

Genes, Cells, and Behavior

A View of Biology Fifty Years Later

by Norman H. Horowitz
Chairman of the Division of Biology

A symposium on "Genes, Cells, and Behavior: A View of Biology Fifty Years Later," marking the 50th anniversary of the founding of the Division of Biology, was held on the Caltech campus on November 1-3, 1978. Eighteen papers, covering topics ranging from molecular genetics of bacteriophage to human behavior, were presented in five sessions. The speakers were all alumni or former members of the Division. Over 700 alumni, students, and friends of the Division attended the symposium, which was moved to Beckman Auditorium after overflowing Ramo.

Social events of the celebration included a reception held in the Athenaeum on November 1 and a Reunion Dinner, also in the Athenaeum, on November 2. The dinner provided the setting for a sentimental journey into the past, since so many alumni and retired faculty members were there.

The original biology faculty consisted of four geneticists, including Thomas Hunt Morgan, its almost legendary chairman. Of the four, only Sterling Emerson, now Professor Emeritus, survives. He and his wife, Mary, were present, as were Phoebe Sturtevant and Florence Anderson, the widows of Alfred Sturtevant and Ernest Anderson, the other members of the original faculty.

Other old timers included Professor Emeritus Henry

Borsook, who came to Pasadena in 1929 as Caltech's first professor of biochemistry, and his wife Lisl; George Beadle, who arrived as a National Research Council Fellow in 1931 and later succeeded Morgan as chairman of the Division; and a number of early alumni, including Donald Poulson (BS '33, PhD '36), Herman Schott (PhD '33), and Charles Schneider (BS '34). Two Chinese alumni, Chia-Chen Tan (PhD '36) and San-Chiun Shen (PhD '51), came from Shanghai to attend the celebration.

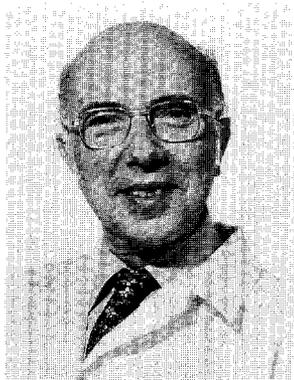
The main event of the evening was an illustrated talk by James Bonner entitled "Caltech and the Origins of Modern Biologists." Since Professor Bonner's Caltech career spans 49 of the Biology Division's 50 years, and since he has nearly total recall, he was in an especially favorable position to recount the history of the Division, which he did with his usual verve. He made the point that Caltech has been not only a source of excellent science and scientists, but also a fountain of inspiration to which its graduates often return — a compliment that we hope we deserve and will continue to deserve as we enter our second half century.

In the following article, we summarize the talks given in the first two sessions of the symposium. Summaries of the last three sessions will appear in the next issue of *E&S*.

Session I — Biology of Cancer

Cell Transformation and Cancer

Dr. Renato Dulbecco
Nobel Laureate
The Salk Institute
La Jolla, California



Renato Dulbecco's work on animal viruses began in Max Delbruck's laboratory at Caltech as an outgrowth of the bacteriophage research that was the major occupation there. He studied polio virus at first, but later he switched to tumor viruses. The latter, unlike polio virus, do not kill cells, but transform them into cancer cells. In this respect they resemble the so-called lysogenic phages, whose DNA is integrated into the DNA of the host bacterium and is thereafter transmitted from one generation to the next as part of the bacterial chromosome.

This analogy is an apt one, because it was subsequently shown that the genetic material of tumor viruses is, in fact,

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integrated into cellular DNA. The integrated viral DNA is transcribed into RNA, just as the cell's own genes are, and viral proteins are made. Transformation is thus seen to be a consequence of the expression of viral genes.

Mutations affecting transformation have been obtained, the most striking of which is in Rous sarcoma virus (a tumor virus of chickens). This mutant is temperature-sensitive: It induces cellular transformations in the usual way at low temperatures (32° C), but at high temperatures (40° C) the transformed cells revert to normal. The gene that gives rise to this mutant, and that is evidently responsible for maintenance of the transformed state, is called *sarc*. Recent work has shown that the product of the *sarc* gene is a protein kinase; that is, it is an enzyme that phosphorylates proteins. This finding may explain the complex action of the virus, since such an enzyme can alter many cell functions by modifying various proteins in the cell. There is some evidence that other tumor viruses may also produce kinases.

What is the relation between spontaneous or chemically induced cancers and viral transformation? Because the former are, as is now clear, caused by mutations in the genetic material of the cell (whereas transformation is induced by foreign genes imported into the cell), they seem at first sight to be different. The difference tends to disappear when the origin of the viral gene is considered, since there is strong evidence that the viral genes are of cellular origin.

Thus the base sequence of the *sarc* gene is very similar to that of a cellular gene, and the same is probably true of the transforming genes of other tumor viruses. These cellular genes may thus be potential cancer genes, normally controlled by repressor genes similar to those known in bacteria. Cancer may result from mutations in the repressor genes inactivating them and releasing the cancer genes. Since two repressor genes would have to be inactivated in a diploid cell, this hypothesis has certain statistical consequences, and these seem to be borne out in the work reported by Alfred G. Knudson (see page 13). Experimental evidence also supports this hypothesis. Thus, all cancer — viral, spontaneous, or chemically induced — can be attributed to the activation of cancer genes which determine enzymes with multiple effects.

Dulbecco concluded: "And so on this 50th anniversary we can contemplate with some satisfaction the progress made in the field of cell transformation and cancer. As in many other fields of biology, the experiments were initiated in these laboratories and then spread to many other places, forming a community of effort that looks at this Division as its alma mater."

RNA Viruses and Cancer

Professor Howard Temin
Nobel Laureate
McArdle Laboratory
for Cancer Research
University of Wisconsin



Retroviruses are those viruses whose genetic material is RNA, not DNA. In the course of their life cycle, retroviruses make a DNA copy of their RNA, and this copy becomes inserted into the DNA of the host cell. Rous sarcoma virus is the prototype of retroviruses. At the beginning of his talk, Temin predicted that there would be much convergence among the speakers at this session concerning the nature of cancer; but he would not say whether this means that we are now approaching the reality of the cancer problem, or whether it merely reflects the common Caltech backgrounds of the speakers.

Rous sarcoma virus is a strongly transforming or rapidly oncogenic (cancer-producing) virus, in contrast to most retroviruses, which take months to produce tumors. Rous virus differs from the weak viruses in having the *sarc* gene (see Dulbecco). It has been shown by DNA-RNA hybridization methods that Rous virus and other strongly transforming viruses arose from weakly transforming viruses, which in turn arose from the cellular DNA of the host. The *sarc* gene is apparently a cellular gene that was incorporated into the weak virus at a later time, making it strongly transforming. Weak viruses become oncogenic by acquiring genes like *sarc* by mutation, which explains why they are so slow to produce cancer. Considerable evidence indicates that weak viruses evolve from the genome of the host species, and various intermediate stages in this process of escape of the virus from the host DNA have been found. Weak viruses persist in nature, but strong ones do not because they kill their host. They are preserved in laboratories, however.

Retroviruses are exceptional in the ease with which they enter and leave the cellular DNA — going in as DNA, coming out as RNA. Much has been learned recently about the integration process. Studies with the spleen necrosis virus, a non-transforming retrovirus otherwise very similar to Rous virus, have shown that the viral DNA is inserted into many different sites of the host DNA. After a period of time during which the virus causes an acute infection, viral DNA disappears from all sites except one, where it

remains during a long period of chronic infection. It thus appears that the behavior of the virus is influenced by the site of integration. By contrast, the integration process involves one specific site in the viral DNA sequence. These various mechanisms can throw light on the mode of evolution of tumor viruses and may also be relevant for the process of differentiation.

Environmental Chemicals Causing Cancer and Mutations

Professor Bruce Ames
Department of Biochemistry
University of California
Berkeley



The problem of exposure to toxic chemicals has taken on new proportions since the 1950's, when large-scale production of modern chemicals began. There are 50,000 chemicals in commerce at the present time, and 1,000 new ones are added every year. Some of these, such as vinyl chloride and ethylene dichloride, are produced at the rate of billions of pounds per year. Although structural considerations alone would suggest that these substances are carcinogens, vinyl chloride was being made at a rate of 4 billion pounds per year before it was tested and found to be carcinogenic. Ethylene dichloride was found to be a potent carcinogen after 100 billion pounds had been manufactured in the United States. It is not proposed that all such chemicals be banned, but it is suggested that they be recognized as dangerous and treated with respect.

The problem in recognizing environmental carcinogens is that their effects in man are delayed for 20 to 25 years. This is the case with cigarette smoking, with radium, with various industrial chemicals, and with the radiation at Hiroshima. The majority of cancers induced by modern chemicals will not appear until the 1980's. It is not known whether low doses of chemicals are safe; the same is true for smoking. Quite possibly some chemicals will give linear response curves, and others will show a threshold.

Animal tests are too expensive and slow to be applied to every new chemical. They are also not very sensitive. A new test devised by Ames is based on the premise that carcinogens are basically mutagens. The test measures the rate of mutation to histidine-independence of a histidine-requiring mutant of the bacterium *Salmonella typhimurium*. The test strain has been modified genetically

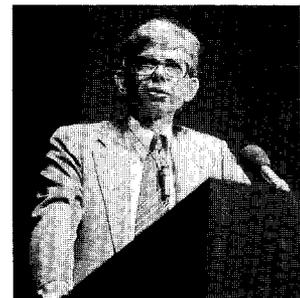
to make it exquisitely sensitive to mutagens. Furthermore, since some chemicals become carcinogenic only after they have been acted on by enzymes in the liver (when they also become mutagens), the Salmonella test incorporates liver extract in the medium. Of 175 known carcinogens assayed, the test detects 90 percent as mutagens. Of the 10 percent missed, some are probably beyond the reach of a bacterial assay because their carcinogenicity is related to metabolic functions not found in bacteria. Griseofulvin, for example, is a carcinogen that deranges mitosis in animal cells by reacting with tubulin; bacteria do not have mitosis. The Salmonella test also fails to pick up carcinogenic glycosides, since they are processed by bacterial glycosidases in the gut before they become carcinogenic. The test is being modified to detect such compounds. The most important class of carcinogens not recognized by the Salmonella test is that of chlorinated hydrocarbons. This may have to do with the short half-life of free radicals through which these compounds act.

Most non-carcinogens are negative in the Salmonella test, but a few are positive. These are probably carcinogens that were missed by animal tests. An example is an antibacterial agent, a nitrofurantoin, that was widely used in Japan as a preservative in *tofu*. This compound had given negative results in animal tests, but it was found to be mutagenic in several systems. More careful animal tests were positive, and the compound is now banned. Other examples are some hair dyes and Tris, a flame-retardant.

Carcinogens are also found in nature. Dietary fat is a known factor in colon and breast cancer. Charred protein, as in charcoal-broiled steak, contains mutagens. Quercetin, a flavonol widely occurring in plants as the glycoside, is also a mutagen. Thus, one should not assume that chemical industry is the only source of dangerous chemicals. To decide on priorities, we first need estimates of the potency of the various carcinogens we come in contact with.

Heredity and Cancer in Man

Dr. Alfred G. Knudson, Jr.
Director
Institute for Cancer Research
Philadelphia



According to some estimates, 80 percent of cancer is of environmental origin and is preventable. The known en-

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environmental agents are irradiation, chemicals, and viruses. Since these agents are also known to be mutagens or to alter the host genes in some way, they provide strong support for the idea that induced cancer arises by mutation. Besides environmentally caused cancer, there is inherited cancer. It is possible, in fact, that most human cancer results from the action of environmental agents on genetically susceptible hosts. Finally, there are cancers in which neither environmental nor hereditary factors seem to play a role. These spontaneous cancers apparently result from spontaneous mutations in the host tissues. Their frequency of occurrence forms a lower limit below which the incidence of cancer cannot fall.

The most important variable of all in cancer is age. The incidence of most cancers rises sharply with age. Cancer of

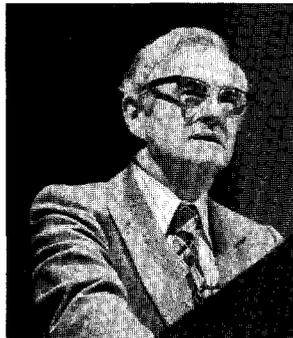
the colon and stomach increases as a power function of age, suggesting that a certain number of mutations must occur in a cell before it is transformed into a cancer cell. This hypothesis explains much that is known about the epidemiology of cancer, and although it cannot be tested directly, indirect tests support the hypothesis. This proposal leads also to the intriguing consideration that, besides mutation, the number of defective genes in a cell can be increased by the chromosomal process called mitotic recombination. The point of major interest is that this mechanism may explain the action of certain chemicals that are not carcinogens by themselves but that are known to enhance the effectiveness of carcinogens. The possibility that these "promoters" act by increasing the frequency of mitotic recombination has some experimental support.

Session II — Phage

ϕ X174

**A Research Odyssey:
From Plaque
to Particle and
Mutant to Molecule**

Dr. Robert L. Sinsheimer
Chancellor
University of California
Santa Cruz



Φ X174 is one of the smallest bacteriophages. Largely owing to that circumstance, more is known about it than about any other DNA-containing virus. The Φ X174 particle has a diameter of 25 nanometers, and it consists of a protein coat enclosing a single-stranded ring of DNA. The coat contains four kinds of proteins, including a small basic protein that is internal and is believed to provide a counter-ion to the negatively charged DNA. After the viral DNA enters a bacterial cell, it is converted into a double-stranded ring. Following this, viral proteins are made from the information contained in the viral DNA; the double-stranded ring is replicated a number of times; single-stranded rings are made by a separate process and packaged into viral coats; and the host cell is lysed, allowing the progeny virus to escape.

The DNA of the virus has recently been completely se-

quenced — i.e., the order of its 5386 nucleotides has been determined — thanks principally to work in Sanger's laboratory in England. Ten genes have been identified. Four of them encode the coat proteins, four are involved in DNA replication, one functions in lysis of the host cell, and one produces a protein whose role is unknown. Examination of the sequence of the viral DNA shows that the genes are not composed of simple segments of DNA, one following on the other around the circle. Rather, there is considerable overlap among the different genes. Gene B, for instance, is completely contained within gene A: The same sequence of nucleotides read in one phase gives protein B; read in another phase it gives a portion of protein A. One segment of the viral DNA is read in three phases — the maximum possible with a triplet code — allowing three different proteins to be encoded in the same segment of DNA. This compression of genetic information is clearly advantageous for a small virus, but it is difficult to see how the system evolved. Further study of the sequence with the feasibility of gene overlap in mind reveals a number of opportunities for the production of proteins that are not known to be made, but that exist as possibilities in the sequence.

Knowledge of the sequence also makes it possible to introduce mutations into any gene or region of the DNA at will, in order to study their effects. Exploitation of this possibility can be expected to enhance our understanding of this virus in the future.

**The Rise and Decline
of T4 Phage Biology
at Caltech:
A Latter-Day View**

Professor William B. Wood
Chairman, Department of
Molecular, Cellular, and
Developmental Biology
University of Colorado



The fundamental genetic problem of biology — how genes replicate and direct the synthesis of proteins — was solved in principle by the early 1960's, but the epigenetic problem remains; that is, how the individual protein products of gene action are organized to form a living organism. To state the question in another way: How does one go from the linear arrangement of information in DNA to a three-dimensional structure? This talk summarized experiments carried out with bacteriophage T4 (most of them in Wood's laboratory) to answer this question.

Compared to simple viruses like Φ X174, T4 is a baroque contraption with a complicated contractile tail that allows it to inject DNA into its host. Experiments with mutants defective in the ability to produce various parts of the phage have shown that T4 is put together from three independent sub-assemblies: head, tail, and tail fibers. Each sub-assembly is made independently of the others. Mutants that are unable to produce one sub-assembly accumulate the other two. The sub-assemblies are intermediates in phage assembly. If extracts of different mutants containing different sub-assemblies are mixed, the parts come together to form complete phage.

Different sets of genes are involved in the production of each sub-assembly. Altogether, about 50 genes participate in phage assembly, or more than the total number of proteins in the phage. Some proteins must have catalytic functions in the assembly process. The pathway of assembly has been worked out, and a strict order of steps is followed. It is clear that T4 construction requires more than spontaneous self-assembly of protein molecules as occurs, for example, in the case of the much simpler tobacco mosaic virus. The temporal order of events implies that the phage exerts kinetic control over the many possible reactions in such a way as to insure the correct outcome. These results show that there are still many intriguing problems to be worked out in bacteria and their viruses.

**The Last of the
T Phages**

Dr. F. William Studier
Department of Biology
Brookhaven
National Laboratory



T7 was the last of the T bacteriophages to be found, and it is the last to receive detailed study. The feature that has attracted Studier's attention is the high degree of variability among stocks of T7 that had originated from a common ancestor isolated in the 1940's. For example, in a study of 17 stocks of the phage being used in different laboratories around the world, Studier observed that only 8 were identical, and 9 were different from these and from each other. The criterion of identity used was the pattern of cleavage of the viral DNA by certain bacterial nucleases (so-called restriction enzymes). This is a very sensitive procedure, easily able to distinguish strains that differ in only a few percent of their DNA basis.

In an extension of this study, Studier collected a number of phages related to T7 and examined them in various ways. Morphologically they are all alike, and they have the same genetic organization — i.e., the order of genes controlling various functions is the same along all the viral chromosomes. Furthermore, the homologous genes are read at the same time following entrance of the phage particle into the host cell, and proteins with the same functions and of similar sizes are produced. These similarities extend to phage T3, a remote but recognizable relative of T7. When DNA's of the various strains were compared, however, they were quite diverse. This suggests that considerable genetic change had gone on beneath the surface, while the basic morphology, genetic structure, and functional organization were conserved. Studier referred to these changes as "neutral" evolution. The extent of divergence possible under this kind of evolution was revealed in a comparison of homologous enzymes of T3 and T7. Enzymes that are identical in function and position on the genetic map have diverged to the point where they perform their functions by different mechanisms.

A preliminary laboratory experiment indicated that T7 accumulates nonlethal mutations at a rate sufficient to account for this evolution. These studies may well throw light on the molecular mechanisms of evolution in general. □