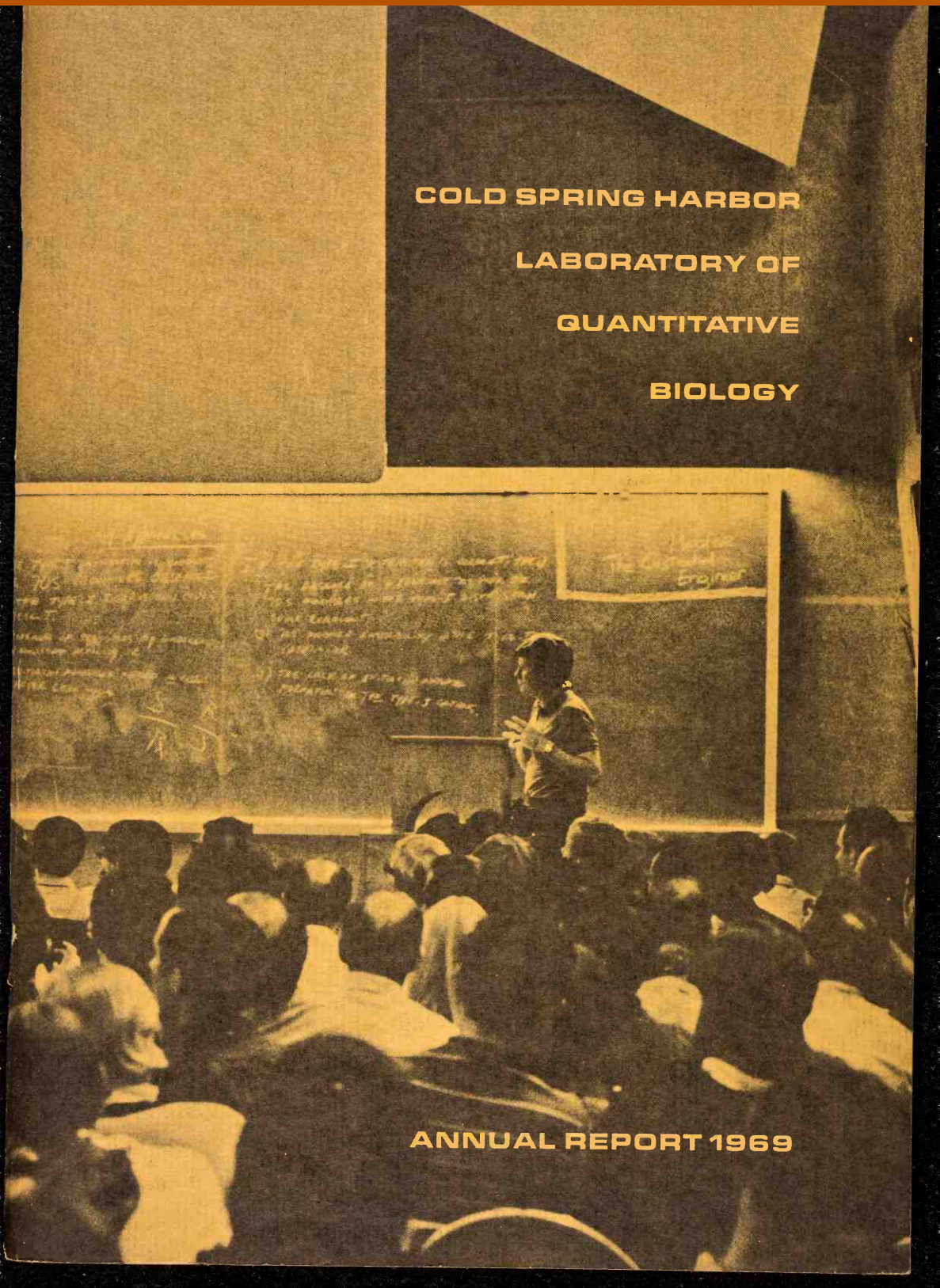

COLD SPRING HARBOR LABORATORY OF QUANTITATIVE BIOLOGY

ANNUAL REPORT 1969

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COLD SPRING HARBOR, LONG ISLAND, NEW YORK

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Cold Spring Harbor, Long Island, New York

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DIRECTOR'S REPORT

The Director of a scientific institution has many allegiances – to his staff, to his board of trustees, to the community in which his institution is situated, to the various individuals and organizations which provide essential financial support, and to the community of scientists whose interests overlap those of his institution. All of these ties were well known to me when I assumed the directorship, partly because they are obvious and very much because of the numerous conversations about Cold Spring Harbor which I had with John Cairns when he was the Director.

Now, as I write this report, I am most conscious of the last of these allegiances – that to the world of molecular biology. Until recently all of us have thought of it as a small group, containing most of our best friends, plus a few dissidents whom, nevertheless, we wanted to talk with if not exchange preprints. Almost suddenly, however, it became clear that we are a very large group. A simple way to grasp our size is to consider the number of people who passed through Cold Spring Harbor during the past summer. The number was slightly over 800. Some of these, of course, stayed only a few hours, but this does not alter the fact that the number of intelligent people now doing sensible, if not important work in molecular biology obviously numbers over 1,000 and, as a conservative estimate, I suggest a world-wide figure slightly over 2,500.

So we are dealing with a number which soon will approximate the number in more classical disciplines like botany, zoology, bacteriology, biochemistry and genetics. And so, correspondingly, we may expect increasing numbers of colleges and universities to add courses if not departments of molecular biology to the stable of disciplines which students may major in. Naturally there are some who decry this tendency, saying with some correctness that our field is not really new but merely a subdivision of a pre-existing field – somewhat in the same way the organic chemists sincerely believed that biochemistry was only a minor subdivision of their field. But I would guess that very few molecular biologists under thirty-five also think of themselves as members of the other disciplines. They may belong to one or another professional society, but generally without passion, and often they obtain a journal whose papers on the whole seem unrelated to the major problems which they wish to solve.

At the same time, I sense no great enthusiasm for a new professional organization to look after the interests of molecular biologists. Compelling to many are arguments like: we are too interested in our research to set up the committees to run such an organization – that setting up a new group would be an unnecessary affront to other societies that we may already belong to, and which we have no desire to upset – that any new body would quickly become rigid and keep going fields which already are out of date. Also a real factor I believe is the existence of Cold Spring Harbor – our Annual Symposium in many years was in effect *the* professional meeting for the molecular biologists. When you came to the Symposium you were certain to see most of your friends and learn the unpublished data which you should know. And if you didn't get to the Symposium you could go to the current phage meeting or give a lecture before the phage course or bacterial genetics course.

Unfortunately, this state of affairs just doesn't exist any more. Now we have space for only just over 300 people at the Symposium and we have made the decision to under-advertise its existence rather than face the almost impossible task of turning down some 300-400 people per year. Attendance is in effect limited to those we invite to speak, scientists in related fields whom our speakers suggest we also should ask, plus a small group whose very persistence and interest overcome our reluctance to let the line at meals grow even more intolerable. And in addition we find the phage meetings themselves,

which up till a few years age numbered less than 100 in attendance, now completely fill our Lecture Hall (280), even when we have divided it (lysogenic vs.lytic) and extended its duration to a week's time.

The inevitable conclusion is that Cold Spring Harbor can effectively retain its former role only by greatly expanding the number of meetings held each year. As a step in this direction, we held a three-day meeting on Tumor Viruses in early August, and during the first week in September those scientists working in the Lactose Operon collected here. This coming year we are considering whether to collect together during the middle of May those people interested in DNA replication, possibly proposing an annual meeting on the topic. And the possibility of still other meetings is under active review. Greatly limiting our freedom of action, however, is the absence of substantial housing and dining facilities when the weather is too cold for operation of Blackford Hall.

Thus a primary goal of mine is the construction of new dormitory facilities. Already substantial financial support has come from Mrs. Max Delbruck, and we are deeply indebted to Manny for leading the way. Unfortunately, other efforts of mine to find enlightened benefactors for such building have run into blank walls. This appears to be the hardest type of money to get since it can be argued that we can congregate at a Hilton Hotel. Of course that is the very point of the matter. By accident molecular biologists are virtually the only professional group which has at its disposal 100 acres of quietly beautiful shore front located near two major airports and without the distraction of good or bad films, drug stores carrying paperbacks,or bars with TV if not topless waitresses. Generally the only diversions at Cold Spring Harbor are going to lectures, talking science, swimming, sailing, or reading in our library. All these items have been of great help in promoting real friendships and helping to alleviate the increasing competitive pace which usually accompanies the entrance of more than one research group into a given field. So we believe that our health and vitality will, in some not minor way, affect the way molecular biology will develop in the United States and Canada if not the world. And we shall do everything possible to see that Cold Spring Harbor remains an institution where graduate students are as welcome as their professors, where bad science is so labelled, and good science encouraged with every resource at our disposal.

Now to quickly summarize some of the more important happenings of the year

Administration

When I took over as Director, I knew that my success would depend much upon my ability to recruit a first rate Administrative Director. Fortunately, we were able to persuade Mr. James L. Brainerd, formerly Vice President for Administration of Robert College, Istanbul, to accept this position and he has been with us since January 1 of this year. Assisting him since May 1 has been Mr. Daniel Hayes, who is in charge of our Business Office. Together they have assumed many of the tasks formerly carried out by Mr. John Philips, who left us to return to New York City. During the seven years Mr. Philips was here, he served the Lab with devotion and good will, helping greatly to tide us over during the trying years of our reorganization.

Salary Support for the Year Round Staff

A most welcomed grant covering the lifetime salary of John Cairns has been received from the American Cancer Society. Beginning as of January 1, 1969, his position is that of American Cancer Society Professor of Molecular Biology. In this capacity he will be able to devote all his time to research, now centered on DNA replication. As will be seen later in this report John quickly seized his regained free time to isolate a new *E. coli* mutant, whose existence may have great impact on molecular biology. This mutant lacks

Arthur Kornberg's famous enzyme DNA polymerase yet appears to grow normally. This suggests that DNA replication may occur in quite a different fashion than generally thought.

Further lifetime support like that now held by John will be indispensable for the long term stability of Cold Spring Harbor. For, lacking an endowment we cannot expect first rate senior scientists to choose Cold Spring Harbor as their homes unless real security can be offered to them and their families.

Hopefully, however, our success will not depend on the need for many such awards since they are very hard to get. Real stability may come if three to five staff members having established tenure can collect together a much larger number of younger scientists who stay here from one to five years. Here we should mention the recent help given us by the award of a Career Development Award from the National Institutes of Health to Ray Gesteland and a similar award to Rudolf Werner from the American Heart Association. Both give much needed salary support for five year intervals.

Tumor Virus Program

Beginning in May, N.I.H. has awarded us a five year grant for 1.6 million dollars to support research on Tumor Viruses in James Laboratory. Very extensive renovations of the first floor area are now occurring, and we hope to begin research there late this year. At the same time architect's plans are now about complete for an addition to James which shall provide office space for the staff working in James, a seminar room, a kitchen, as well as an area for my office to which I shall move as soon as the annex is complete. Over the past several years the Nichols administration staff has been growing and many offices and other facilities are already too crowded for efficient work. Even more important, I believe the Director of a scientific institution should always be a scientist, not an administrator. At times this is impossible to believe, but at other times it is necessary for sanity.

Fortunately we have been able to entice several highly competent animal virologists to join our James group. All already have had extensive experience in the tumor virus area. Drs. Joseph Sambrook, Carel Mulder, and Henry Westphal all are from Dulbecco's group at the Salk Institute, while Dr. Bernhard Hirt comes from the Institute for Cancer Research at the University of Lausanne in Switzerland. In addition several younger people have joined us as research assistants, hoping simultaneously to work for Ph.Ds at neighboring universities. We have thus already allocated most of the space currently available for tumor virus research. With the completion of the annex the space situation will open up slightly, permitting us to accept several new postdoctoral fellows.

Electron Microscope Facility

Late in the summer Dr. Hajo Delius, from the University of Geneva, arrived to take charge of a new Siemans electron microscope. Its purchase was made possible by a recent grant from the National Institutes of Health. It is being installed as I write this report and hopefully will soon assist the research efforts of virtually all our staff.

Summer Courses

This year about 100 students took one or more of our various courses. Lecturing to them were 75 specialists in many aspects of molecular and cellular biology. On the average there were only some 40-50 people attending each lecture and so the informality of the past was maintained. But the very high quality of virtually all the teaching makes us wonder whether we shouldn't try to encourage more people to attend the lectures. However, this point seems largely academic until new housing space becomes available. The size of the audience will reflect largely the number of beds available to house our

guests. And even our most optimistic plans for new dormitories will probably only increase the number of guests by 32. And as all of them won't come to any given talk, the students in the courses, for whom the lectures are directly intended should continue to be able to closely question and talk with all our speakers.

Next year the projected increase in our N.I.H. training grant will allow us to offer, for the first time, a course on the Molecular Biology and Genetics of Yeast. Because it will be a new course and because we are so short of housing it will be limited to 10 students for the first year. All the other courses given this year will be repeated and hopefully (see below) Max Delbruck will return to teach again a course on Sensory Transducers. We must note with pride that this coming year will be the 25th consecutive year that the Phage Course has been given. A large fraction of the leading figures in molecular biology today have come to Cold Spring Harbor at some time to attend it and it must rank among the most influential courses taught anywhere in the world during this century, perhaps comparing in influence to the courses at the Bauhaus.

We suspect, however, that in the future it, and its companion Bacterial Genetics course, will have much much less impact unless means are found to improve the Davenport facilities where these courses have always been given. Already many of the experiments depend upon the existence of equipment only available in other buildings and which is likely to be hopelessly over-employed as our current year round program grows in size. So we have made tentative plans for an addition facing to the south which will approximately double our working space, giving for the first time rooms for modern centrifuges, spectrophotometers and scintillation counters. We would like construction to start next September as soon as the courses end.

Neurobiology

We are in the process of applying to several agencies and foundations for funds which shall permit us to have a continuing neurobiology program over the next several years. In the late 1920s and early 1930s Cold Spring Harbor was one of the most active centers for imaginative work on the nervous system. So it was most refreshing when Max Delbruck in 1965 reintroduced summer interest in this area. Optimally, the very makeshift conditions now existing in the Animal House can be thoroughly modernized and many new books and journals added to a library totally deficient in modern information about how nervous systems are put together. Expansion here is even more dependent upon the acquisition of living quarters for summer guests and hopefully some enlightened body will see the advantages of having a small but talented group of neurobiologists coexisting with many of our better molecular biologists. How these two groups will influence each other is still very debatable but I would be surprised if the inter-action did not have a real payoff.

Support from the Participating Institutions

Indispensible for the various improvements carried out over the past year was the $42,000 contributed by the twelve universities and research institutions which sponsor us. Without this money none of the improvements made to the library, to the teaching lab in James, to the Lecture Hall and the various course labs could have been made. Since we lack endowment, we would have to stay in an essentially static state if our income were largely restricted to research grants. No business manager, no matter how astute, can collect enough overhead to permit expansion. Thus the continued financial support of our current sponsors plus the addition of new sponsoring universities and industrial organizations will be necessary ingredients for innovative direction.

Construction of the new Osterhout

To conclude I must mention that my wife and I moved into the newly completed Osterhout early in June. We are very pleased with the final product, a design of the imaginative New York architect, Harold Edelman. Though much of its external appearance retains a colonial flavor it is a thoroughly modern house with marvelous views out on the Harbor. Now as I wander through some of our less renovated buildings it becomes much harder to avoid the occasional notion that I am a slum landlord. Much of our ancient furniture must thus go to the dump. When our summer visitors arrive next year I hope that they may be in for some colorful surprises.

All in all it has been a very good year.

J. D. Watson

As we go to press, we happily note that the Nobel Prize in Medicine and Physiology for 1969 has been awarded to Al Hershey for his very distinguished contributions to our knowledge of bacterial viruses. That Al should someday be so honored has been obvious to many since his famous 1952 blender experiment which showed that DNA carried the genetic specificity of phage. But this did not lessen our delight when we learned that the obvious had happened, and the fact that we possess a great scientist of exceptional talents, both theoretical and experimental, became of more open knowledge to the world.

Especially pleasing to us is the news that Al shares his award with Max Delbruck and Salvador Luria. For, since 1941 Max and Salva have had very close associations with Cold Spring Harbor – many of their most important experiments have been conceived, if not performed, during the many summers they have spent with us. The Nobel Committee, by honoring its founders, tells the world that the "phage group" has played a unique role in the history of biology. As the spiritual, if not the real home of many of its members, we feel also honored.

Alfred Day Hershey, Nobel Laureate – October, 1969

THE REPLICATION OF DNA

Laboratory of
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J. Gross
M. Gross
W. Hazeltine
D. Kuhn
P. De Lucia

Kinetics of DNA Synthesis and the Precursor Pools

Last year we reported briefly our studies on the flow of thymine and thymidine into bacterial DNA. Since then we have pursued this investigation only to find that the results became increasingly ambiguous the closer they were scrutinized; similarly, an attempt during the summer to check an experiment, done by Zamenhof two years ago, on the kinetics of labeling DNA-phosphorus with P^{32}, has given results that are hard to interpret. So we have turned to other methods for dissecting the mechanism of DNA synthesis.

The location of DNA Polymerase in E. coli

As a prelude to a search for mutants of the *E. coli* polymerase, we investigated various methods for extracting this enzyme. It turns out that the enzyme can be readily extracted from *E. coli* by treatment with lysozyme, and the non-ionic detergent Brij-58. Since this procedure does not detach the enzyme from DNA in vitro nor from cells that have been subject to ultraviolet irradiation, and yet liberates virtually all the enzyme from replicating cells, we conclude that normally the enzyme is not attached to the cell's DNA. This conclusion supports the idea that this enzyme is used for repair synthesis rather than for normal replication.

Mutants of the DNA Polymerase

Armed with this simple method of extraction, we proceeded to check lines of cells from a mutagenized stock of *E. coli* for enzyme, in the belief that we should eventually find a line deficient in enzyme and therefore, by investigating the properties of such a line, determine what function is served by this enzyme. After some months of work, we found our mutant. With the aid of Julian Gross (who had just finished teaching the course on Bacterial Genetics) and Charles Richardson (Harvard Medical School) we were able to show: (a) that the mutant is an amber mutation which results in loss of the polymerase action but preservation of the 5' exonuclease action of the Kornberg enzyme; (b) that the mutant strain is abnormally sensitive to ultraviolet light and certain radiomimetic drugs as a result of the mutation, but otherwise grows normally and can support the multiplication of various phages including, interestingly enough, ØX174; (c) that the gene lies close to metE on the genetic map; and (d) that the mutation proves to be recessive in merogenotes. From all this we conclude that the only DNA polymerase, so far recorded in *E. coli*, is an enzyme used for the repair of DNA, and not for the normal synthesis of DNA.

MESSENGER RNA AND PROTEIN SYNTHESIS

Laboratory of
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J. Mickelbank

Bacteriophage R-17

Studies have continued on the RNA bacteriophage R-17, taking advantage of specific RNA fragments produced by the action of RNase IV. Limited treatment with this enzyme produces a single cut in the 3300 nucleotide molecule to give a 60% fragment (2,000 nucleotides) from the original 3' end of the RNA and a 40% fragment (1300 nucleotides) from the 5' end. These fragments have been used to study the structure of the RNA and to direct *in vitro* protein synthesis in order to study the details of translation of this polycistronic messenger.

Protein synthesis

Our previous studies using polyacrylamide gel electrophoresis show that the 60%, 3' fragment directs synthesis of the RNA synthetase molecule (requiring about 1400 nucleotides of information). These results were inconclusive concerning the other two proteins: coat and A or maturation.

In order to increase the sensitivity and specificity of such experiments we have employed the system described by Lodish to label only the amino terminal peptides made *in vitro* using S^{35}f-met-sRNA. Native R-17 RNA stimulates initiation of about 3-4 coat protein molecules (f-met-ala-. . .) per initiation of each RNA synthetase molecule (f-met-ser-. . .) and much less A protein initiation which we have not been able to identify in the products. The 60% fragment stimulates initiation of synthetase almost as efficiently as the native RNA, as expected, but also initiates coat protein, although at about the same frequency as the synthetase. That is, the 60% fragment is about 3-4 fold less efficient at initiating coat than the native RNA. Careful analysis of the RNA fragment

preparations make unlikely the possibility that the coat initiation by the 60% piece is due to contamination or to a cut in the other position on the RNA molecule that would produce the opposite 60% and 40% pieces. This, however, is not conclusive since only a fraction of the RNA molecules of any preparation actually participate in *in vitro* protein synthesis. The dependence of translation of the synthetase gene on that of the coat gene disappears with the 60% fragment as shown by the loss of polarity in 60% fragment from the polar coat protein amber mutant of R-17. This supports the notion that a new ribosome initiation site is exposed upon fragmentation as if the three dimensional structure of the RNA is responsible for polarity and the accessibility of the initiation sites.

The 40% fragment is relatively inactive in the initiation assay, as it is for total amino acid incorporation, and does not produce a consistent or identifiable pattern of initiation peptides. Dr. H. Lodish has found, however, that in extracts from *B. stearothermophilus* the 40% fragment is much more active than the 60% fragment. He has previously shown that these extracts, in contrast to those of *E. coli*, initiate predominantly the A protein. Preliminary results indicate that the 40% fragment also initiates predominantly the A protein.

The results of the protein synthesis experiments imply a gene order of:

5' – A protein	coat protein	Synthetase – 3'
1000 nuc.	400 nuc.	1400 nuc.

but the positioning of the coat gene depends on the rather inefficient coat initiation by the 60% fragment.

Sequence information

Defined fragment should be of great value to work on the nucleotide sequence of R-17; however these experiments require very high specific activity P^{32} containing RNA. In order to make good fragments the starting RNA must be intact, which is difficult because of the radioactivity. We have found that P^{32} labeled phage can be purified using DEAE chromatography in a method that prevents much of the secondary radiation damage. This permits routine production of intact RNA of at least 10^6 cpm/μgm. Examination of the size distribution of the T-1 oligonucleotides shows that there are obvious composition differences between the 60% and 40% fragments in the larger nucleotides (9 and greater). We are working out thin layer electrophoresis and chromatography techniques to use in looking at specific oligonucleotides.

Dr. Joan Argetsinger-Steitz (M.R.C., Cambridge, England) has used these highly labeled fragments to look for the ribosome initiation sequences that she has identified in the native RNA. Her results are consistent with the above picture, but she finds coat initiation sequences in both the 40% and 60% fragments, with more in the 40%. One obvious interpretation that is consistent with all of our data is that the RNase IV enzyme cleaves at more than one place within a small region, producing on the average 40% and 60% pieces and that sometimes the coat initiation site is left on one fragment and sometimes on the other. This is being checked by looking for known oligonucleotides and by identifying the newly produced end sequences of the fragments.

Viral DNA dependent protein synthesis

Work is continuing on the *in vitro* synthesis of T-4 bacteriophage mRNA, in particular to see if the newly discovered polymerase associated factors will enable the polymerase to make active T-4 lysozyme messenger RNA.

Using a system from *E. coli*, various DNAs have been tested as templates for coupled transcription and translation. The system is dependent on added RNA polymerase (σ-containing complex) and exogeneous DNA. The DNAs used stimulated amino acid incorporation in the order: T-4 100, fd-RF 50, SV-40 15, polyoma 10. In all cases most of the polypeptide synthesized was between 10,000 and 20,000 molecular weight, as estimated by polyacrylamide gel electrophoresis, with no clear indication of virus specific differences. In the absence of added DNA there was no detectable material of this size. It is not clear whether the similarity of the patterns is due to some peculiarity of the system or is an artifact, such as stimulation of endogeneous synthesis. This should be resolved by the characterization of the tryptic peptides now being done.

DNA REPLICATION IN BACTERIOPHAGE AND BACTERIA

Laboratory of
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Replication of Incomplete Phage T4 Chromosomes

Some small particles of phage T4 contain incomplete chromosomes measuring two-thirds of the length of normal T4 chromosomes. They cannot produce progeny phage after single infection because their incomplete chromosomes lack random segments of the genetic map. We have investigated the replication of DNA following single infection with small particles. Our results indicate that approximately two-thirds of the incomplete chromosomes can initiate DNA replication and apparently extend the process from a genetically fixed origin to one end of the molecule. However, few if any of the incomplete chromosomes can initiate a second round of replication. It seems that circularity is only needed for continuation of DNA replication and for initiation of secondary growing points. The reason for this requirement is unclear at the moment.

DNA Replication in Gene 32 – Defective Phage T4

Phage T4 containing an amber mutation in gene 32 is defective in the formation of joint

"recombinant" molecules (Tomizawa et al., 1966). Only 50% of the parental DNA is replicated (Kozinski & Felgenhauer, 1967), which suggests a necessary recombinational event in normal DNA replication. This event is presumably the circularization of the molecule, since a similar replication pattern is observed in solitary incomplete T4 chromosomes containing the origin but lacking terminal redundancy. Initiation of secondary rounds of replication in gene 32 mutants seems to be repressed similarly as in incomplete chromosomes. However, the rate of initial replication is much slower than in incomplete chromosomes which replicate at normal rate independently of the presence or absence of gene 32. It is possible that the protein fragment produced by a defective gene 32 somehow interferes with the replication process. These studies will be continued with other mutants of gene 32 in the hope of finding out more about the mechanism of initiation of DNA replication in general.

Precursors for DNA Replication in vivo

Protoplasts of *E. coli* or cells treated with tris are permeable to deoxyribonucleoside triphosphates (Buttin & Kornberg, 1966). In the presence of a carbon source (energy source) these cells incorporate ^{3}H-TTP at the same rate as they incorporate ^{3}H-thymidine. Phage-infected cells produce viable phage particles, indicating that real DNA replication and not just repair synthesis takes place. In sucrose gradients the ^{3}H-labeled DNA is large and cosediments with the bulk of chromosomal DNA. In the absence of a carbon source, little if any incorporation of ^{3}H-TTP occurs (Buttin & Kornberg, 1966), thus suggesting that the energy contained in the triphosphates is not sufficient for DNA replication. However, if ^{32}P-labeled TPP is used as precursor, no ^{32}P-counts ever appear in DNA even in the presence of glucose. This surprising result can mean either that triphosphates cannot enter the cell without phosphate exchange or that the α-phosphate is exchanged in the process of DNA replication, possibly because TTP is not the immediate precursor of DNA. Two observations suggest that the latter is true. If ^{32}P-labeled TMP is fed to tris-treated cells, some of the ^{32}P-label can be extracted from the cells as TTP, which indicates that TMP has entered the cells intact and has been converted to TTP. Furthermore, if *E. coli* 15T^{-} is starved of thymine and then fed ^{32}P-TTP, ^{32}P-label does appear in DNA. This DNA, however, is of low molecular weight.

These results indicate that normal DNA replication either does not use deoxyribonucleoside triphosphates as precursors or that the α-phosphate is exchanged during the polymerization reaction. However, cells engaged in repair synthesis, as thymine-starved cells are, do incorporate deoxyribonucleoside triphosphates without α-phosphate exchange.

Intergenic suppression of T4 ligase mutants

According to the model of discontinuous DNA replication proposed by Okazaki et al. (1968), the presence of polynucleotide ligase should be obligatory for DNA replication. However, Kozinski (1968) has recently shown that phage T4 DNA is synthesized in bacteria infected with T4am H39X, a mutant defective in the ligase gene, if chloramphenicol is added between 3 and 5 minutes after infection. Under these conditions about 200 molecular equivalents of phage DNA are accumulated per cell, and when chloramphenicol is removed, about 10% of this phage DNA becomes incorporated into viable phage particles. Kozinski interpreted this result as indicating that ligase is primarily needed to repair breaks in the T4 DNA made by a T4-induced endonuclease; if the synthesis of this enzyme is prevented by chloramphenicol, the presence of polynucleotide kinase does not seem to be required. If this interpretation is correct, it should be possible to find mutants of this endonuclease that "suppress" the ligase mutation. Among "revertants" of T4am H39X we have found many such double mutants that were able to grow on *E. coli* B and still contained the amber allele. Most of these mutants turned out to be rII mutants. In addition, we have found some mutants mapping outside the rII region that were recessive in mixed infection with amH39X. Such behavior is expected from endonuclease-ligase double mutants. We are testing now whether these mutants show a decreased frequency of recombination.

RNA POLYMERASE FROM MAMMALIAN CELLS

Laboratory of
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C. Goff
W. Sugden

We are interested in whether the RNA polymerases from *E. coli* and mammalian cells recognize the same DNA sequences for initiation and termination of RNA chains. Since no good method exists for the isolation of RNA polymerase from tissue culture cells, we devised the following method of isolating and purifying RNA polymerase from HeLa cells.

Cells were washed and suspended at 0°C in three volumes of 0·6M sucrose, 0·001M $mgcl_2$, 0·005M β-mercaptoethanol 0·01M Tris pH 7·8 prior to breaking with 10 strokes of a Potter-Elvejhelm homogenizer. The isolated nuclei were collected by centrifugation and washed in the homogenization medium containing 0·5% Triton X – 100. Finally, the purified nuclear pellet was suspended in 3 volumes 0·05M Tris pH 7·9 0·01M $(NH_4)_2So_4$, 0·006M $mgcl_2$, 0·001M EDTA, 0·005M β-mercaptoethanol, 30% glycerol.

During overnight storage of the nuclear suspension at 0°C, the RNA polymerase leached out of the nuclei. At this stage the enzyme was still attached to DNA, but it became soluble and dependent upon added DNA by treatment with 0·5M$(NH_4)_2So_4$, 0·001M EDTA, 0·05M Tris pH 8·0, glycerol 30% (buffer B). The free enzyme was then purified by centrifugation through a 10 – 30% glycerol gradient in buffer B in a SW 41 rotor. The enzyme activity appeared as a sharp peak with a sedimentation constant of about 15 S.

The enzyme was then concentrated and further purified on a DEAE-cellulose column, from which all the RNA polymerase eluted at 0·1M $(NH_4)_2So_4$. The enzyme at this stage was completely dependent on added DNA and was sensitive to Actinomycin D.

The method works for all types of tissue culture cells tested (3T3, SV3T3, BSC-1).

Currently we are attempting to purify the enzyme completely and determine its polypeptide composition on polyacrylamide SDS-urea gels.

DNA PHENOTYPES

Laboratory of
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A. Skalka
H. Yamagishi

Perhaps the most puzzling aspect of DNA phenotypes has to do with the distribution of nucleotides within the molecules. Since recent discussions of this subject (Skalka, Burgi, and Hershey, *Journal of Molecular Biology*, 1968; *Carnegie Institute Year Book 67*, pp. 558-560) are already out of date, I recapitulate here the main historical facts before presenting some recent results obtained by Yamagishi and Skalka.

Perhaps the best way to state the problem is to describe the methods of study. Owing to the base-paired structure of DNA, the average composition of a molecule or fragment can be expressed by a single number, the molar fraction of guanine plus cytosine (G + C), which is equivalent to the fraction of guanine-cytosine pairs. The remaining fraction, if we neglect exceptional bases, represents adenine-thymine pairs.

The distribution of nucleotides within molecules can be determined by breaking them into fragments of known size, separating the fragments into classes of diverse composition, and measuring the G + C content in each class. Since the distribution is necessarily dependent on size of fragments, the analysis has to be repeated with fragments of various sizes. This sort of analysis has now been carried out for a few phage and bacterial species.

The nature of the problem could be seen only dimly in 1953, when interest was first focused on base sequence as the clue to the genetic message. The DNA species known at that time contained about 44% G + C, which seemed reasonable in a way since an efficient language would use all letters with similar frequency. This thought was short lived because Lee, Wahl, and Barbu (1956) and Belozersky and Spirin (1958) reported a number of bacterial DNA species whose G + C contents ranged from 26% to 74%. Thus it appeared that DNA language, like human language, was not designed primarily for efficient communication.

The discovery that the buoyant density of DNA is strongly dependent on composition (Rolfe and Meselson, 1959; Sueoka, Marmur, and Doty, 1959) yielded the first results concerning nucleotide distribution in DNA. For instance, Rolfe and Meselson found that the standard deviation of G + C content among fragments of *E. coli* DNA (fragment length probably about 10^4 nucleotide pairs) was less than ±3%, to be compared with the 48% range covered by variations among species. Thus diverse bacterial species, surely possessing many functions in common, do not contain many DNA segments of similar composition. Rolfe and Meselson concluded that the compositions of protein and DNA could not be directly related to each other by a universal code.

Sueoka (1961) studied directly the relation between composition of DNA and composition of protein by analyzing the whole cellular protein of a number of microbial species. He found that the frequencies of the amino acids leucine, valine, and threonine showed no correlation with the G + C content of DNA. However, glycine, alanine, and arginine showed a weak positive correlation, and lysine, glutamic acid, and isoleucine showed a weak negative correlation. Sueoka's results can now be interpreted in terms of the degeneracy of the genetic code, in which 61 codons specify one or another of just 20 amino acids (Crick, *Cold Spring Harbor Symp. on Quant. Biol.* Vol. 31, 1966). Thus there are four valine triplets each containing either one or two guanine or cytosine residues, and the abundance of valine could not be favored by either extreme of DNA composition. Alanine triplets contain two or three guanine or cytosine residues, and lysine triplets zero or one, in agreement with Sueoka's results. Sueoka's main conclusion, that compositions of DNA and protein are not strongly correlated, is also consistent with the coding dictionary, which allows a stretch of DNA specifying one each of 15 frequently occurring amino acids to vary in G + C content between 29% and 67%. Furthermore, both mutational study of individual proteins and comparative analysis of homologous proteins from different species show that functional requirements do not impose severe restrictions on the composition of proteins. Therefore the observed variations in composition of DNA cannot signify diverse requirements with respect to the composition or function of proteins.

Having reached the conclusion just stated, Sueoka (1962) and Freese (1962) proposed that the composition of DNA was determined mainly by the genetically determined rates of mutational interconversion between guanine-cytosine pairs and adenine-thymine pairs. These authors also assumed that DNA composition as such had no functional significance and therefore could not respond to selective pressures. The latter assumption was perhaps superfluous to their main proposal because, however divergence in composition of DNA among different species may arise, one might expect it to be accompanied by coadaptive variation in mutational habit.

If DNA composition in a given species were determined primarily by mutational habit, guanine-cytosine pairs should be distributed at random among DNA fragments of gene size or larger. Recent analyses of several phage and bacterial DNA's by Yamagishi and Skalka show that the distributions are never random (see below). One must conclude either that DNA composition does reflect specialized functional adaptations or that interspecific genetic recombination is frequent with

respect to the evolutionary time scale. Perhaps both possibilities should be considered likely. In any case, the hypothesis of domination by mutational equilibria loses its force.

Last year Yamagishi and Skalka proposed that an asymmetric distribution of G + C in bacterial DNA in the vicinity of λ prophage might be designed to favor the types of genetic recombination that give rise to transducing phage (*Year Book 67*, pp. 558-560). They are no longer enthusiastic about this hypothesis for two reasons. First, it now appears that the *bio*-transducing phage analyzed last year is atypical, having picked up bacterial DNA not proper to the *bio* region of *E. coli*. Its structure may not be relevant to the hypothesis under test. Second, the recognition that unselected fragments of *E. coli* DNA are rather dissimilar in composition neutralizes the significance of departures from the average composition in the vicinity of prophage insertion sites.

Miyazawa and Thomas (1965) first demonstrated that the DNA of *E. coli* contains segments of dissimilar composition. Yamagishi has carried the analysis further, and some of his results indicate that fragments of the order of size of individual genes range in G + C content from 39% to 56%. The distribution is asymmetrical, with an average at 51%. The distribution is nevertheless rather compact: its standard deviation is ±3.8 percentage units in G + C content, as compared with ±6.7 units for λ DNA (Skalka, Burgi, and Hershey, 1968).

Yamagishi also examined *E. coli* DNA fragments of other lengths. His results show that stretches of the extreme composition 39% G + C range in length up to about 35,000 nucleotide pairs and comprise 3% of the total DNA. The asymmetry of the distribution is characteristic, and signifies that long stretches of low G + C content are more numerous than long stretches of high G + C content.

The DNA of *Bacillus subtilis* is generally similar to that of *E. coli* except that its fragments range from 35% to 50% in G + C content, with an average of 44%. In collaboration with I. Takahashi of McMaster University, Yamagishi could show by genetic tests that regions of exceptional G + C content in *B. subtilis* include typical bacterial genes. Therefore local variations in composition do not reflect merely temporary residents in the bacterial chromosome such as prophages.

Yamagishi also analyzed several specific segments of *E. coli* DNA recovered from various φ80 transducing phage lines. Here the content of bacterial genes can be identified by genetic tests, and the corresponding DNA can be recognized by fractionation with respect to density combined with hybridization tests to distinguish between components of phage and bacterial origin. A segment containing the tryptophan operon consists of DNA ranging in G + C content from 45% to 57%. A segment containing lactose genes is more homogeneous, with an average G + C content of 54%. Among the various segments examined, only the *gal* region contains DNA corresponding to the average for the entire chromosome, 51% G + C (Yamagishi and Skalka, *Year Book 67*, p. 559).

Skalka has examined a number of phage DNA species by density analysis of molecular halves and smaller fragments (about 2000 nucleotide pairs). By this method λ DNA molecules are readily shown to consist of dissimilar halves and to be made up of four or more distinct segments containing 37%, 43%, 48.5%, and 57% G + C (Skalka, Burgi, and Hershey, 1968). The closely related phages 434, 82, and 21 are very similar to λ except that the 37% G + C section is absent in phage 21. Phage φ80 related to λ, and the unrelated phage 186 resemble each other in containing only two distinct segments, the molecular halves, measuring approximately 50% and 55% G + C respectively. The DNA of phage P2 also contains dissimilar halves, and resolves into three widely dissimilar segments. The DNA of phage P22 contains at least two dissimilar segments. Molecular halves of this DNA have the same composition, presumably because the molecules come with circularly permuted nucleotide sequences. Unlike the others, phages T5, T7, and P1 contain DNA's that are strikingly uniform in composition, though not absolutely so, because small fragments exhibit asymmetrical density distributions. Phage P1 contains 5% of DNA of only 37% G + C.

Two conclusions emerge. First, all DNA's so far examined contain relatively long segments that differ in composition. Second, the phage DNA's so far examined fall into two classes. DNA molecules from phages λ, 186, P2, and probably P22 are composed of a few long segments of dissimilar composition. Since the effect is to produce dissimilar halves, these may be called asymmetric DNA's. By contrast, phages T5, T7, and P1 contain DNA's of relatively uniform composition. The grouping suggests that phage λ may be taken as representative of a class. If so, asymmetry of DNA structure, clustering of genes of related function in the genetic map, and propensity toward interspecific genetic recombination form a seemingly harmonious set of class characteristics.

COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

June 5th to June 12th, 1969

XXXIV. THE MECHANISM OF PROTEIN SYNTHESIS

A science comes of age when the principles on which it was founded have been vindicated and are replaced, as an occupation, by the accumulation of detail – detail from which, of course, further principles will eventually arise. Molecular biology is now entering the stage of detail. However, the detail is so formidable and the techniques for disclosing it are so powerful that successive symposia have had to be restricted to smaller and smaller sectors of the field if they are to remain even partly digestible by the participants. Six years ago, we could hold a symposium on the synthesis of biological macromolecules. This year, it was scarcely possible to do justice to the mechanism of protein synthesis.

Some 150 distinct macromolecules are now known to be involved in the formation of peptide bonds, showing that the workings of the ribosome and its immediate environs are even more complicated than most people had expected. On another front, the gargantuan efforts to determine the entire base sequence of a natural messenger, such as an RNA phage, have shown unexpectedly that much of this sequence is so arranged that the message will tend to fold upon itself and in this way influence the gross ordering of events during its translation. In all this there is the promise of a great elegance of detail and therefore the assurance that an end to the detail is not yet in sight.

The meeting was held from the 5th to the 12th of June, and was attended by about 350 people including 35 from overseas. As in the past, the program was supported by the National Institutes of Health, United States Public Health Service; the National Science Foundation; and the United States Atomic Energy Commission.

THE PROGRAM

RIBOSOME STRUCTURE

ZAMECNIK, P. C.: An Historical Account of Protein Synthesis with Current Overtones – A Personalized View

KURLAND, C. G., P. VOYNOW, S. J. S. HARDY, L. RANDALL, and L. LUTTER: Physical and Functional Heterogeneity of *E. coli* Ribosomes

TRAUT, R. R., H. DELIUS, C. AHMAD-ZADEH, T. A. BICKLE, P. PEARSON, and A. TISSIERES: Ribosomal Proteins of *E. coli:* Stoichemistry and Implications for Ribosome Structure

STAEHELIN, T., D. MAGLOTT, and R. E. MONRO: On the Catalytic Center of Peptidyl Transfer: A Part of the 50S Ribosome Structure

NOMURA, M., S. MIZUSHIMA, M. OZAKI, P. TRAUB, and C. V. LOWRY: Structure and Function of Ribosomes and their Molecular Components

TRAUB, P., and M. NOMURA: Studies on the Assembly of Ribosomes in vivo

GUTHRIE, C., H. NASHIMOTO, and M. NOMURA: Studies on the Assembly of Ribosomes in vivo

SYPHERD, P. S., D. M. O'NEILL, and M. M. TAYLOR: The Chemical and Genetic Structure of Bacterial Ribosomes

SMITH, I., C. GOLDTHWAITE, and D. DUBNAU: The Genetics of Ribosomes in *Bacillus subtilis*

SMITH, F. A., and R. HASELKORN: Proteins Associated with Ribosomes in T4 Infected *E. coli*

BOLLEN, A., T. HELSER, T. YAMADA, and J. DAVIES: Altered Ribosomes in Antibiotic-Resistant Mutants of *E. coli*

GORINI, L. C.: The Contrasting Role of *str A* and *ram*-Gene Products in Ribosomal Functioning

MODOLELL, J. and B. D. DAVIS: A Unitary Mechanism for the Several Effects of Streptomycin on the Ribosome

APIRION, D., S. L. PHILLIPS, and D. SCHLESSINGER: Approaches to the Genetics of *E. coli* Ribosomes

CRAVEN, G. R., R. GAVIN, and T. FANNING: The tRNA Binding Site of the 30S Ribosome and the Site of Tetracycline Inhibition

MONIER, R., J. FEUNTEUN, B. FORGET, B. JORDAN, M. REYNIER, and F. VARRICCHIO: 5S RNA and the Assembly of Bacterial Ribosomes

STRUCTURE AND BINDING OF tRNA

BOCK, R. M., J. D. YOUNG, M. LABANAUSKAS, and P. G. CONNORS: X-Ray Diffraction Studies of Crystalline Transfer RNA

KIM, S-H, P. SCHOFIELD, and A. RICH: Transfer RNA Crystals Studied by X-Ray Diffraction

DUBE, S. K., P. S. RUDLAND, B. F. C. CLARK, and K. A. MARCKER: A Structural Requirement for Codon-Anticodon Interaction on the Ribosome
IGARASHI, K., H. ISHITSUKA, Y. KURIKI, and A. KAJI: Interaction of tRNA with Ribosomes-Binding and Release of tRNA
SWAN, D., G. SANDER, E. BERMEK, W. KRAMER, T. KREUZER, C. ARGLEBE, R. ZOLLNER, K. ECKERT, and H. MATTHAEI: On the Mechanism of Coded Binding of Aminoacyl-tRNA to Ribosomes: Number and Properties of Sites
SPIRIN, A. S.: A Model of the Functioning Ribosome: Locking and Unlocking

RIBOSOME CYCLING

KAEMPFER, R. and M. MESELSON: Studies of Ribosomal Subunit Exchange
SUBRAMANIAN, A. R., B. D. DAVIS, and R. J. BELLER: The Ribosomal Dissociation Factor and the Ribosome-Polysome Cycle
SCHLESSINGER, D., C. GURGO, L. LUZZATTO, and D. APIRION: Polyribosome Metabolism in Growing and Non-Growing *E. coli*
PHILLIPS, L. A. and R. M. FRANKLIN: The in vivo Distribution of Bacterial Polysomes, Ribosomes, and Ribosomal Subunits
FREIDMAN, H., P. LU, and A. RICH: An in vivo Block in the Initiation of Protein Synthesis

INITIATION

REVEL, M., M. HERZBERG, and H. GREENSHPAN: Initiator Protein Dependent Binding of Messenger RNA to the Ribosome
THACH, R. E., J. W. B. HERSHEY, D. KOLAKOFSKY, K. F. DEWEY, and E. REMOLD-O'DONNELL: Purification and Properties of Initiation Factors F_1 and F_2
WAHBA, A. J., Y. B. CHAE, K. IWASAKI, R. MAZUMDER, M. J. MILLER, S. SABOL, and M. A. G. SILLERO: Initiation of Protein Synthesis in *E. coli*. I. Purification and Properties of the Initiation Factors
WAHBA, A. J., K. IWASAKI, M. J. MILLER, S. SABOL, M. A. G. SILLERO, and C. VASQUEZ: Initiation of Protein Synthesis in *E. coli*. II. Role of the Initiation Factors in Polypeptide Synthesis
DUBNOFF, J. S. and U. MAITRA: Protein Factors Involved in Polypeptide Chain Initiation in *E. coli*
BRAWERMAN, G.: Role of Initiation Factors in the Translation of Messenger RNA
WILSON, D. B. and H. DINTZIS: Initiation of the Alpha Chain of Rabbit Hemoglobin

TRANSLOCATION AND ELONGATION

RAVEL, J. M., R. L. SHOREY, C. W. GARNER, R. C. DAWKINS, and W. SHIVE: The Role of an Aminoacyl-tRNA-GTP-Protein Complex in Polypeptide Synthesis
HARDESTY, B., W. CULP, and W. McKEEHAN: The Sequence of Reactions Leading to the Synthesis of a Peptide Bond on Reticulocyte Ribosomes
MOLDAVE, K., W. GALASINKI, P. RAO, and J. SILER: Studies on the Peptidyl tRNA Translocase from Rat Liver
MONRO, R. E., T. STAEHELIN, M. L. CELMA, and D. VASQUEZ: The Peptidyl Transferase Activity of Ribosomes
VASQUEZ, D., E. BATTANER, R. NETH, and R. E. MONRO: The Function of 80 S Ribosomal Subunits and Effects of Some Antibiotics
PARMEGGIANI, A. and E. M. GOTTSCHALK: Isolation and Some Properties of the Amino Acid Polymerization Factors from *E. coli*
KAZIRO, Y., N. INOUE, Y. KURIKI, K. MIZUMATO, M. TANAKA, and M. KAWAKITA: Purification and Properties of Factor G
PESTKA, S.: Translocation, Aminoacyl – Oligonucleotides, and Antibiotic Action
LEDER, P., A. BERNARDI, D. LIVINGSTONE, B. LOYD, D. ROUFA, and L. SKOGERSON: Protein Biosynthesis: Studies Using Synthetic and Viral mRNA's
WEISSBACH, H., N. BROT, D. MILLER, M. ROSMAN, and R. ERTEL: Interaction of Glucose Triphosphate with *E. coli* Soluble Transfer Factors
LOCKWOOD, A. H., S. HATTMAN, and U. MAITRA: The Nature of T-Factor-Guanine-Nucleotide Complexes
LENGYEL, P., SKOULTCHI, ONO, and WATERSON: Peptide Chain Elongation

LUCAS-LENARD, J., P. TAO, and A-L HAENNI: Further Studies on Bacterial Polypeptide Elongation
TOCCHINI-VALENTINI, G. P., L. FELLICETTI, and G. M. RINALDI: Mutants of *E. coli* Blocked in Protein Synthesis: Mutants with an Altered G Factor

TERMINATION

CAPECCHI, M. R. and H. A. KLEIN: Characterization of Three Proteins Involved in Polypeptide Chain Termination
CASKEY, T., E. SCOLNICK, R. TOMKINS, J. GOLDSTEIN, and G. MILMAN: Peptide Chain Termination, Codon, Protein Factor, and Ribosomal Requirements
CHAPEVILLE, F., P. YOT, and D. PAULIN: Enzymatic Hydrolysis of N-Acyl-Aminoacyl-tRNA's
PHILLIPS, S. L., D. SCHLESSINGER, and D. APIRION: Temperature Dependent Suppression of UGA and UAA Codons in a Temperature-Sensitive Mutant of *E. coli*
CARBON, J., C. SQUIRES, and C. W. HILL: Genetically Altered tRNA GLY Subspecies in *E. coli*



SALSER, W., M. FLUCK, and R. EPSTEIN: The influence of the Reading Context Upon the Suppression of Nonsense Codons, III
WILCOX, M.: γ-Phosphoryl Ester of G-tRNA GLN as an Intermediate in *Bacillus subtilis* Glutaminyl-tRNA Synthesis
SOFFER, R. L., H. HORINISHI, and M. J. LEIBOWITZ: The Aminoacyl tRNA-Protein Transferase

MAMMALIAN SYSTEMS

PENMAN, S., C. VESCO, R. WEINBERG, and E. ZYLBER: The RNA Metabolism of Nucleoli and Mitochondria in Mammalian Cells
ADAMSON, S. D., G. A. HOWARD, and E. HERBERT: The Ribosome Cycle in a Reconstituted Cell-Free System from Reticulocytes
BAGLIONI, C., C. VESCO, and M. JACOBS-LORENA: The Role of Ribosomal Subunits in Mammalian Cells
RABINOVITZ, M., M. L. FREEDMAN, J. M. FISHER, and C. R. MAXWELL: Translational Control in Hemoglobin Synthesis
HUNT, J. A. and D. G. LAYCOCK: Characterization of Messenger RNA for Hemoglobin
ANDERSON, W. F. and J. M. GILBERT: Translational Control of in vitro Hemoglobin Synthesis
COLLIER, R. J. and J. A. TRAUGH: Interactivation of Aminoacyl Transferase II by Diphtheria Toxin
GILL, D. M., A. M. PAPPENHEIMER, Jr., and J. B. BASEMAN: Studies on Transferase II Using Diphtheria Toxin
HONJO, T., Y. NISHIZUKA, O. HAYAISHI: Adenosine Diphosphoribosylation of Aminoacyl Transferase II by Diphtheria Toxin
GOOR, R. S. and E. S. MAXWELL: A Proposed Mechanism for ADP Ribosylation of Aminoacyl Transferase II by Diphtheria Toxin

SEQUENCES OF mRNA

ADAMS, J. M., P. G. N. JEPPESEN, F. SANGER, and B. G. BARRELL: Nucleotide Sequences from Fragments of R 17 Bacteriophage RNA
STEITZ, J. A.: The Nucleotide Sequences of the Ribosomal Binding Sites of Bacteriophage R 17 RNA
BILLETER, M. A., J. E. DAHLBERG, H. M. GOODMAN, J. HINDLEY, and C. WEISSMANN: Nucleotide Sequence Analysis of an Enzymatically Synthesized RNA Corresponding to the 5' Terminal Region of Qβ RNA
HO, N. W. Y., T. UCHIDA, F. EGAMI, and P. T. GILHAM: A New Specific Cleavage Method for the Study of Nucleotide Sequences in RNA

TRANSLATION OF mRNA

BRETSCHER, M. S.: Ribosome Initiation and the Mode of Action of Neomycin in the Direct Translation of Single-Stranded fd DNA
LODISH, H. F. and H. D. ROBERTSON: Regulation of in vitro Translation of Bacteriophage f2 RNA
WEBSTER, R. E., H. D. ROBERTSON, and N. D. ZINDER: The 5' Terminus of f2 RNA and the Coat Protein Gene
SUGIYAMA, T.: Translational Control of MS2 RNA Cistrons
FIERS, W., M. VAN MONTAGU, R. DeWACHTER, G. HAEGEMAN, W. MIN JOU, E. MESSENS, E. REMAUT, A. VANDENBERGHE, and B. VAN STYVENDAELE: Studies of the Primary Structure and the Replication Mechanism of Bacteriophage RNA
GESTELAND, R. F. and P. F. SPAHR: Translation of R17 RNA Fragments
KEDES, L. H., B. HOGAN, G. COGNETTI, S. SELVIG, P. YANOVER, and P. R. GROSS: Regulation of Translation and Transcription of Messenger RNA During Early Embryonic Development
MORSE, D. E., R. D. MOSTELLER, and C. YANOFSKY: Dynamics of Synthesis, Translation, and Degradation of *trp* Operon mRNA in *E. coli*
BALTIMORE, D., M. F. JACOBSON, J. ASSO, and A. S. HUANG: The Formation of Poliovirus Proteins
BALTIMORE, D., D. REKOSH, and H. F. LODISH: Translation of Poliovirus RNA by an *E. coli* Cell-Free System
ZUBAY, G. and D. A. CHAMBERS: A DNA-Directed Cell-Free System for β-Galactosidase Synthesis; Characterization of the De Novo Synthesized Enzymes and Some Aspects of the Regulation of Synthesis
SCHWEIGER, M. and L. M. GOLD: DNA-Dependent in vitro Synthesis of Bacteriophage Enzymes
SALSER, W., R. F. GESTELAND, and B. RICARD: Characterization of Lysozyme Messenger and Lysozyme Synthesized in vitro
NISHIZUKA, Y., K. UEDA, K. YOSHIHARA, H. YAMAMURA, M. TAKEDA, and O. HAYAISHI: Enzymic Adenosine Diphosphoribosylation of Nuclear Proteins
ILAN, J.: The Role of tRNA in Translational Control of Specific mRNA During Insect Metamorphis
WILHELM, J. M. and R. HASELKORN: In vitro Synthesis of T4 Proteins: Lysozyme and the Products of Genes 22 and 57
HEYWOOD, S. M.: Synthesis of Myosin on Heterologous Ribosomes
KLEINKAUF, H., W. GEVERS, and F. LIPMANN: Nonribosomal Polypeptide Synthesis: The Biosynthesis of Cyclic Peptide Antibiotic, Gramicidin S
KURAHASHI, K., M. YAMADA, K. MORI, K. FUTIKAWA, M. KAMBE, Y. IMAE, E. SOTO, H. TAKAHASHI, and Y. SAKAMOTO: Biosynthesis of Cyclic Oligopeptide

LENGYL, P.: Summary

POST GRADUATE TRAINING COURSES

Summer 1969

Many new fields have been developing in biology during the last ten years that do not fall into any particular subject but equally involve biochemistry, biophysics and genetics. As a result, most research workers have had to enlarge the extent of their professional competence: the biochemist has at last been forced to familiarize himself with genetics, and the geneticist has had to learn some biochemistry. This process of re-education, which could only be carried out with difficulty in most universities, tied as these are to a rigid curriculum, is being accomplished through a series of courses for qualified scientists held each summer at Cold Spring Harbor. The courses are given by a staff drawn from institutions all over the world and have already been attended by many hundreds of scientists drawn from disciplines as far apart as medicine and nuclear physics. In conjunction with these courses, the Laboratory invites many investigators as seminar speakers. This program of seminar speakers provides an extensive review of current research in these fields.

This year the summer courses were expanded to meet the growing interest in tissue culture techniques for investigation of higher cells and their viruses.

In addition to the three courses dealing with these subjects, the courses on bacterial genetics and bacterial viruses were again presented.

1) QUANTITATIVE MICROBIOLOGY OF ANIMAL AND PLANT CELLS IN CULTURE – June 15-July 7, 1969

The purpose of this course was to acquaint scientists from a wide variety of fields with the modern techniques and developing areas of animal and plant cell culture. The uses of functionally differentiated cells, such as hormone-secreting or hormone-responsive cells, and recently developed techniques in cell hybridization and heterokaryon formation were particularly emphasized. Laboratory exercises included such techniques as growth of cells in both suspension and monolayer cultures, primary culture preparation, autoradiography, tumor transplantation, assays for differentiated function, (such as micro-complement fixation assays of protein hormones), selection of differentiated cells exhibiting such function for growth in culture, cell hybridization, and plant cell culture. The laboratory was supplemented by lectures given by prominent biologists working in cell culture.

INSTRUCTORS:

Pfeiffer, Steven E., Ph.D., University of Connecticut Medical School, Farmington, Conn.
Sato, Gordon, Ph.D., Brandeis University
Watkins, J. F., M.D., Oxford University, Oxford, England

VISITING INSTRUCTORS:

Krikorian, A. D., Ph.D., State University of New York at Stony Brook
Meiss, Harriet, Ph.D., Public Health Research Institute of the City of New York, Inc.

ASSISTANT:

Carlin, Steven C., B.S., Brandeis University

KITCHEN STAFF:

Nancy Isomura, Jennifer Meek, Donald Ruthig

STUDENTS:

Alberts, Bruce M., Ph.D., Princeton University
Bargellesi, Antonio, M.D., Albert Einstein College of Medicine
Chaykin, Sterling, Ph.D., University of California, Davis
Coffino, Philip, Albert Einstein College of Medicine
Dannies, Priscilla, B.S., Brandeis University
David, Charles N., Ph.D., Max-Planck-Institut fur Virusforschung, Tubingen, W. Germany
Gupta, Naba K., Ph.D., Dept. of Chemistry, University of Nebraska, Lincoln
Llanes, Barbara, Ph.D., Princeton University
Makover, Shraga, Ph.D., Cold Spring Harbor Laboratory and Genetics Research Unit, Carnegie Inst. of Washington, D.C.
Marshall, Richard E., M.D., University of Washington, Seattle
Orth, David N., M.D., Vanderbilt University
Oyer, Philip E., University of Chicago
Pagoulatos, Gerassimos, D.Sc., Albert Einstein College of Medicine
Rosenberg, Roger N., M.D., National Institutes of Health
Skalka, Anna Marie, Ph.D., Genetics Research Unit, Carnegie Institution of Washington, D.C., and La Roche Institute, Nutley, New Jersey

Terry, Thomas M., Ph.D., Albert Einstein College of Medicine
Terzi, Mario, Dr., International Laboratory of Genetics and Biophysics, Naples, Italy
Tsai, Randolph L., University of Illinois, Urbana
Wolff, Jan, M.D., National Institutes of Health
Yamagishi, Hideo, Ph.D., Genetics Research Unit, Carnegie Institution of Washington, D.C. and Cold Spring Harbor Laboratory

SEMINARS: ANIMAL AND PLANT CELL CULTURE

H. Eagle, Dept. of Cell Biology, Albert Einstein College of Medicine, *"Biochemistry of Cultured Mammalian Cells."*
G. Tompkins, Dept. of Biochemistry, Univ. of Calif. School of Medicine, San Francisco, *"Enzyme Regulation in Cultured Cells."*
A. Krikorian, Dept. of Biology, S.U.N.Y. Stony Brook, *"Plant Cell Culture"*
J. Littlefield, Dept. of Pediatrics, Harvard Medical School, *"Cell Hybridization"*
R. Perry, Institute for Cancer Research, *"RNA Metabolism"*
J. Darnell, Biology Dept., Columbia Univ., *"RNA Metabolism"*
J. German, Rockefeller University, *"Human Cytogenetics"*
B. Mintz, Institute for Cancer Res., Philadelphia, Pa. *"Aliphenic Mice"*
H. Green, Dept. of Cell Biology, New York Univ. School of Medicine, *"Cell Division, Cell Contact, and Oncogenic Viruses"*
B. Cunningham, Rockefeller University, *"Immuno Globulin Structure"*
V. Allfrey, Rockfeller University *"On Histones"*
B. Papermaster, Roswell Memorial Park Institute, *"Lymphocyte Culture"*
H. Holtzer, Dept. of Anatomy, Univ. of Penn. *"Myosin Biogenesis"*
S. Gordon, Rockefeller University, *"Studies on Mouse Microphages in Heterokaryons"*

2) BACTERIAL GENETICS — July 8-July 29, 1969

Dilution and plating techniques; mode of origin of bacterial variants; induction of mutation; isolation and characterization of auxotrophs, mutagen specificity and reversions; sexual recombination and genetic mapping in *Escherichia coli*; transduction and determination of the linear order of mutational sites in *Salmonella typhimurium*; abortive transductions; characterization of suppressors and reversions by transduction; isolation and characterization of transforming DNA; transformation in *B. subtilis*; individual projects on the genetics of the lac operon.

INSTRUCTORS:

Joseph S. Gots, Ph.D., University of Pennsylvania
Julian Gross, Ph.D., University of Edinburgh, Scotland
Benno Mueller-Hill, Ph.D., University of Cologne, Germany

ASSISTANTS:

Marilyn Gross, Ph.D., University of Edinburgh
Jeffrey Miller, A.B., Harvard University
Sheila Ehlinger, A.B., New York University

KITCHEN STAFF:

V. Cairns, Peter Strode, Arlene McKenzie

STUDENTS:

Batt, Ellen R., Ph.D., Columbia College of Physicians & Surgeons
Bellino, Francis L., M.S.E.E., State University of New York at Buffalo
Benson, Charles E., M.S., Dept. of Microbiology, Bowman Gray School of Medicine, Winston-Salem, North Carolina
Berliner, Lawrence J., Ph.D., Dept. Chemistry, Stanford University
Biswas, Debajit, Ph.D., Dept. Bacteriology & Immunology, Harvard Medical School
Blasi, Francesco, M.D., Lab. of Chemical Biology, NIAMD, National Institutes of Health
Brown, Arnold, Ph.D., Stanford University
Chou, Iih-Nan, B.S., Biochemistry Division, University of Illinois, Urbana
Cronan, John E., Ph.D., Dept. of Biochemistry, Washington University School of Medicine
Datta, Naomi, M.D., Royal Postgraduate Medical School, London, England
Ebrey, Thomas C., Ph.D., Dept. of Biological Sciences, Columbia University
Erbe, Richard, M.D., National Institutes of Health, National Cancer Institute
Harris, Maureen I., Ph.D., NIAMD, National Institutes of Health
Henderson, Ellen J., B.S., Dept. of Biochemistry, Purdue University
Martin, Thomas F. J., A.B., Harvard University
Mordoh, Jose, M.D., Instituto de Investigaciones Bioquimicas, Buenos Aires, Argentina
Singer, Britta, A.B., University of Connecticut, Storrs
Skogerson, Lawrence E., Ph.D., National Institutes of Health
Willecke, Klaus, Ph.D., Biology Dept., Princeton University
Wu, Anna Fang, Ph.D., Dept. Biochemistry, College of Physicians & Surgeons, Columbia University

SEMINARS:

Charles E. Helmstetter, Roswell Park Memorial Institute, "Regulation of replication and transcription of the *E. coli* genome."

Philip E. Hartman, The Johns Hopkins University, "An introduction to control of the histidine operon."
David Freifelder, Brandeis University, "*E. coli* sex factors; Genetic and physical properties and their usefulness in studying DNA function."
Robert F. Goldberger, National Institutes of Health, "Relationship between feedback inhibition and repression in the histidine system of *Salmonella typhimurium.*"
Roy Curtiss, III, Oak Ridge National Laboratory, "Conjugation with mini cells of *E. coli.*"
Brooks Low, Yale University School of Medicine, "Studies on recombination deficiency in *E. coli.*"
Robert H. Rownd, University of Wisconsin, "Control of the replication of bacterial episomes."
Paul Margolin, Public Health Research Institute of the City of New York, Inc., "Initiation of expression of tryptophan genes."
Noboru Sueoka, Princeton University, "Chromosome replication in *B. subtilis.*"
Evelyn M. Witkin, State University of New York, Brooklyn, "UV Mutagenesis and DNA repair."
Jonathan R. Beckwith, Harvard University, "Mechanisms of transcriptional control of the *lac* operon."
David Zipser, Columbia University, "Polypeptide reinitiation in the *lac* operon."

3) ANIMAL VIRUSES – July 8-July 29, 1969

Preparation of primary and secondary chick and mouse embryo cell cultures; chick embryo cultures of heart fibroblast, lung, kidney and iris epithelium; HeLa, BHK, and L-cell growth in mass culture and in clones; isolation of clonal sublines; tests and elimination of PPLO in cell cultures. Spinner cell culture. Karyotype analysis. DNA and RNA synthesis in the cell life cycle – an introduction to quantitative autoradiography. Synthesis of viral-RNA: coverslip technique. Growth and purification of Newcastle disease virus (NDV). Assay of NDV by plaque formation, hemagglutination, hemadsorption, and cell-killing. The hemadsorption-negative plaque test and intrinsic interference: detection of noncytopathic viruses. Assay and properties of Sindbis virus, vesicular stomatitis virus and the pox viruses. Quantitative neutralization of viruses with antibody. One-step growth curve. Effect of antimetabolites on viral and cell growth. Preparation, assay, and the mechanism of action of interferon. Histochemistry and fluorescent antibody techniques in virus infection. Phenotypic mixing of myxovirus, genetic recombination of pox viruses, complementation with Sindbis virus. Morphologic transformation of cells by Rous sarcoma virus and polyoma. Synchronous growth in cell culture. Hybridization of cells.

INSTRUCTORS:

Marcus, Philip I., Ph.D., The University of Connecticut, Storrs
Summers, Donald F., M.D., Albert Einstein College of Medicine

VISITING INSTRUCTOR:

Sambrook, Joseph, Ph.D., Cold Spring Harbor Laboratory

ASSISTANTS:

Cross, Rise K., B.S. Albert Einstein College of Medicine
Weiss, Helene, B.S., New York University
Rosenblum, Margaret, B.S., Albert Einstein College of Medicine

STUDENTS:

Banerjee, Amiya K., Ph.D., Dept. Molecular Biology, Albert Einstein College of Medicine
Barlati, Sergio C., Dr., Dept. Genetics, Stanford Medical School
Burgess, Ann, B.S., Biological Laboratories, Harvard University
David, Anshel E., Ph.D., Albert Einstein College of Medicine
Godson, Godfrey N. Ph.D., Dept. Radiobiology, Yale University
Herrick, Glenn, M.S., Program in Biochemistry, Frick Chemistry Lab, Princeton University
Huang, Wai Mun, Ph.D., Albert Einstein College of Medicine
Leder, Philip, M.D., National Institutes of Health
Lenard, Jean M. L., Ph.D., Rockefeller University
Levine, Myron, Ph.D., Dept. Human Genetics, University of Michigan
Miggiano, Vincenzo C., M.D., Dept. of Genetics, Stanford University, School of Medicine
Paul, Dietrich, Ph.D., Salk Institute for Biological Studies
Schwartz, Maxime S., Ph.d., Harvard University Biological Laboratories
Scolnick, Edward M., M.D., Lab of Biochemical Genetics, National Heart Institute, National Institutes of Health
Sekellick, Margaret J., M.S., Dept. Animal Genetics, University of Connecticut, Storrs
Signer, Ethan R., Ph.D., Dept. of Biology, Massachusetts Institute of Technology
Skalka, Anna Marie, Ph.D., Genetics Research Unit, Carnegie Institution of Washington, Cold Spring Harbor
Ward, David C., M.Sc., Rockefeller University
Wolf, Barbara, B.A., Massachusetts Institute of Technology
Zweerink, Hendrik, Ph.D., Dept. Microbiology & Immunology, Duke University

SEMINARS:

H. Eagle, Dept. of Cell Biology, Albert Einstein College of Medicine, "Biochemistry of Cultured Mammalian Cells"
E. Robbins, Dept. of Cell Biology, Albert Einstein College of Medicine, "Mammalian Cell Ultrastructure and the Cell Cycle"

J. Maizel, Dept. of Cell Biology, Albert Einstein College of Medicine, "Virus Structure and the Replication of Adenovirus"
R. Wagner, Dept. of Microbiology, University of Virginia School of Medicine, "Interferon: Induction and Action"
B. Burge, Dept. of Biology, Massachusetts Institute of Technology, "Membrane Glycoprotein of Enveloped Viruses"
H. Temin, McArdle Laboratory, University of Wisconsin, "Rous Sarcoma Virus"
H. Koprowski, The Wistar Institute of Anatomy and Biology, "Slow Viruses"
A. Graham, The Wister Institute of Anatomy and Biology "Replication of Reovirus"
T. Benjamin, Public Health Research Institute of the City of New York "Polyoma Virus"
D. Summers, Department of Microbiology, Albert Einstein College of Medicine, "The Replication of Poliovirus"
G. Todaro, Viral Carcinogenesis Branch, National Cancer Institute "SV_{40} Virus"
J. Sambrook, Cold Spring Harbor Laboratory "Poxviruses: Genetics and Replication"
P. Choppin, The Rockefeller University, "Parainfluenza Virus SV5: Structure, Replication, and Interactions with Cell Membranes"
D. Kingsbury, St. Jude Children's Research Hospital, "Large RNA Viruses"
P. I. Marcus, Department of Microbiology, Albert Einstein College of Medicine, "Intrinsic Interference: The Action of Sindbis Virus Polymerase Proteins"

4) TUMOR VIRUS WORKSHOP – July 31-August 15, 1969

The course was comprised of two weeks, the first week being on DNA tumor viruses, finishing with a two-day meeting. The second week dealt with RNA tumor viruses. There was no formal practical work, but a small number of demonstrations were included. For the most part the workshop consisted of extended discussions led by the lecturers listed below. Detailed notes of the discussions were taken by students and typed up as a manual which was distributed to all the participants.

ORGANIZERS:

L. V. Crawford, Ph.D., Imperial Cancer Research Foundation, London, England
Joseph Sambrook, Ph.D., Cold Spring Harbor Laboratory

TYPISTS:

Diane Jordan and Linda Grill

STUDENTS:

Burge, Bovce W., Ph.D., Dept. of Biology, Massachusetts Institute of Technology
Champoux, Jim, Ph.D., Dept. Biochemistry, Stanford University School of Medicine
Estes, Mary C. K., B.A., Dept. of Bacteriology & Immunology, University of North Carolina
Keller, Walter, M.D., Dept. of Biophysics, Johns Hopkins University
Levine, Arnold J., Ph.D., Dept. of Biology, Princeton University
Levine, Myron, Ph.D., Dept. Human Genetics, University of Michigan
Scher, Charles D., M.D., National Cancer Institute. National Institutes of Health
Sorof, Sam, Ph.D., Institute for Cancer Research, Fox Chase, Philadelphia, Pa.
Van der Eb, Alex J., Division of Chemistry and Chemical Engineering, California Institute of Technology
Weimann, Bernd-Jurgen, Ph.D., Salk Institute for Biological Studies
Vogt, Volker M., B.S., Biological Laboratories, Harvard University
Lebowitz, J., Ph.D., Department of Medicine, Yale University
Miggiano, Vincenzo C., M.D., Dept. of Genetics, Stanford University School of Medicine
Ward, Tom, B.S., Harvard University

TUMOR VIRUS WORKSHOP LECTURERS:

M. Green
Bernhard Hirt
W. Murakami
Paul Berg
L. Gross
Marguerite Vogt
Michael Fried
Basil Smith
Kenneth Takemoto
G. Todaro
Tom Benjamin
Vittorio Defendi
S. Nandi
Walter Doerfler
Walter Eckhart
Fred Rapp
Allan Granoff
W. Henle
Robert Pollack
David Baltimore
Will Robinson
R. Ting
Michael Bratt
Raymond Erikson
Peter Vogt
H. Hanafusa
H. Temin
R. Huebner
Paul Black

5) BACTERIAL VIRUSES – July 31-August 21, 1969

Genetic, physiological, and biochemical techniques used in work with bacterial viruses. The life cycle of the temperate virus λ was the principal object of study. The processes of repression, induction, lysogenization, recombination and transcriptional control were taken up in detail.

INSTRUCTORS:

Gottesman, Max, National Institutes of Health
Kourilsky, P. H., Institute de Biologie Physico-Chimique, Paris, France
Yarmolinsky, Michael, National Institutes of Health
Weisberg, Robert A., Oak Ridge National Laboratory

STUDENTS:

Bendis, Ina K., B.S., Albert Einstein College of Medicine
Black, Paul H., M.D., Massachusetts General Hospital, Boston
Cerutti, Peter A., Ph.D., Dept. of Chemistry, Princeton University
Colli, Walter, M.D., Public Health Research Institution of the City of New York Inc.
Cormack, Douglas V., Ph.D., The Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada
Cronan, John E., Ph.D., Dept. of Biochemistry, Washington University School of Medicine
De Lisle, Allan L., Ph.D., Dept. of Microbiology, University of Maryland School of Dentistry
Erbe, Richard W., M.D., Biophysics Research, Harvard Medical School
Garro, Anthony M., Ph.D., Dept. of Biochemistry, Albert Einstein College of Medicine
Juni, Elliot, Ph.D., Dept. of Microbiology, University of Michigan
Krisch, Henry M., M.A., Dept. of Biophysics, Johns Hopkins University
LaMontagne, John R., M.A., Tulane University
Martin, Thomas F. J., A.B., Harvard University
Mojica, Tobias, B.A., Dept. of Genetics, McGill University, Montreal, Canada
Mordoh, Jose, M.D., Instituto de Investigaciones Bioquimicas, Buenos Aires, Argentina
Rieber, Manuel, Ph.D., Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela
Roozen, Kenneth J., M.A., Oak Ridge National Laboratory
Saz, Arthur K., Ph.D., Georgetown University, Washington, D.C.
Schmitt, Rudiger W., Ph.D., Institut fur Mikrobiologie, Universitat Erlangen-Nurnberg, Germany
Tsai, Mei-Huei, M.A., Temple University, Philadelphia, Pennsylvania

SEMINARS:

Rudolf Werner, "Structural requirements of DNA replication as studied with phage T4"
Andrew Travers, "Isolation of T4-specific σ-factor"
Joseph F. Speyer, "Role of DNA polymerase in mutagenesis and recombination of T4"
Charles A. Thomas, "The chromosomes of phage and fish"
E. Peter Geiduschek, "Transcription in phage development"
Raymond Gesteland, "Regulation of T4 development: *in vitro* protein synthesis"
Myron Levine, "DNA synthesis and maturation; phage morphogenesis"
William Sly, "The mechanism of virulence in λ (2 lectures)"
Nancy Hopkins, "Phage repressor and repression; N-independent mutants of λ"
William Dove, "Replication control in λ"
Dale Kaiser, "λDNA cohesive ends, structure and function"
Dale Kaiser, "λdv, a miniature λ"
W. Studier, "T7"
Harrison Echols, "Integration, excision, and recombination"
Philippe Kourilsky, "Studies on λ mRNA"
Julius Marmur, "Phages of *B. subtilis* with particular reference to the defective phages"
Robert Webster, "RNA phages"
Jeffrey Roberts, "A termination factor for RNA synthesis"
Norton Zinder, "The bacteriophage f1"

SUMMER MEETINGS

For many years we have sponsored two summer meetings each year, the Symposium and the Bacteriophage Meetings. This summer we expanded our program by organizing a Tumor Virus Meeting, held in conjunction with the Tumor Virus Workshop, and a meeting on the Lactose Operon. Both were highly successful, we felt, largely because many of the best people in the fields were able to attend. The resulting papers and discussions should thus help advance the dates when important new discoveries in these subjects occur.

Another project initiated this year is the Annual Abstracting Service for Meetings held at Cold Spring Harbor. Until now, abstracts of these meetings have been sent out on request but, as many people are unaware of this service, the Laboratory now offers to mail quickly abstracts of the five meetings described below.

During the period July 1969 to June 1970, at least five books of abstracts will be sent. They will cover material presented at the *August 1969 Tumor Virus Meeting*, the annual *Late August Phage Meetings*, material presented at the *September European Phage Meetings* to be held in Sussex, the abstracts of a meeting on the *Lactose Operon*, and the abstracts of the annual *Cold Spring Harbor Symposium (1970)*. The possibility exists that several other small meetings will also be held here (or elsewhere) and their proceedings distributed in this series.

THE TUMOR VIRUS MEETING

Attended by 81 participants

THURSDAY, AUGUST 7 – 7:30 P.M.

SV40: TRANSFORMATION AND RECOVERY
Chairman: L. V. Crawford, Imperial Cancer Research Laboratories, London, England

S. Aaronson and G. J. Todaro, National Institutes of Health, Bethesda, Maryland: "Simian Virus 40 DNA: *In Vitro* Transformation of Human Cells."

S. Kit and M. Brown, Baylor College of Medicine, Houston, Texas: "Rescue of SV40 from Cell Lines Transformed at Low Input Multiplicities by Unirradiated or UV-Irradiated SV40."
Malcolm A. Martin and David Axelrod, National Institutes of Health, Bethesda, Maryland: "SV40 Gene Activity During Lytic Infection and in Transformed Animal Cells."
George Khoury and J. van der Noordaa, Harvard University, Cambridge, Massachusetts, and University of Amsterdam: "Absence of Infectious Virus from a Line of SV40-Transformed Human Liver Cells."
Miriam Margalith, Eva Margalith, Tamar Nasialski and N. Goldblum, Hebrew University, Hadassah Medical School, Jerusalem, Israel: "Studies on the State of SV40 Virus in BSC_1 Transformed Cells."
Yosef Aloni, Ernest Winocour, Leo Sachs and Judith Torten, Weizmann Institute of Science, Rehovoth, Israel: "Hybridization Between SV40 and Cellular DNA's."

FRIDAY, AUGUST 8 – 10:30 A.M.

POLYOMA AND SV40 MUTANTS

Chairman: J. F. Sambrook, Cold Spring Harbor Laboratory, New York

Carel Mulder and Marguerite Vogt, Salk Institute for Biological Studies, San Diego, California: "Pattern of Oligomeric Polyoma DNA in TSA-3T3 Cells at 31°C."
Michael N. Oxman, Walter Eckhart and Kenneth K. Takemoto, Children's Hospital, Boston, Mass., Salk Institute for Biological Studies, San Diego, California and National Institutes of Health, Bethesda, Maryland: "Polyoma T Antigen Formation by Temperature-Sensitive Mutants of Polyoma Virus."
Thomas L. Benjamin, The Public Health Research Institute of the City of New York: "Isolation of Host Range Mutants of Polyoma Virus."
Fred Rapp and Sidney C. Trulock, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania: "Replication of a Defective SV40 Genome in Simian Cells Transformed by SV40."
Harvey L. Ozer and Kenneth K. Takemoto, National Institutes of Health, Bethesda, Maryland: "Host Restriction of Coat Mutants of SV40."

FRIDAY, AUGUST 8 – 2:00 P.M.

VIRAL DNA AND ITS TRANSCRIPTION

Chairman: Paul Berg, Stanford University, California

Maurice Green and Magdalena Pina, Institute for Molecular Virology, St. Louis University School of Medicine, Missouri: "Integration of the Viral Genome in Adenovirus Transformed Cells."
K. Ohe and S. M. Weissman, Yale University, New Haven, Connecticut: "Properties of a Low Molecular Weight RNA from Cells Infected with Adenovirus."
H. Westphal, Salk Institute for Biological Studies, San Diego, California: "SV40 DNA Strand Selection by *E. coli* RNA Polymerase."
Pierre Bourgaux, Salk Institute for Biological Studies, San Diego, California: "Evidence for a Nicked Replicative Intermediate of Polyoma Virus DNA."
H. V. Thorne, University de Sherbrooke, Canada: "Heterogeneity of Component I Polyoma Virus DNA."
M. E. Blackstein, C. P. Stanners and A. J. Farmilo, University of Toronto, Canada: "Heterogeneity of Polyoma Virus DNA: Isolation and Characterization of Non-Infectious Small Supercoiled Molecules."
David A. Goldstein, Roger Weil and James Hudson, Scripps Clinic and Research Foundation, La Jolla, California and Swiss Institute for Cancer Research, Lausanne: "RNA Metabolism and Transcription in Polyoma-Infected Mouse Kidney Cell Cultures."
P. E. Branton and R. Sheinan, University of Toronto, Canada: "DNA Synthesis in Polyoma Virus Infected Cells."

SATURDAY, AUGUST 9 – 10:00 A.M.

RNA TUMOR VIRUSES

Chairman: David Baltimore, Massachusetts Institute of Technology, Cambridge, Mass.

R. L. Erikson, University of Colorado, Denver: "The RNA Isolated from RNA Tumor Viruses."
John P. Bader, Theodore L. Steck and Tsuyoshi Kakefuda, National Institutes of Health, Bethesda, Maryland: "The RNA of RNA-Containing Tumor Viruses."
M. A. Baluda, UCLA School of Medicine, Los Angeles, California: "Replication of Avian Myeoblastosis Virus RNA."
R. Emmanoil-Ravicovitch, Institute de Recherches sur les Maladies du Sang, Hopital Saint-Louis, Paris, France: "Studies of the Homology of RNA from Rauscher Mouse Leukemia Virus and DNA from Different Mice Strains."
S. R. Opler, Stanford University School of Medicine, California: "Biological Studies on Cavian Leukemia Virus."

SATURDAY, AUGUST 9 – 2:00 P.M.

POLYOMA VIRUS/CELL INTERACTION

Chairman: Robert Pollack, New York University Medical Center

Claudio Basilico, New York University School of Medicine: "Interaction of Polyoma Virus with Mouse-Hamster Somatic Hybrid Cells."

VIRAL ANTIGENS AND MEMBRANE CHANGES

Robert C. Nowinski, LLoyd J. Old, Sloan-Kettering Institute for Cancer Research: "Mouse Mammary Tumor Virus: Identification of Viral Antigens."
Erwin Fleissner, Sloan-Kettering Institute for Cancer Research: "Virus-Specific Antigens in Hamster Cells Transformed by Rous Sarcoma Virus."
Rose Sheinin and Kazikuyo Onodera, University of Toronto, Canada: "Viral Oncogenesis and a Surface Component of Mouse Cells."

Max M. Burger, Princeton University, New Jersey: "Proteolytic Enzymes Producing a Change of the Cell Surface Similar to that Seen After Transformation."
Peter T. Mora, Roscoe O. Brady and Richard Smith, National Institutes of Health, Bethesda, Maryland: "Glycolipids in SV40 and Polyoma Virus Transformed Mouse Cell Lines."
G. Greering and L. J. Old, Sloan-Kettering Institute for Cancer Research, New York: "Shared Viral Antigens of Mammalian Leukemia Viruses."

THE 1969 BACTERIOPHAGE MEETINGS

Attended by 293 participants

SECTION I — LYSOGENIC PHAGES

W. Spiegelman, Stanford Univ., Stanford, Calif.: "Expression of Early Lambda Genes in a Defective Lysogen".
W. S. Sly, K. Rabideau & A. Kolber, Washington University School of Medicine, St. Louis, Mo.: "The λ Vir Antirepressor: Role in the Mechanism of Classical λ Virulence.
M. J. Bronson & M. Levine, University of Michigan, Ann Arbor: "Virulent Mutants of P22".
G. W. Ordal, Stanford University, Palo Alto, Calif.: "Two New Virulence Mutations in λ".
M. Lieb, Univ. of Southern California School of Medicine, Los Angeles: "Repressor-less λ: Plasmids and Prophages".
J. Cowlishaw & W. Ginoza, Oakland University, Rochester, Michigan, & Penn State University, University Park, Pa.: "Effect of Nalidixic Acid on λ Repression".
C. M. Radding & D. M. Carter, Yale University, New Haven, Conn.: "The Action of Exonuclease and β Protein of Phage λ in Genetic Recombination".
D. Botstein & M. J. Matz, Mass. Inst. Technology, Cambridge, Mass.: "Recombination function Essential to the Growth of Phage P22".
S. Elsevier & W. Dove, McArdle Lab., Univ. of Wisconsin, Madison: "The Basis of Restoration Mutagenesis in Lambda".

MONDAY, AUGUST 25 — 9:00 A.M.

W. Dove, E. Hargove, M. Ohashi, & C. McLeester, McArdle Lab., Univ. of Wisconsin, Madison: "Replication Control in Lambda".
S. Makover, Carnegie Institute of Washington, Genetics Research Unit, Cold Spring Harbor, N.Y.: "The Origin and Direction of λ DNA Replication."
E. C. Cox, Dept. of Biology and the Program in Biological Sciences, Princeton University: "The Replication of λ DNA and Mutator Gene Action."
M. Levine, M. J. Bronson, & M. Charavorty, Univ. of Michigan, Ann Arbor: "Repressor Action and Replication Inhibition in P22 Lysogens."
R. C. Shuster, Emory University, Atlanta, Georgia: "The Breakdown of Twisted Circular Phage by C17 Mutants".
L. Hallick, R. P. Boyce, & H. Echols, Univ. of Wisconsin, Madison: "Membrane Association of λ DNA and its Control".
A. Kolber & W. Sly, Division of Medical Genetics, Departments of Pediatrics & Medicine, Washington U. School of Medicine, St. Louis, Mo.: "The Formation of a Fast-Sedimenting λ DNA-Host Cell Component Complex".
C. F. Gunsalus, A. M. Chakrabarty & I. C. Gunsalus, Univ. of Illinois, Urbana: "Transduction Analysis and Characteristics of a Mutator Gene in Pseudomonas Putida".
M. Ohashi & W. Dove, McArdle Laboratory, Univ. of Wisconsin, Madison: "Correlates of the Loss of Replication Inhibition in Lambda".

MONDAY, AUGUST 25 — 7:30 P.M.

J. W. Roberts, Harvard University, Cambridge, Mass.: "In Vitro Synthesis of Lambda Messenger RNA".
M. Konrad, Univ. of California, Los Angeles: "Messenger RNA Synthesis at Early Times After Induction".
P. Kourilsky, Institut de Biologie Physico-Chimique, Paris: "Very Early Kinetics of λ Prophage Induction, and the Regulation of Transcription of Immunity Region".
H. J. Nijkamp, W. Szybalski & K. Bovre, Univ. of Wisconsin, Madison: "Leftward Transcription of the J-b2-*aa'* Region upon Induction of λ Lysogens".
H. J. Nijkamp, W. Szybalski & W. F. Dove, McArdle Lab., Univ. of Wisconsin, Madison: "Effect of Constitutive Mutations in the *x*-P Region on Rightward and Leftward Transcription in λ Lysogens".
H. J. Nijkamp, Y. Saturen & W. Szybalski, McArdle Lab., Univ. of Wisconsin, Madison: "Controls of the Leftward Transcription in Coliphage λ and its Mutants".
A. Guha, Y. Saturen & W. Szybalski, Univ. of Toronto & Univ. of Wisconsin: "Divergent Orientation of Transcription from the Biotin Operon of *E. Coli*".
T. C. Jones & W. Dove, McArdle Lab., Univ. of Wisconsin, Madison: "Sensitization of the Capacity for Genetic Transcription by Incorporation of DUdR into Lambda DNA".

TUESDAY, AUGUST 26 — 9:00 A.M.

D. Court, K. Sato & S. Adhya, Stanford Univ., Stanford, Calif.: "Mutants of Phage λ Able to Grow Without Gene N".
J. Pero, Harvard Univ., Cambridge, Mass.: "Location of the Phage λ Gene Responsible for Turning off λ-Exonuclease Synthesis".
R. Hendrix, Harvard Univ., Cambridge, Mass.: "Early Lambda Proteins".
M. Fiandt, D. M. Zuhse, J. E. Khorana & W. Szybalski, McArdle Lab., Univ. of Wisconsin, Madison: "Insertion of DNA of Unknown Origin in the Right Arm of λ*sus*N7N53".
B. W. Holloway & J. Pemberton, Dept. of Genetics, Monash Univ., Clayton, Victoria, Australia: "Recombination Deficient Mutants of Pseudomonas Aeruginosa with Altered Properties of Lysogenisation".
R. Calendar, B. Lindqvist, G. Sironi & A. J. Clark, Univ. of Calif., Berkeley: "Characterization of Rep Mutants and their Interaction with P2 Phage".

G. Lindahl, G. Sironi, H. Baily & R. Calendar, Karolinska Institutet, Stockholm, Sweden; Univ. of California, Berkeley: "Exclusion of λ by P2 Prophage".
Naomi Franklin, Biology Dept., Stanford University, Stanford, Calif.: "Fusion Between N-controlled Operon of λ and Tryptophan Operon of *E. coli*."

TUESDAY, AUGUST 26 – 7:30 P.M.

Ira Herskowitz & E. R. Signer, MIT, Cambridge: "A Site Essential for Expression of all Late Genes in Phage Lambda".
S. Adhya, K. Sato & D. Court, Stanford Univ., Stanford, Calif.: "A Site on Phage λ Required for Transactivation of Gene Q".
K. Sato & A. Campbell, Stanford Univ., Stanford, Calif.: "Infectious Particle Production by Lambda Phage Deleted for Gene Q".
G. Kayajanian, Stanford Univ.: "Packaging of Phage λ Genomes".
V. C. Bode, Univ. of Maryland Medical School, Baltimore, Md.: "The Inactivation of Lambda Phage Heads by Micrococcal Nuclease".
D. P. Harrison & V. C. Bode, Univ. of Maryland Medical School, Baltimore, Md.: "Purification and in vitro Joining of λ Phage Heads and Tails".
E. W. Six & B. Lindqvist, University of Iowa, Iowa City and Univ. of Stockholm, Sweden: "Helper Dependent Reproduction of Coliphage P4".
J. Ebel, D. Botstein & M. S. Fox, MIT, Cambridge, Mass: "Physical Studies of Generalized Transduction in *S. Typhimurium*".

WEDNESDAY, AUGUST 27 – 9:00 A.M.

P. D. Harriman, Dept. of Biochemistry, Duke University, Durham, N.C.: "Production of P1 Transducing Particles in *E. coli*."
A. Skalka, Carnegie Institution of Washington, Genetics Research Unit, Cold Spring Harbor, *and* the Roche Institute of Molecular Biology, Nutley, N.J.: "Comparisons on the Distribution of Nucleotides in Various Phage DNA's."
G. Guarneros & H. Echols, Univ. of Wisconsin, Madison: "Bacteriophage Mutants Unable to Excise from the Host Chromosome".
R. Weisberg, NIH, Bethesda, Md.: "Requirements for Prophage Excision".
A. Folkmanis & D. Freifelder, Brandeis Univ., Waltham, Mass.: "Physicochemical Studies on Integration of Coliphage λ".
D. Freifelder, Brandeis University, Waltham, Mass.: "Physicochemical Studies of Excision of λ Prophage from a Sex Factor".
J. Little, M. Gottesman & R. Weisberg, NIH, Bethesda, Md. "Defective λ Particles Produced by Abnormal Excision".
L. R. Bullas & R. L. Nutter, Loma Linda University, Loma Linda, Calif.: "A New Host Modification System Involving the Growth of Salmonella Phages in *E. coli*".

WEDNESDAY, AUGUST 27 – 7:30 P.M.

SECTION II – VIRULENT PHAGES

E. F. Rossomando, NIDR, N.I.H., Bethesda, Md.: "Thermal Stability of Bacteriophage f1".
D. A. Marvin & B. Hohn, Yale University, New Haven, Conn.: "Macromolecular Synthesis during Early Stages of Infection with the Filamentous Phage FD".
D. S. Ray, Molecular Biology Institute, University of California, Los Angeles: "Properties of M13 Replicative Form DNA Synthesized in the Presence of Chloramphenicol".
A. B. Forsheit & D. S. Ray: UCLA, Los Angeles: "Replication of Bacteriophage M13: Early Events in Infection".
S. Hilliker & D. L. Wulff, University of California, Irvine: "Interactions of M13 Phage with its Host".
S. Tonegawa & M. Hayashi, Salk Institute, San Diego, & Dept. of Biology, Univ. of Calif., San Diego, La Jolla: "Intermediates in the Assembly of φX-174".
L. B. Dumas & R. L. Sinsheimer, Calif. Institute of Technology, Pasadena: "Temperature Effects on in vitro Synthesis of φX174 RF DNA".
M. Iwaya and David Denhardt, Biological Laboratories, Harvard Univ.: "DNA Replication in Several Mutants of φX 174".
L. L. Greenlee, Univ. of Utah, Salt Lake City: "A New Structure of φX-174 RF-DNA".
A. Puga & R. Shleser, Purdue University, Lafayette, Indiana, and D. Kohne, Carnegie Institution, Washington, D.C.: "Homology Between Phage S13 and *E. coli* C122".

THURSDAY, AUGUST 28 – 9:00 A.M.

V. L. Chan, S. Shugar & K. Ebisuzaki, Univ. of Western Ontario, London, Canada: "Intergenic Suppression of Amber Polynucleotide Ligase Mutation in Bacteriophage T4".
H. Berger & A. Kozinski, Johns Hopkins Univ., Baltimore, Md. & The University of Pa. Medical School, Phila.: "Suppression of T4D Ligase Mutations by *rIIA* and *rIIB* Mutations".
J. D. Karam, Sloan-Kettering Institute, New York, N.Y. "A Role for the rII Gene in T4 DNA Metabolism".
R. Wernèr and J. Bowen, Cold Spring Harbor Laboratory: "Suppression of the T4D Ligase Mutations".
K. Hercules & J. S. Wiberg, Univ. of Rochester, N.Y.: "A T4 Mutation Near Gene 33 which Partially Suppresses Mutations in Genes 46 and 47".
E. Kutter, Univ. of Virginia, Charlottesville, & J. Wiberg, Univ. of Rochester, N.Y.: "Intragenic Complementation between T4 Mutants Defective in Deoxycytidylate Hydroxymethylase (HMase)".
L. D. Simon & Judith Swan, Institute for Cancer Research, Phila.: "Functional Defects in T4 Bacteriophages Lacking the Gene 11 and Gene 12 Products".
W. C. Benz & E. B. Goldberg, Tufts Univ. School of Medicine, Boston, Mass.: "Infection of Spheroplasts by Urea-treated T4 Base-plate Mutants".
E. Meezan & W. B. Wood, Calif. Inst. Tech., Pasadena: "Ordering of the steps involved in T4 tail core assembly".

N. Sternberg & S. P. Champe, Rutgers Univ., New Brunswick, N.J.: "The Genetic Determinant of an Internal Peptide of Bacteriophage T4".
G. W. Howard Jr. & S. P. Champe, Institute of Microbiology, Rutgers Univ., New Brunswick, N.J.: "Internal Proteins of Phages T4D & T2H".
D. J. Cummings & G. L. Forrest, Univ. of Colorado Medical Center, Denver: "Characterization of Head Proteins of T-even Bacteriophage".

THURSDAY, AUGUST 28 – 7:30 P.M.

G. Mosig, Vanderbilt University, Nashville, Tenn.: "A Preferred Origin and Direction of T4 DNA Replication".
R. C. Miller Jr., Univ. of Pennsylvania, Phila: "Production of Long Single Strands During T4 Infection".
J. D. Childs, Univ. of Washington, Seattle: "Permuted Partial Phage Within Single Burst of *Escherichia Coli* Infected with a T4D Mutant".
S. G. Dennis & R. Werner, Cold Spring Harbor Laboratory: "DNA Replication in Gene 32-Defective Phage T4".
A. W. Kozinski, Dept. of Medical Genetics, Univ. of Pa.,Phila.: "Non-Random Intracellular Breakage of T4 DNA".
G. Hall & D. MacDonald Green, Univ. of New Hampshire, Durham: "Natural Breaks and the Renaturation of Biological Activity in SP82G Bacteriophage DNA."
R. A. Schlegel & C. A. Thomas Jr., Harvard Medical School, Boston, Mass.: "Fractionation of Intracelluiar T7 DNA on Benzoylated-naphthoylated DEAE Cellulose".
R. Barzilai & C. A. Thomas Jr., Harvard Medical School, Boston, Mass.: "Spontaneous Renaturation of Replicating T7-DNA"
W. T. McAllister, Univ. of New Hampshire, Durham: "Entry of Markers in SP82G Bacteriophage Infection".
M. Zweig, H. S. Rosenkranz & C. Morgan, Dept. of Microbiology, Columbia University, N.Y.: "Effect of Inhibitors of DNA Synthesis on T5 Development".
Y. C. Yeh & Irwin Tessman, Univ. of Arkansas, Little Rock, and Purdue Univ., Lafayette, Indiana: "Control of Purine Ribonucleotide Reduction by Phage T4".
M. Marcus & M. Newlon, MIT, Cambridge, Mass.: "DNA Synthesis in ϕe-Infected *Bacillus Subtilis*".

FRIDAY, AUGUST 29 – 9:00 A.M.

M. Linial & M. Malamy, Tufts Univ. School of Medicine, Boston, Mass.: "Characterization and Properties of Bacteriophage ϕII and ϕII Infected Cells".
N. Agabian-Keshishian, D. Button, R. Bevill & L. Shapiro, Albert Einstein College, New York: "A Caulobacter DNA Bacteriophage and Phage-Resistant Mutants".
H. Drexler, Bowman Gray School of Medicine, Wake Forest Univ.,Winston-Salem, N.C.: "Transduction by Bacteriophage T1".
A. W. Kozinski, Univ. of Pa., Philadelphia: "Synthesis of Phage DNA in Cell Free Complexes Adsorbed on Fiberglass Filters".
L. A. McNicol & E. B. Goldberg, Tufts Univ. School of Medicine, Boston, Mass.: "Some Applications of the Interaction Between T4 DNA and its Specific Antibody".
P. J. Buckley & R. C. Miller, Univ. of Pa., Philadelphia: "Newly Synthesized Proteins in the T4 DNA Complex".
D. H. Gelfand, R. N. Bryan & M. Hayashi, Univ. of Calif., San Diego, La Jolla, Calif.: "In Vitro Viral DNA Directed Protein Synthesis".
L. M. Gold & M. Schweiger, Rockefeller Univ., New York, N.Y.: "Synthesis of T4 Lysozyme in a DNA-Dependent in vitro System".

FRIDAY, AUGUST 29 – 7:30 P.M.

W. Paranchych, Univ. of Alberta, Edmonton, Canada: "Phage R17 Interaction with F-Piliated *E. Coli*."
H. D. Robertson, Rockefeller Univ., New York, N.Y.: In Vitro Regulation of Phage RNA Synthesis by Coat Protein".
M. Osborn, K. Weber, Harvard Univ., Cambridge, Mass, and H. Lodish, MIT, Cambridge, Mass.: "Amino terminal peptides of RNA phage proteins".
R. Radloff & P. Kaesberg, Univ. of Wisconsin, Madison: "Temperature Sensitive Mutants of Qβ Virus".
I. K. Bendis & L. Shapiro, Albert Einstein College of Medicine, New York: "The Structure of a Caulobacter RNA Bacteriophage".
A. S. Vanderbilt & I. Tessman, Purdue Univ., Lafayatte, Indiana: "The Ubiquity of UGA Suppressors".
B. Molholt & T. Phillip Franklin, Univ. of Kansas, Lawrence: "The Restriction-Sensitive Site in T2 Phage DNA".
H. R. Revel & C. P. Georgopoulos, M.I.T., Cambridge, Mass.: "P1 Restriction of Nonglucosylated T-Even Bacteriophages".
S. M. Michalek & I. Tessman, Purdue Univ., Lafayette, Indiana: "High Photoreactivability of UV-Inactivated S13".
J. Levy, Univ. of Washington, Seattle: "Aspects of the p^{32} Suicide Phenomenon in Phage T4D."
J. M. Boyle & P. A. Swenson, Oak Ridge Natl. Lab., Oak Ridge, Tenn.: "Loss of the Capacity of UV-Irradiated Bacteria to Support Phage Growth".

SATURDAY, AUGUST 30 – 9:00 A.M.

J. J. Dunn & E. K. F. Bautz, Rutgers University, New Brunswick, N.J.: "Studies on the Role of σ in RNA Chain Initiation on DNA".
W. C. Summers & R. B. Siegel, Radiobiology Laboratories, Yale University, New Haven, Conn.: "Transcription of Coliphage T7 in vivo".
R. B. Siegel & W. C. Summers, Radiobiology Laboratories, Yale University, New Haven, Conn.: "Control of Template Specificity of *E. coli* RNA Polymerase by a T7 Phage-Coded Protein".
R. Marsh & G. Mosig, Dept. of Molecular Biology, Vanderbilt Univ., Nashville, Tenn.: "Regulation of Phage T4 Gene Expression".

R. Jayaraman & E. B. Goldberg, Tufts Univ., Boston, Mass.: "A Genetic Assay for mRNAs of Phage T4".
G. Guthrie, Indiana University, Bloomington: "Stringent Control of T4 mRNA Synthesis and the Effect of Chloramphenicol and Puromycin on mRNA Metabolism".
D. Kennell, Washington University School of Medicine, St. Louis, Mo.: "Effect of T4 Infection on Host mRNA-Polysomes".
J. Baker & S. Hattman, Univ. of Rochester, New York: "Exclusion of M13 by T4".
S. Hattman, Univ. of Rochester, New York: "Influence of T4 Superinfection on the Formation of RNA Phage Coat Protein".
D. Duckworth, Univ. of Virginia, Charlottesville: "A New Look at Phage Ghosts".

THE 1969 LACTOSE OPERON MEETING

Attended by 108 participants

The enzyme β-galactosidase has played a central role in the understanding of the control of protein synthesis in bacteria. Originally, most of the important ideas originated in Paris, but now many of the students of Monod and Jacob work in many different laboratories all over the world. To bring together virtually all of the people now doing serious work on the system, we organized a week-long meeting in early September. The pace was leisurely, much like our earlier less crowded symposia, and it was pleasantly obvious that this enzyme and its partners in the lactose operon still are at the center of much of what is interesting in biology today.

MONDAY, SEPTEMBER 1 – 7:30 P.M.

Opening Remarks: Jacques Monod, Institut Pasteur, Paris

THE LAC REPRESSOR

Chairman: Walter Gilbert, Harvard Univ., Cambridge, Mass.

K. Beyreuther & A. Klemm, Inst. f. Genetik, Universitat zu Koln, Germany: "Some Properties of the Lac Repressor".
S. Bourgeois & A. Jobe, The Salk Institute for Biological Studies, and the University of California at San Diego, La Jolla: "Superrepressors of the Lac Operon".
S. Tomino & K. Paigen, Roswell Park Memorial Institute, Buffalo, N.Y.: "β-Galactoside Binding Proteins of *E. coli*".
G. Myers & J. R. Sadler, University of Colorado Medical Center, Denver: "Analysis and Significance of Repressible Constitutivity in the Lac Operon".

TUESDAY, SEPTEMBER 2 – 9:30 A.M.

LAC CONTROLLING ELEMENTS

Chairman: A. D. Pardee, Princeton University

J. H. Miller, Harvard Medical School, Boston, Mass. & T. Platt, Biological Laboratories, Harvard University, Cambridge, Mass.: "A Deletion Mutant which Fuses the Lac i-Gene to the Lac Promoter".
T. Platt & K. Weber, Harvard University, Cambridge, Mass. and J. Miller, Harvard Medical School, Boston, Mass.: "Isolation and Purification of a Repressor Molecule Altered by a C-Terminal Deletion".
J. R. Sadler, Dept. of Biophysics, University of Colorado Medical Center, Denver: "Dominant Constitutives of the Lac System".
W. S. Reznikoff, J. H. Miller, A. E. Silverstone, D. H. Mitchell, E. Signer & J. R. Beckwith, Harvard Medical School, Boston, Mass. and M.I.T., Cambridge, Mass.: "*Trp-lac* fusion strains: Studies of the *lac* Controlling Elements".
L. Eron, J. Beckwith, & F. Jacob, Harvard Medical School, Boston, Mass. & Institut Pasteur, Paris: "Characterization of 0-2 Deletions in the Lac Operon".

TUESDAY, SEPTEMBER 2 – 7:30 P.M.

β-GALACTOSIDASE

Chairman: Francois Gros, Institut de Biologie Physico Chimique, Paris

R. P. Erickson & E. Steers Jr., N.I.A.M.D., N.I.H., Bethesda, Md.: "Protein Chemistry of β-Galactosidase".
G. R. Craven, Laboratory of Molecular Biology & Department of Genetics, University of Wisconsin, Madison: "On the Mechanism of Folding of β-Galactosidase".
M. E. Goldberg, Dept. of Cellular Biochemistry, Institut Pasteur, Paris: "On the structure of wild-type and ω-complemented B-D-galactosidase".
A. Ullmann & J. Monod, Dept. of Molecular Biology, Institut Pasteur, Paris: "On the Kinetics of in vitro Complementation between Peptide Fragments Produced by Deletion Mutants of the z Gene".
M. B. Rotman, Division of Medical Sciences, Brown University, Providence, R.I.: "Partial Loss of Activity of Individual Molecules of Aged β-Galactosidase".
W. Messer & F. Melchers, Max-Planck-Institut fur Molekulare Genetik, Berlin, W. Germany: "The Activation of Mutant β-galactosidase by Specific Antibodies".

WEDNESDAY, SEPTEMBER 3 – 9:30 A.M.

INDUCTION AND TRANSPORT

Chairman: Adam Kepes, Institut Pasteur, Paris.

C. A. Homewood, M. Levine, W. D. Stein, I. West, Dept. of Biological Chemistry, The University, Manchester, England: "Differential Labelling of the Lactose Permease".

C. F. Fox, Dept. of Biochemistry, University of Chicago, Illinois: "Induction of the β-Galactoside Transport System".
W. Goad, Los Alamos Scientific Laboratory & J. Sadler, Univ. of Colorado, Denver: "Concerning Quantitative Interpretation of Induction Phenomena".
L. Adamson, C. Gross & A. Novick, University of Oregon, Eugene: "Induction Lag at Low Rates of Synthesis".
M. K. Rumley, J. B. Armstrong & E. P. Kennedy, Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.: "Direct Measurement of Binding of Thiodigalactoside to M Protein in Cell Free Fractions of *Escherichia Coli*.

WEDNESDAY, SEPTEMBER 3 – 2:00 P.M.

TRANSCRIPTION AND TRANSLATION OF THE LAC MESSAGE

Chairman: Boris Magasanik, M.I.T., Cambridge, Mass.

S. Kumar & W. Szybalski, McArdle Lab, Univ. of Wisconsin, Madison: "Counterclockwise Orientation of Transcription of the Lac Operon and its Repressor Gene i as Determined by RNA-DNA Hybridization".
G. Contesse, M. Crepin & F. Gros, Service de Physiologie Microbienne, Institut de Biologie Physico-Chimique, Paris: "Contribution to the Study of the Transcription Mechanism in the lac Operon of *E. coli*".
D. Dutting, Max-Planck-Institut fur Virusforschung, Tubingen, Germany: "Synthesis of Lac-Messenger RNA in an Extreme Polar Mutant of the Z-Gene".
J. Shapiro, L. Eron, L. MacHattie, K. Ippen, G. Ihler, J. Beckwith, Harvard Medical School, Boston, Mass.: "Purification of Lac Operon DNA".
S. Bourgeois, The Salk Institute for Biological Studies, La Jolla, Calif.: "The Lac Repressor-Operator Interaction".
G. Zubay & D. A. Chambers, Dept. of Biological Sciences, Columbia Univ., N.Y.: "Cell-Free Studies on the Regulation of the Lac Operon".

THURSDAY, SEPTEMBER 4 – 9:30 A.M.

LAC GENETICS

Chairman: Suzanne Bourgeois, Salk Institute, San Diego, California

M. H. Malamy, Dept. of Molecular Biology & Microbiology, Tufts University, Boston, Mass.: "Insertion Mutations in the Lactose Operon".
R. K. Herman, Dept. of Genetics & Cell Biology, Univ. of Minnesota, St. Paul: "Effect of Lac Induction on the Rate of ICR 191 Mutagenesis in the Lac Region".
R. D. Brock, CSIRO, Canberra, Australia: "Mutation of Active versus Inactive Genes".
A. B. Pardee, Biochemical Sciences Program, Princeton Univ., Princeton, N.J.: "β-Galactosidase as an Indicator of Bacterial Gene Function and Replication".
S. D. Barbour, Virus Laboratory, Univ. of California, Berkeley: "Conjugational Transfer of the Lactose Operon in *E. coli*".
L. Norkin & D. Zipser, Columbia University, N.Y. "Recombination within the 2 Gene".
J. H. Campbell, Dept. of Anatomy, School of Medicine, Univ. of Calif., Los Angeles: "A Second Gene for β-Galactosidase in *E. coli*".

THURSDAY, SEPTEMBER 4 – 2:00 P.M.

GLUCOSE EFFECTS

Chairman: Eugene Kennedy, Harvard Medical School, Boston, Mass.

I. Pastan, H. Varmus, B. deCrombrugghe & R. L. Perlman, National Institutes of Health, Bethesda, Md.: "Regulation of Lac Operon Expression by Cyclic Adenosine 3',5'-Monophosphate (Cyclic AMP)".
A. E. Silverstone & B. Magasanik, M.I.T., Cambridge, Mass, & R. Arditti, Harvard Medical School, Boston: "Target Site for Catabolite and Transient Repression".
H. V. Rickenberg, D. Monard & J. Janecek, National Jewish Hospital & Dept. of Microbiology, Univ. of Colorado School of Medicine, Denver: "The Regulation of the Expression of the Lac Operon by Catabolite Repression".
R. Musso & I. Zabin, Dept. of Biological Chemistry, School of Medicine & Molecular Biology Institute, Univ. of California, Los Angeles: "A Physiological Difference Between Thiogalactoside Transacetylase Plus and Minus Strains of *E. coli*".

FRIDAY, SEPTEMBER 5 – 9:30 A.M.

MORE GLUCOSE EFFECTS

Chairman: Jon Beckwith, Harvard Medical School

R. L. Perlman & I. Pastan, National Institutes of Health, Bethesda, Md.: "Regulation of the Lac Operon in Phosphotransferase Mutants of *E. coli*".
B. Tyler & B. Magasanik, Dept. of Biology, M.I.T., Cambridge, Mass.: "Mechanism of Transient Repression".
V. Moses, Laboratory of Chemical Biodynamics, Univ. of California, Berkeley: "Genetic Control of Catabolite Repression".
M. D. Yudkin, Dept. of Biochemistry, Univ. of Oxford, England: "The Use of *Lac* Diploids in Studies of Catabolite Repression".

UNDERGRADUATE RESEARCH PARTICIPATION PROGRAM

Summer 1969

The laboratory was able to take on ten undergraduates for the summer, sponsored by the National Science Foundation's Undergraduate Research Participation Program. The object of this program is to provide increased opportunities for the scholarly development of outstanding undergraduates who may pursue careers in science. Each undergraduate is therefore made a member of one of the research teams working at Cold Spring Harbor during the summer, and is given a particular project. At the same time, he has the opportunity of attending the lectures given in the various courses in microbial genetics, and of joining in the singularly unstratified society that is so characteristic of the place. This program for undergraduates has proved to be very successful in the past, as witness the number of molecular biologists who began their careers as undergraduates at Cold Spring Harbor.

The following students participated in this program during the summer of 1969:

Josephine Bowen, Notre Dame, Calif. *Supervisor:* R. Werner	Intergenic suppression of T4 ligase mutations
Stephen Dennis, Mass. Inst. of Technology *Supervisor:* R. Werner	Replication of T4 mutants defective in gene 32
Charles Gilbert, Amherst College *Supervisor:* R. Gesteland	Nucleotide sequence of phage R17 RNA
John F. King, Harvard College *Supervisor:* J. D. Watson	Isolation of UGA mutants in phage R17
David N. Kuhn, Mass. Inst. of Technology *Supervisor:* J. Cairns	Phosphate precursor pools of DNA
Sondra Lazarowitz, Mass. Inst. of Technology *Supervisor:* R. Webster	Mechanism of killing of *E. coli* K38 upon infection with amber mutants of phage f1
Michael Link, Columbia Univ. *Supervisor:* J. Marmur	Isolation of conditional lethal mutants in phage SPO2
Palma J. Longo, St. Bonaventure U. *Supervisor:* R. Gesteland	*In vitro* synthesis of phage T4 glucosyl transferase
Patricia Stanley, Cornell Univ. *Supervisor:* J. Davies	Difference between cytoplasmic and mitochondrial protein synthesis in yeast
Joan Stephenson, Duke University *Supervisor:* J. Marmur	Prophage site of *B. subtilis* phage SPO2

SUMMER GUEST INVESTIGATORS

Originally conceived as an informal summer haven, where scientists may meet their colleagues, the laboratories at Cold Spring Harbor continue to play host to a group of active workers who spend the summer here. They come to teach the courses, pursue independent projects, write, and collaborate with others in related fields.

In the informal summer atmosphere at Cold Spring Harbor, the scientific activities are enhanced intellectually by the presence of this group.

SUMMER GUEST INVESTIGATORS AND ASSOCIATES

Lionel V. Crawford, Imperial Cancer Research Fund, London, England
J. Davies, University of Wisconsin; P. Stanley, R. Levitt
J. Marmur, Albert Einstein College of Medicine
R. E. Webster, Rockefeller U.
Harvard Group; N. Axelrod, C. Goff, W. A. Haseltine, M. Kaplan, E. G. Minkley, W. Sugden, A. Travers, T. Ward

MUTANTS AFFECTING PROTEIN SYNTHESIS

J. Davies
P. Stanley
R. Levitt

It is well established that yeast cells contain two different protein synthetic systems, cytoplasmic and mitochondrial. The use of antibiotics to differentiate between these two systems has been studied and we sought to extend the useful range of antibiotics, since only inhibitors of 50S ribosomal function have been used so far. We tested a large number of antibiotics which are known to affect protein, RNA, and DNA synthesis in bacteria for their effects on mitochondrial function in *Saccharomyces cerevisae* and found that spectinomycin and rebramycin (inhibitors of 30S function) and nalidixic acid (an inhibitor of DNA synthesis) showed promise of being specific inhibitors of mitochondrial function and we obtained mutants resistant to these drugs. In addition, temperature sensitive mutants of yeast were obtained which survived exposure to lethal concentrations of amino acid analogues at high temperature. These mutants are being analysed further and it is hoped that this selection technique will enrich for mutants in the protein synthetic machinery.

Pat Stanley, in addition to collaborating in much of the above work, isolated a number of mutants of yeast which are osmotically sensitive. These mutants only grow in the presence of sorbitol and we are interested in obtaining such mutants which lyse completely on osmotic shock. Some half dozen good candidates were obtained and their lysis behavior in the presence of various detergents was examined – we plan to use these mutants in studying the synthesis of macromolecules in yeast, under various sets of conditions.

Rhonda Levitt continued her work on the isolation of episomes carrying the genes which direct the synthesis of ribisomal proteins in *E. coli.* Several different Hfr's were crossed into suitably marked rec⁻ females and the "recombinants" scored for the presence of episomes. We hope to obtain a variety of episomes of various lengths which will enable us to characterize ribosomal DNA and which will be very useful in studying the synthesis of ribosomes in *E. coli*.

BACILLUS SUBTILIS PHAGES

Julius Marmur
Michael Link
Joan Stephenson

Continuing the work from last summer, additional experiments were carried out with the temperate *B. subtilis* phage, SP02. One project was concerned with its site of insertion on the chromosome and the other with the construction of a vegetative genetic map. The location of the SPO2 prophage was determined by DNA mediated transformation and PBS1-mediated, generalized transduction. The donor strain was resistant to several antibiotics and lysogenized with wild type SPO2, while the recipient strain was sensitive to the antibiotics and lysogenized with a defective mutant of SPO2. Transductants and transformants were then examined for the presence of wild type prophages. In this manner it was shown that SPO2 is inserted close to the erythromycin resistance marker, and most likely to the right of it, but to the left of the spectinomycin resistance marker. Attempts to obtain specialized transducing lysates were unsuccessful.

In order to obtain a genetic map of SPO2, nitrosoguanidine treated phage were examined for temperature sensitive (*ts*) and suppressor sensitive (*sus*) mutants. These mutants were then examined for this ability to complement one another under restrictive conditions. Approximately 25 *ts* and 10 *sus* mutants were examined and found to fall, thus far, into 11 complementation groups.

STUDIES ON BACTERIOPHAGE f1

Robert E. Webster
Sondra Lazarowitz
Chase Churchill

Normal infection of *E. coli* with the wild-type f_1 does not lyse or kill the host bacterium. However, infection of a non-permissive host with amber mutants of the phage results in a stoppage of bacterial growth. Careful examination of this phenomenon in f_1 showed that infection by phage carrying amber mutants in genes 1, 3, 4, 5, 6, 7 all killed the cells to a similar degree.

In an attempt to further examine this phenomenon, the growth of non-permissive *E. coli* were partially synchronized using a sucrose gradient technique and infected at various times during their growth cycle with both wild-type and amber mutant phage. It was found that infection of the bacteria with various amber mutants immediately after division resulted in no further division of the bacteria. If the bacteria were infected midway between the time of division, one division would occur at the normal time before further growth of the bacteria ceased. In contrast, infection of the bacteria at both times with wild-type f_1 resulted in the lengthening of the division cycle from 40 to 55 minutes. Studies on the rates of DNA, RNA and protein synthesis in such infected synchronized cells were in progress at the end of the summer.

In addition to these studies, an attempt was made to isolate mutants of the phage requiring both an amber (UAG) and an UGA suppressor for growth. Amber mutant phage growing in a strain carrying both suppressors were mutagenized with either 2-aminopurine or nitrosoguanidine. Preliminary screening only yielded double mutants which had unsatisfactory reversion rates.

LABORATORY OF
J. D. Watson
N. Axelrod
C. Goff
W. A. Haseltine
M. Kaplan
E. G. Minkley
W. Sugden
A. Travers
T. Ward

Several students from J. D. Watson's laboratory at Harvard worked on a variety of projects at Cold Spring Harbor during the summer;

Chris Goff spent the summer working with Joe Sambrook, attempting to purify DNA-dependent RNA polymerase from rat liver and Hela cells. The goal was to develop a simple procedure which would permit the large-scale preparations necessary in order to do protein chemistry on the mammalian RNA polymerase. By modifying techniques already worked out for the bacterial enzyme, they succeeded in freeing the polymerase from DNA (the major problem encountered) and achieved a several-hundred fold purification.

Martin Kaplan made studies on the RNA polymerase sigma factor using streptolydigin, an antibiotic known to inhibit transcription. Streptolydigin was shown in this project to promote the irreversible binding of RNA polymerase to DNA; it was then used in experiments toward determining whether there were qualitatively "correct" and "incorrect" sites on template T_4 DNA. These experiments used streptolydigin to lock core or complete enzyme molecules at their initiation sites. A streptolydigin-resistant complete enzyme was then unable to transcribe the DNA pre-incubated with the complete sensitive enzyme but was unaffected by pre-incubation with the core enzyme. These results support the model of DNA sites seen only by a specific core enzyme + sigma complex.

Edwin Minkley worked on the genetic construction of a strain of *E. coli* from which a transducing phage for DNA-dependent RNA polymerase could be isolated. The technique used employs the lysogenization of a ϕ80met transducing phage at the $met_{B,F}$ locus and the selection from a UV-induced lysate of this strain of a phage carrying the fairly closely linked locus for rifamycin resistance. Since at least four types of mutations in RNA polymerase (resistance to rifamycin, streptovaricin, and streptolydigin, and a temperature sensitive mutant) map near argH, it seemed reasonable to hope for a phage carrying the genetic information for all of the subunits of RNA polymerase. However, the report of a temperature sensitive mutant mapping near streptomycin resistance makes this unlikely.

Nancy Axelrod and *John King* looked for UGA mutants in the RNA phage R17, hoping to find mutants in 1) the coat protein cistron, thus providing an experimental system in which to determine the amino acid(s) inserted by bacterial UGA suppressor strains, and/or 2) the hypothetical fourth cistron of the phage. From a phage stock mutagenized with nitrosoguanidine, about 20,000 phage were tested for their ability to grow on the UGA suppressor strain CAJ70 but not on the Su^- strain *E. coli* C. Six such mutants were found, and characterization of them is in progress.

William Haseltine, working with John Cairns on the Kornberg enzyme (DNA polymerase) demonstrated that the deoxynucleotide triphosphate incorporating activity extracted from cells by Brij lysis has the same sedimentation velocity as the Kornberg enzyme, suggesting that this extraction procedure is an effective method for isolating the enzyme. He also worked with Julian Gross on the mapping of a mutation which lacks this enzymatic activity.

NATURE STUDY COURSES

Children of Ages 6 to 16

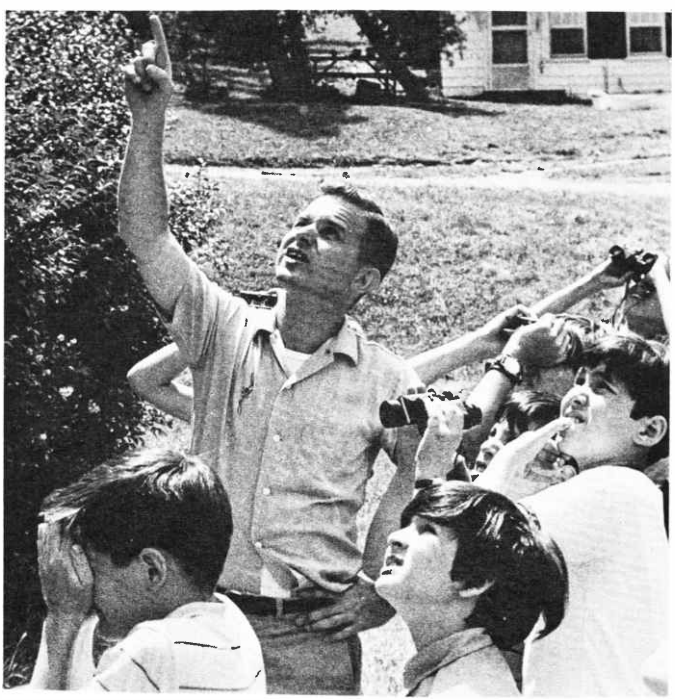
During the summer of 1969, 22 courses in Nature Study were conducted in two monthly sessions. The enrollment this year was 463 students. The course offerings included:

General Nature Study (ages 6, 7)
General Ecology (ages 8, 9)
Plant-Insect Relationships (ages 8, 9)
Elementary Geology (ages 8, 9)
Bird Study (ages 10, 11)
Fresh-Water Life (ages 10, 11)
Seashore Life (ages 10, 11)
Insect Study (ages 10, 11)
Animals with Backbones (ages 10, 11)
Geology (ages 10, 11)
Ichthyology-Herpetology (ages 12-16)
Plant Ecology (ages 12-16)
Advanced Geology (ages 12-16)
Animal Ecology (ages 12-16)
Oceanography (ages 12-16)

The Laboratory gratefully acknowledges the tenth year contribution of the Huntington Federal Savings and Loan Association. This provided nature study scholarships for 12 students of the Huntington elementary schools.

INSTRUCTORS:

Mr. Otto A. Heck, M. S., Assistant Professor of Biology at Trenton State College, Trenton, N. J.
Mrs. Barbara Church, M.Ed., Science Substitute Teacher at Central High School, Dist. #3.
Miss Virginia Jones, M.S., Graduate Student in Nature and Conservation Education, Michigan State University.
Mr. Alex Pepe, M.A., Science Teacher, K-6, East Side School, Cold Spring Harbor, N.Y.
Mr. Larry Roel, Junior at Princeton University and. former student and assistant of the Children's Nature Study Program, Cold Spring Harbor Laboratory.
Mr. Ronald Roel, Freshman at Princeton University, and former student and assistant of the Children's Nature Study Program, Cold Spring Harbor Laboratory.
Mr. Richard L. Rosenman, M.A., Chemistry and Biology Teacher, Cold Spring Harbor High School.



NATURE STUDY WORKSHOP FOR TEACHERS

The 14th annual Workshop in Nature Study was offered from June 27th to July 25th, 1969. This program is designed to familiarize elementary and secondary school teachers with the natural environment of the Long Island area, including the animals and plants living there; and those aspects of the environment which affect these organisms. The course consisted of field trips to ponds, stream, seashore, woodlands, field and other natural habitats, for purposes of collecting and first-hand study, with indoor laboratory work-time divided between lectures and practical work. The experiences of the course are designed to help teachers in their classroom science activities.

Fourteen teachers attended the Workshop. Upon satisfactory completion of the requirements of the course, teachers were entitled to four points of in-service credit authorized by the New York State Education Department, Division of Higher Education. The instructor for the summer of 1969 was Mr. Otto A. Heck.

LABORATORY STAFF 1969

COLD SPRING HARBOR LABORATORY

Margaret L. M. Anderson
Charles Anderson
John Cairns
Elspeth Cairns
Annamarie Coschignano
Donald Caldarelli
Anne Carhart
Mary D. Conant
Patricia Crouch
Robert Crouch
Gloria Danielson
Hajo Delius
Paula Delucia
Carmen Dewan
Anne Eastlake
Donald Eckels
Barbara Eggers
Leonora Frisch
Ray Gesteland
Maryalice Gladding
Zebedee Harris
Daniel Hayes
June Henry
Harriet Hershey
Arlene Hildebrandt
Bernhard Hirt
Betty Jacques
Walter Jürgensen
Robert Klipera
Kathryn W. Knight
Anthony Little, Jr.
Joseph McDonald
William McDonald
Agnes Meyer
Jaima Mickelbank
Blanche Mrazek
Carel Mulder
Dorothy Nilsson
Elaine Oates
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FINANCIAL REPORT

For the period May 1, 1967—April 30, 1968

BALANCE SHEET

April 30, 1969

ASSETS

Current Funds:			
General			
Cash		$ 16,734.83	
Investments in Government obligations (at cost which approximates market)		70,231.48	
Accounts Receivable (net of reserve)		55,362.51	
Inventory of books (at cost less reserve for obsolescence)		63,674.87	
Prepaid expenses		4,589.76	
Total General			$ 210,593.45
Restricted:			
Cash and Grants Receivable			1,403,514.26
Total Current Funds			$1,614,107.71
Plant Funds			
Unexpended			
Cash	$ 7,281.48		
Invested in Government obligations (at cost which approximates market)	39,758.24	$ 47,039.72	
Investment in Plant		567,668.71	
			$ 614,708.43
			$2,228,816.14

LIABILITIES AND FUND BALANCES

Current Funds:			
General:			
Accounts Payable and Accrued Expenses		$ 42,641.32	
Deferred income		5,737.50	
Fund Balance			
Appropriated for scientific staff salaries	$ 17,403.61		
Unappropriated	144,811.02	162,214.63	
Total General			$ 210,593.45
Restricted:			
Fund Balance			1,403,514.26
Total Current Funds			$1,614,107.71
Plant Funds:			
Unexpended		$ 47,039.72	
Invested in Plant		567,668.71	
			614,708.43
			$2,228,816.14

STATEMENT OF CURRENT INCOME

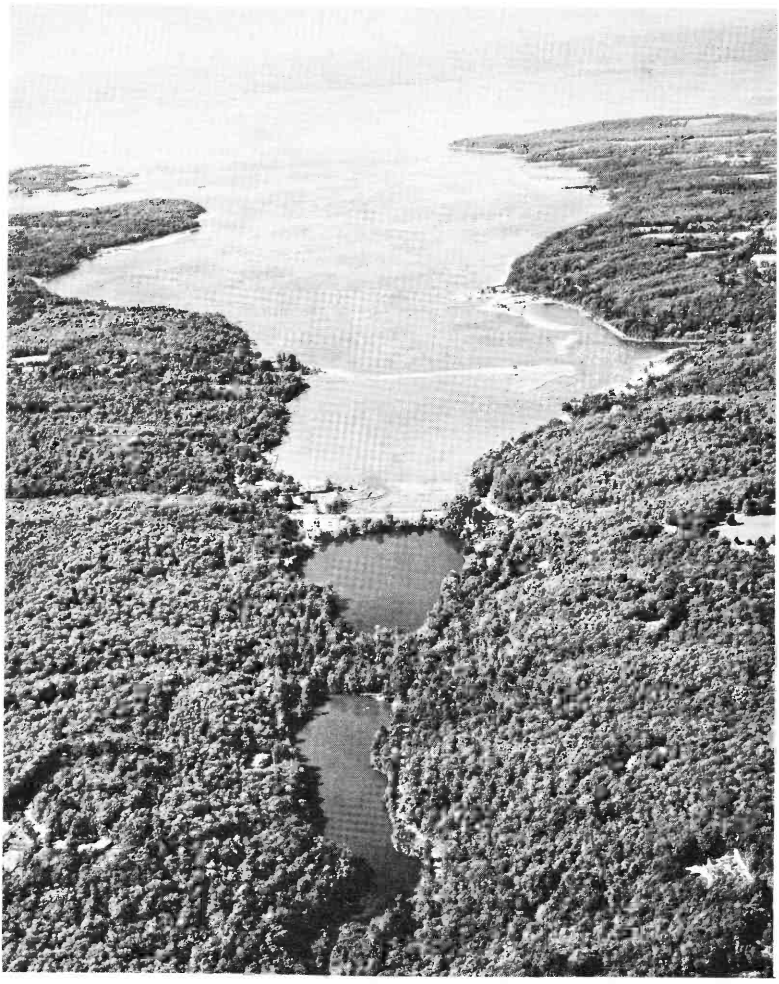
Year ended April 30, 1969

Income:		
Expendable gifts and grants		$191,206.61
Indirect cost allowance on grants		26,630.59
Contributions		123,927.00
Symposium registration fees		14,759.50
Summer programs		33,765.00
Laboratory rental		2,500.00
Investment income		4,139.78
Other sources		1,804.09
Auxiliary enterprises:		
Symposium book sales	$143,376.25	
Dining hall	47,823.51	
Rooms and apartments	43,302.66	234,502.42
Total income		$633,234.99
Expenditures:		
Research and Education Programs		$183,523.62
Annual symposium		30,169.29
Library		11,182.84
Operation and maintenance of physical plant		158,271.16
General and administrative		104,310.98
Scholarships		250.00
Auxiliary enterprises:		
Symposium book	$ 59,898.28	
Dining hall	40,845.05	
Rooms and apartments	13,370.71	114,114.04
Total expenditures		$601,821.93
Excess of income over expenditures		$ 31,413.06

GRANTS AND CONTRACTS

May 1, 1968 to April 30, 1969

Grantor	*Investigator or Program*	*Total Award*	*Grant Number*	*Duration of Grant*
Research Grants				
National Science Foundation	Dr. Cairns	$ 43,800.	GB-7671	5/1/68-8/31/69
National Institute of Health	Dr. Watson Tumor Virus	519,713.	1-RO2-CA11 43201	5/1/69-4/30/70
National Institute of Health	Dr. Gesteland Electron Microscope	82,000.	5-RO1-GM 16093-01	1/1/69-12/31/69
Salary Support Grants				
American Cancer Society	Dr. Cairns	581,352.	ACS PRP-37	1/1/69-6/30/89
National Institute of Health	Dr. Gesteland	15,000.	1/04-9M-18029-01	1/1/69-12/31/69
American Heart Association	Dr. Werner	75,000.	69-113	7/1/69-6/30/74
Training Grants				
National Institute of Health	Summer Courses	47,865.	5 TO1-GM 890-13	1/1/69-12/31/69
National Institute of Health	Summer Courses Supl.	45,836.	5 TO1-GM 890-13S1	1/1/69-12/31/69
National Science Foundation	Undergraduate Research	12,960.	GM 5976	1/1/69-10/31/69
Symposium Grants				
National Science Foundation		5,000.	6B 8673	4/1/69-6/30/69
National Institute of Health		17,000.	2RB-CM 02809-14	4/1/69-3/31/70
Atomic Energy Commission		7,000.	AT(49-7) 3032	5/1/69-7/30/69
Continuing Grants and Contracts				
Research Grants		*Remaining in Award*		
National Science Foundation	Dr. Gesteland	$ 26,182.	GB-7209X	12/1/67-11/30/69
National Science Foundation	Dr. Werner	30,101.	GB-7222	1/1/68-12/31/69



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