns, Bruce Ames, Bernard Weinstein, Richard Peto, Joyce McCann, Fred de Serres of the National Institute of Environmental Health Sciences, Alexander Hollaender of Associated Universities, Inc. and with such representatives of industry as John Burns of Hoffmann-La Roche, Inc. As the conversations proceeded, the subject of biological risk assessment divided itself into such salient topics as the technology of short-term testing, problems of human data collection, industrial carcinogens, consumer health risks, and diet and human cancer. In developing the Banbury program, it became clear that our topics are a logical extension of the territory pioneered by the Laboratory's 1976 Conference on the Origins of Human Cancer. It also became clear that every department of the laboratory was ready to help the new center get under way—and that this help was vital.

ASSESSING CHEMICAL MUTAGENS: The Risk to Humans **May 15–May 17**

Session 1

- R.N. Hill, Office of Toxic Substances, Environmental Protection Agency, Washington, D.C.: Introduction.
- J.V. Neel, Department of Human Genetics, University of Michigan Medical School, Ann Arbor: Mutation and disease in humans.
- W.G. Flamm, Division of Toxicology, Bureau of Foods, Food and Drug Administration, Washington, D.C.: Strengths and weaknesses of tests for mutagenesis.

Session 2

- E. Eisenstadt, Departments of Microbiology and Physiology, Harvard University School of Public Health, Boston, Massachusetts: Bacterial mutagenicity testing: Some practical considerations.
- G. Walker, Biology Department, Massachusetts Institute of Technology, Cambridge: Theory and design of short-term bacterial tests for mutagenesis.
- R. Setlow, Biology Department, Brookhaven National Laboratory, Upton, New York: DNA repair.
- J. G. Brewen, Biology Division, Oak Ridge National Laboratory, Tennessee: Cytogenetic studies and risk assessment for chemicals and ionizing radiation.

Session 3

- L. R. Valcovic, Public Health Service, Bureau of Foods, Food and Drug Administration, Washington, D.C.: General aspects of comparative mutagenesis.

Session 4

- J.W. Baum, Safety and Environmental Protection Division, Brookhaven National Laboratory, Upton, New York: Radiation-induced cancer.
- D. Hoel, Biometry Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Low-dose and species-to-species extrapolation for chemically induced carcinogenesis.
- S. Abrahamson, Department of Zoology, University of Wisconsin, Madison: Estimating radiation-induced genetic disease burdens.
- L. Ehrenberg, Wallenberg Laboratory, Stockholm University, Sweden: Risk assessment of ethylene oxide and other compounds.
- W.B. Lee, Jr., Department of Zoology, Louisiana State University, Baton Rouge: Dosimetry of alkylating agents.
- V. Ray, Medical Research Laboratory, Pfizer, Inc., Groton, Connecticut: Are benzene effects limited to the chromosomal level?

Sessions 5, 6, 7

Discussions

LABORATORY STAFF

December 1978

DIRECTOR
J. D. Watson

ADMINISTRATIVE DIRECTOR
William R. Udry

**ASSISTANT DIRECTOR
FOR RESEARCH**
Joseph Sambrook

DIRECTOR—BANBURY CENTER
Victor K. McElheny

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Ahmad Bukhari
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Diana O'Loane
Rebecca Pashley
Christine Paul
Patricia Reichel
Vicki Rowland-Guarascio
Nora Sarvetnick
Mark Schwartz
John Scott
Leslie Smith
Susan Smith
Margaret Wallace
Susanne Weirich
Jeanne Wiggins
Gail Wong



Welcoming the first day of Spring, Dr. Walter Schaffner, costumed in bark mask and branches, chides critics of recombinant DNA research.

SUPPORTING STAFF

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Louisa Dalessandro
Bruce DeTroy
Joyce Dorso
August Dulis
Steven Eagles
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BANBURY CENTER

Beatrice Toliver

GENETICS RESEARCH UNIT CARNEGIE INSTITUTION OF WASHINGTON

Alfred Hershey
Barbara McClintock

*After a busy summer, the
Lab staff unwinds with a
tug-of-war game at the annual
Fall picnic.*



FINANCIAL STATEMENT

BALANCE SHEET

year ended December 31, 1978

with comparative figures for 12 months ended October 31, 1977

ASSETS	<u>1978</u>	<u>1977</u>	LIABILITIES AND FUND BALANCES	<u>1978</u>	<u>1977</u>
CURRENT FUNDS			CURRENT FUNDS		
<i>Unrestricted</i>			<i>Unrestricted</i>		
Cash	\$ 312,756	\$ 269,437	Accounts payable	\$ 149,608	\$ 68,291
Accounts Receivable	174,199	89,366	Mortgage Payable	166,500	—
Prepaid expenses	23,742	68,704	Due to plant funds	377,531	417,178
Inventory of books	135,838	121,937	Fund balance	<u>69,533</u>	<u>199,544</u>
Due from restricted fund	101,653	135,569			
Due from Banbury Center	14,984	—			
Total unrestricted	<u>763,172</u>	<u>685,013</u>	Total unrestricted	<u>763,172</u>	<u>685,013</u>
<i>Restricted</i>			<i>Restricted</i>		
Grants and contracts receivable	<u>2,063,598</u>	<u>2,396,218</u>	Due to unrestricted funds	101,653	135,569
Total restricted	<u>2,063,598</u>	<u>2,396,218</u>	Fund balance	<u>1,961,945</u>	<u>2,260,649</u>
Total current funds	<u>\$ 2,826,770</u>	<u>\$ 3,081,231</u>	Total restricted	<u>2,063,598</u>	<u>2,396,218</u>
			Total current funds	<u>\$ 2,826,770</u>	<u>\$ 3,081,231</u>
ENDOWMENT FUNDS			ENDOWMENT FUNDS		
<i>Robertson Research Fund</i>					
Cash	25,073	96,492			
Accrued interest	—	45,400			
Marketable securities					
(quoted market 1978—\$8,427,386;					
1977—\$8,122,321)	<u>7,978,726</u>	<u>7,814,204</u>			
Total Robertson Research Fund	<u>8,033,799</u>	<u>7,956,096</u>			

<i>Olney Memorial Fund</i>		
Cash	412	550
Marketable Securities		
(quoted market 1978—\$22,496; 1977—\$20,951)	24,123	21,510
Total Olney Memorial Fund	<u>24,535</u>	<u>22,060</u>
Total endowment funds	<u>\$ 8,058,334</u>	<u>\$ 7,978,156</u>

PLANT FUNDS

Investments	219,358	84,508
Due from unrestricted fund	377,531	270,362
Land and improvements	935,530	719,545
Buildings	5,493,511	5,171,784
Furniture, fixtures and equipment	857,891	792,690
Books and periodicals	365,630	365,630
Construction in progress	65,012	24,136
	<u>8,314,463</u>	<u>7,428,655</u>
Less allowance for depreciation and amortization	1,545,396	1,143,102
Total plant funds	<u>6,769,067</u>	<u>6,285,553</u>

BANBURY CENTER

Cash	9,304	4,178
Accrued interest	—	18,181
Marketable securities		
(quoted market 1978—\$1,539,539; 1977—\$1,530,097)	1,493,754	1,515,548
Total operating funds	<u>1,503,058</u>	<u>1,537,907</u>
Land	772,500	772,500
Buildings	412,672	384,234
Furniture, fixtures, and equipment	160,700	147,887
Construction in progress	8,856	—
	<u>1,354,728</u>	<u>1,304,621</u>
Less allowance for depreciation	86,886	45,211
Total plant funds	<u>1,267,842</u>	<u>1,259,410</u>
Total Banbury Center	<u>\$ 2,770,900</u>	<u>\$ 2,797,317</u>
Total—All funds	<u>\$20,425,071</u>	<u>\$20,142,257</u>

Fund balance	<u>\$ 8,058,334</u>	<u>\$ 7,978,156</u>
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PLANT FUNDS

Investments	219,358	84,508
Due from unrestricted fund	377,531	270,362
Land and improvements	935,530	719,545
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Furniture, fixtures and equipment	857,891	792,690
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Less allowance for depreciation and amortization	1,545,396	1,143,102
Total plant funds	<u>6,769,067</u>	<u>6,285,553</u>
Fund balance	<u>\$ 6,769,067</u>	<u>\$ 6,285,553</u>

BANBURY CENTER

Due to unrestricted fund	14,984	—
Fund balance	<u>1,488,074</u>	<u>1,537,907</u>
Total operating funds	<u>1,503,058</u>	<u>1,537,907</u>
Land	772,500	772,500
Buildings	412,672	384,234
Furniture, fixtures, and equipment	160,700	147,887
Construction in progress	8,856	—
	<u>1,354,728</u>	<u>1,304,621</u>
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Total plant funds balance	<u>1,267,842</u>	<u>1,259,410</u>
Total Banbury Center	<u>\$ 2,770,900</u>	<u>\$ 2,797,317</u>
Total—All funds	<u>\$20,425,071</u>	<u>\$20,142,257</u>

CURRENT REVENUES, EXPENDITURES AND TRANSFERS
 year ended December 31, 1978
 with comparative figures for 12 months ended October 31, 1977

COLD SPRING HARBOR LABORATORY			BANBURY CENTER		
	<u>1978</u>	<u>1977</u>		<u>1978</u>	<u>1977</u>
REVENUES			REVENUES		
Grants and contracts	\$2,826,545	\$2,133,568	Endowment income	\$ 83,424	\$107,291
Indirect cost allowances on grants and contracts	1,299,409	957,464	Grants & contributions	100,000	15,000
Contributions			Conference fees	970	—
Unrestricted	100,660	37,529	Rooms and apartments	16,700	12,750
Restricted	10,000	25,000	Transfer from Cold Spring Harbor Laboratory	<u>12,900</u>	<u>61,043</u>
Long Island Biological Association	31,000	194,000	Total revenues	<u>213,994</u>	<u>196,084</u>
Robertson Research Fund Distribution	379,167	325,000			
Summer programs	207,838	183,744	EXPENDITURES		
Laboratory rental	21,796	20,554	Conferences	10,179	—
Marina rental	39,900	38,000	Operation and maintenance of plant	81,929	56,665
Investment income	45,424	10,430	Program administration	86,949	12,545
Publications sales	649,041	383,196	Capital plant	<u>49,826</u>	<u>169,825</u>
Dining hall	272,845	238,300	Total expenditures	<u>228,883</u>	<u>239,035</u>
Rooms and apartments	183,575	151,601			
Other sources	<u>59,175</u>	<u>8,234</u>	Excess of expenditures over revenues	<u>\$ 14,889</u>	<u>\$ 42,951</u>
Total revenues	<u>6,126,375</u>	<u>4,706,620</u>			
EXPENDITURES					
Research*	2,583,374	2,014,119			
Summer programs*	518,570	342,544			
Library	111,479	100,927			
Operation and maintenance of plant	796,998	638,316			
General and administrative	598,880	497,863			
Publications sales*	530,834	303,829			
Dining hall*	<u>228,767</u>	<u>211,647</u>			
Total expenditures	<u>5,368,902</u>	<u>4,109,245</u>			

TRANSFERS		
Plant funds	759,855	515,483
Banbury Center	<u>12,900</u>	<u>61,043</u>
Total transfers	<u>772,755</u>	<u>576,526</u>
Total expenditures and transfers	<u>6,141,657</u>	<u>4,685,771</u>
Excess (deficit) of revenues over expenditures and transfers	<u>\$ (15,282)</u>	<u>\$ 20,849</u>

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

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.

GRANTS

January 1, 1978—December 31, 1978

NEW GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
National Institutes of Health	Dr. Watson—advanced bacterial genetics course	\$ 40,000	6/30/78– 4/30/79
	Dr. Watson—transfer RNA meeting	5,000	7/1/78– 6/30/79
	Dr. Watson—hormones and cell culture meeting	25,000	8/1/78–12/31/78
	Dr. Gingeras—fellowship	26,400	1/1/78–12/31/79
	Dr. Hicks—research	263,400	7/1/78– 6/30/81
	Dr. Klar—research	253,000	7/1/78– 6/30/81
	Dr. D. Zipser—fellowship training	618,000	7/1/78– 6/30/83
	Dr. Albrecht-Buehler—research	73,612	9/1/78– 8/31/80
	Dr. Blose—research	169,237	12/1/78–11/30/81
National Science Foundation	Dr. Watson—neurobiology courses	45,000	6/15/78– 5/31/79
	Dr. Watson—hormones and cell culture meeting	2,000	8/1/78– 7/31/79
	Dr. Watson—transfer RNA meeting	5,000	7/1/78– 6/30/79
	W. Udry—Symposium support	5,000	5/15/78–10/31/78
	Dr. Burrige—equipment	120,440	7/1/78–12/31/79
	Dr. Hicks—equipment	24,000	8/15/78– 1/31/80
	Dr. B. Zipser—research	120,000	11/1/78– 4/30/82
	Dr. D. Zipser—research	136,000	2/15/78– 7/31/81
American Cancer Society	Dr. Lewis—research	53,250	7/1/78– 6/30/79
	Dr. Garrels—fellowship	21,075	9/1/78– 8/31/80
	Dr. Zain—research	67,908	1/1/78–12/31/79
Canadian Medical Research Council	Dr. Chaconas—fellowship	12,000	10/1/78– 9/30/79
Department of Energy	W. Udry—Symposium support	8,000	5/1/78– 4/30/79
The Camille and Henry Dreyfus Foundation	Dr. Watson—undergraduate research participation	20,000	12/1/78– 9/30/79
International Union Against Cancer	Dr. Graessman—institutional allowance	1,000	4/1/78–10/31/78

NEW GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
Leukemia Society of America	Dr. Engler—fellowship	25,000	11/1/78—10/31/80
Muscular Dystrophy Association	Dr. Burridge—research	25,142	7/1/78— 6/30/79
	Dr. Gordon—fellowship	15,500	1/1/78—12/31/78
	Dr. D. Zipser—neurobiology courses	15,000	6/1/78— 9/30/78
Alfred P. Sloan Foundation	Dr. D. Zipser—Biological Information Processing Workshop	17,800	5/1/78— 4/30/79
Damon Runyon-Walter Winchell Cancer Fund	Dr. Brooks—fellowship	28,560	1/1/78—12/31/79
	Dr. Hu—fellowship	25,800	7/1/78— 6/30/80
	Dr. Kilpatrick—fellowship	30,000	1/1/78—12/31/79
	Dr. Stow—fellowship	30,000	10/1/78— 9/30/80
	Dr. Strathern—fellowship	29,980	4/1/78— 3/31/80

BANBURY CENTER

Environmental Protection Agency	conference on chemical mutagens	29,000	5/1/78—11/10/78
Esther A. and Joseph Klingenstein Fund	program support	100,000	3/1/78— 2/29/80
Alfred P. Sloan Foundation	program support	100,000	8/1/78— 7/31/80

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
National Institutes of Health	Dr. Watson—Cancer Research Center	\$13,500,000	1/1/77—12/31/81
	Dr. Watson—general research support	114,761	4/1/76— 3/31/79
	Dr. Watson—Symposium support	96,000	4/1/74— 3/31/79

CONTINUING GRANTS

COLD SPRING HARBOR LABORATORY

<i>Grantor</i>	<i>Principal investigator and program</i>	<i>Total award</i>	<i>Duration of grant</i>
	Dr. Watson—summer workshops	400,620	4/1/77— 3/31/82
	W. Udry—cancer research facility	1,411,000	9/15/77—indefi- nite
	W. Udry—advanced bacterial genetics course	35,500	6/29/77— 6/30/78
	Dr. Broach—research	144,320	8/1/77— 7/31/79
	Dr. Bukhari—career development	148,961	5/1/75— 4/30/80
	Dr. Bukhari—research	121,351	9/9/77— 8/31/80
	Dr. Chow—research	238,186	4/1/77— 3/31/80
	Dr. Galloway—fellowship	28,000	12/1/76—11/30/78
	Dr. Zain—research	34,572	9/30/77— 7/31/79
	Dr. D. Zipser—research	355,657	5/1/74— 4/30/79
National Science Foundation	W. Udry—Symposium support	\$ 10,000	6/1/77— 5/31/78
	Dr. Bukhari—research	130,000	8/1/76— 1/31/79
	Dr. Gesteland—research	168,000	1/1/75— 8/31/78
	Dr. McDougall—research	45,274	8/1/77—10/31/78
	Dr. Roberts—research	130,000	4/1/77—10/31/79
	Dr. Zain—research	65,000	8/15/77— 1/31/80
	Dr. D. Zipser—research	160,000	1/1/74— 8/31/78
Rita Allen Foundation	Dr. Lewis—research	150,000	8/1/76— 7/31/81
American Cancer Society	Dr. Lewis—research	45,500	7/1/77— 6/30/78
American Heart Association	Dr. Chen—research	12,000	7/1/77— 6/30/78
Jane Coffin Childs	Dr. Gluzman—fellowship	24,000	9/16/77— 9/15/79
Energy Research and Development Administration	W. Udry—Symposium support	\$ 8,000	5/1/77— 4/30/78
Eppley Foundation	Dr. Watson—neurobiology courses	30,000	1/1/76—12/31/78
Muscular Dystrophy Association	Dr. Burridge—research	18,900	7/1/77— 6/30/78
	Dr. Chen—research	20,000	7/1/77— 6/30/78
Alfred P. Sloan Foundation	Dr. Watson—neurobiology courses	165,000	1/1/75—12/31/78
Damón Runyon-Walter Winchell Cancer Fund	Dr. Sciaky—fellowship	28,980	3/1/77— 2/28/79

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FINANCIAL SUPPORT OF THE LABORATORY

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

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

Methods of contributing to Cold Spring Harbor Laboratory

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities

- (1) Your broker or bank may sell the securities and remit the proceeds directly to the Laboratory.
- (2) If you wish to send stock directly to the Laboratory, either (a) endorse the certificate(s) by signing your name on the back, leave the space for the transferee's name blank, have your signature guaranteed on the certificate(s) by your bank or broker, and send the certificate(s) by *registered mail* to the Laboratory, or (b) send unsigned certificate(s) with a covering letter and send under separate cover a stock power executed in blank, with signature guarantee, for each certificate, and also a copy of the covering letter (use first-class mail). Depreciated securities should be sold to establish a tax loss, then the contribution to the Laboratory should be made by check.

Bequests

Probably most wills need to be updated. Designating Cold Spring Harbor Laboratory as a beneficiary ensures that a bequest will be utilized as specified for continuing good.

Appreciated real estate or personal property

Sizeable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Conversion of private foundation to "public" status on termination

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

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

LONG ISLAND BIOLOGICAL ASSOCIATION



Carnegie Dormitory

The Laboratory was founded in 1890 by several local philanthropists and the Brooklyn Institute of Arts and Sciences. The first chairman of the Board of Managers of the Laboratory was Eugene G. Blackford, who served from 1890 until his death in 1904. William J. Matheson succeeded him, serving until 1923.

In that year, when the Brooklyn Institute of Arts and Sciences withdrew from Cold Spring Harbor, the local supporters of the research formalized their efforts by incorporating as the Long Island Biological Association. Colonel T.S. Williams became the first Chairman of the new group. Jointly with the Carnegie Institution of Washington, LIBA continued to support and direct the research at Cold Spring Harbor Laboratory.

In 1962 the Laboratory was reorganized as an operating organization and LIBA relinquished its management responsibilities. During the past 16 years, LIBA's chief function has been to widen the interest of the community in the Laboratory and to help support it financially.

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