GENES, CELLS, AND BEHAVIOR

Edited by

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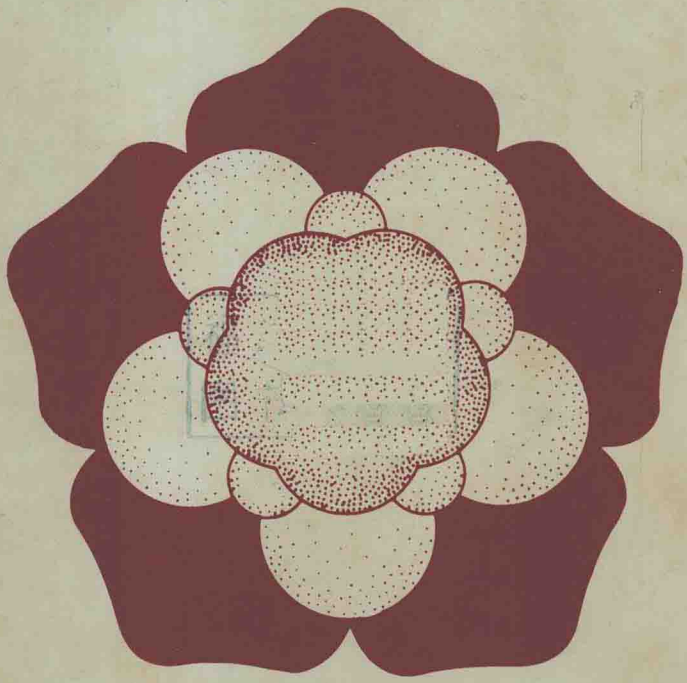

GENES, CELLS, AND BEHAVIOR:

A View of Biology Fifty Years Later

50th Anniversary Symposium

Division of Biology
California Institute of Technology

Edited by **Norman H. Horowitz** and **Edward Hutchings, Jr.**

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GENES, CELLS, AND BEHAVIOR

A Series of Books in Biology

Cedric I. Davern, Editor

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Preface

The papers in this book were originally presented in a symposium of the same title, held in November, 1978, on the campus of the California Institute of Technology. The occasion was the celebration of the 50th Anniversary of the founding of the Division of Biology at Caltech. The Division was formed in 1928 by Thomas Hunt Morgan, its first chairman. The original faculty consisted entirely of geneticists: A. H. Sturtevant, E. G. Anderson, Sterling Emerson, and Morgan. Calvin Bridges was a research associate, and Theodosius Dobzhansky was a research fellow. With such a beginning, it is not surprising that genetics in its various manifestations--classical, microbial, biochemical, and now molecular--has remained central to the interests of the Division, although cell biology and, especially, neuroscience have also become important disciplines at Caltech in recent years.

For the Jubilee celebration, we desired to have a reunion and also a scientific event worthy of the occasion. To these ends, it was decided to organize a symposium on current problems in biology in which all the speakers would be alumni or former members of the Division. The result is presented in this book. The reader will find here a collection of overviews of their current research by a group of notable biologists, written for a general biological audience. My only regret is that it was impossible within the limits of a $2\frac{1}{2}$-day symposium to include in the program a larger number of our distinguished alumni, many of whom were in the audience.

The order of papers as presented here follows the sequence of the symposium. One paper, that of David Hogness, was unavailable. The chairmen's introductions were somewhat modified for publication, but Delbrück's account of the history of phage research at Caltech is essentially as it was presented. Eventually, as the river of science flows on, this book will go out of date, but it will remain an historical document; perhaps it will be quoted at the Centennial celebration in 2028 to show what some of us were thinking and doing in the 1970's. I hope so.

I wish to express my thanks to the authors and chairmen for their cooperation in making this publication possible. Thanks are due also to Connie Katz and Bernita Larsh for typing the manuscript, to Geraldine Cranmer and Elizabeth Koster for proofreading it, and to David Asai, Larry Johnson, and Donna Livant for preparing the Indexes.

N. H. Horowitz, Chairman
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GENES, CELLS, AND BEHAVIOR

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SESSION I: BIOLOGY OF CANCER

Introduction

R. D. Owen

Professor of Biology, Caltech

In celebrating 50 years of biology at Caltech, it may seem capricious to begin with selections from contributions of Divisional people to "cancer research." For the most part, the Division has prided itself through its history on its devotion to basic experimental biology, not to the solution of practical human problems, however pressing. The general impression has been fostered that meeting the intellectual challenges of understanding life and its processes at primary levels will lead to eventual control of processes at all levels, including those from abnormal cellular behavior to organismal disease. The implication has been that "applied" developments are to be left to others elsewhere, not to distract us from the "basic" research we have tried to do well and have thought it best for us to do.

The four papers in this section represent a step toward the "applied" against this background, because they deal with a compelling human problem, cancer. In different ways, they illustrate the power of the principles upon which the Biology Division at Caltech has been built. When Renato Dulbecco came to Caltech as a Senior Research Fellow in 1949, to join Delbrück's group after an interval with Luria, high excitement characterized the use of bacteriophage as a tool for understanding primary attributes of life. There was at the time, I am sure, in the back of many minds an idea that what was being found out about viral infections of E. coli would relate, in time, to practically important matters of plant and animal viral disease. But that set of possibilities was not evident as a prime motivation for the research; the deepening insight, being able to put biological phenomena into a quantitative framework of understanding, was immediate reward enough. And when Dulbecco turned to the animal viruses on the basis of the phage experience, including the very provocative aspects of the incorporation of lysogenic phages into the host cell's genome, it was with a sense of extension of that same kind of excitement. First with polio viruses, and adapting with modification the quantitative methods of phage research using animal cells in culture and derivatives of plaque-counting techniques, and then with tumor viruses, Dulbecco established a laboratory at Caltech in the forefront of one very important aspect of cancer research. His move to the Salk Institute in 1963 and later to become a Deputy Director of The Imperial Cancer Research Fund in London, now back again at the Salk Institute and a Professor of Pathology at the University of California, San Diego, Medical School, was a loss to Caltech but not to Science.

In the first paper in this section, he gives an up-to-date account of the incorporation of the DNA of a class of animal tumor viruses into the genomes of their host cells, and of subsequent processes of transformation of these cells into cancer cells. He discusses evidence, in turn, that the viral genes themselves are ultimately of cellular origin, genes that were initially controlled by repressor mechanisms similar to those known in bacteria, but escape from repression, possibly by mutation in the controlling genes. The hypothesis provides a basis for understanding the provocative relationship between spontaneous or induced cancers and viral transformation.

If Dulbecco's paper can be described as a child of the phage group, Temin's is a grandchild. Three generations of Nobel Prize work--Delbrück to Dulbecco

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to Temin! Temin's paper deals primarily with a kind of virus that was not envisioned in the early phage work--tumor viruses whose primary genetic material is RNA rather than DNA. Temin was, of course, a graduate student in Dulbecco's group, who came to Caltech after taking his Bachelor's degree at Swarthmore in 1955. Four years later, with a Caltech Ph.D., he joined the staff of the McArdle Laboratory for Cancer Research at the University of Wisconsin, where he is currently an American Cancer Society Professor of Viral Oncology and Cell Biology. I remember Temin's fascination, as a graduate student, with the RNA viruses and his speculations about how they might operate. In his paper, he describes how the "retroviruses," RNA viruses including the rapidly oncogenic Rous sarcoma virus, make DNA copies of their RNA, which become inserted in the DNA of the host cells. He deals with his hypothesis, for which there is substantial evidence, that the "weak" viruses, which are relatively slow to transform cells, evolved from genes in the host species, and he describes how such viruses may develop, evolve, and escape. Their ability to enter cells as RNA, to integrate into the host genome as DNA, and to come out again as RNA has provided not only an intellectually fascinating subject for investigation, but is also conceptually an important part of the revolution in genetics associated with the control of recombinant DNA. No doubt the most important potential contribution from this kind of research is still to come--an understanding not only of the abnormal growth we call cancer but of some of the processes of normal differentiation and development as well.

Although the environment the Caltech Biology Division has provided its students has always included easy interactions among people with various interests, it is fair to say that Bruce Ames' immediate surroundings were considerably different from those in the virology group, when Ames was a graduate student here. He came to work with Herschel Mitchell in 1950, after having taken his Bachelor's degree at Cornell University, and he received his Caltech Ph.D. in 1953. Then he went to the National Institutes of Health, returning to California, the University of California at Berkeley, as a Professor of Biochemistry in 1968. As a student in Mitchell's laboratory, his intellectual lineage therefore traces through Mitchell to Beadle, and the active traditions of biochemical genetics, especially of Neurospora. Mitchell's style with graduate students is relatively permissive; he is always there, attentive and helpful, but he has not chosen to restrict the problems his students might undertake to a narrow and intense range of topics in which he was himself most immediately active at the time. I suspect that this tolerance for diversity is related to how well Mitchell's students have, on the whole, done so many different things when they left Caltech for the outside world. Ames' primary research interests over the years have been closely relevant to those of traditional biochemical genetics--histidine biosynthesis, the regulation of metabolism and protein synthesis, and bacterial biochemical genetics. His paper, however, describes an excursion from this frontier, into territory most directly and importantly relevant to human health, the identification of environmental carcinogens. His expertise in bacterial biochemical genetics led him to develop laboratory strains of Salmonella sensitive to mutagens, and most importantly, able to distinguish a variety of mechanisms for mutagenesis. The strong overlap between carcinogenesis and mutagenesis tempted him to apply these quantitative techniques to the identification of carcinogens. And because important classes of carcinogens in man are not themselves carcinogenic in the form in which they exist in the environment, but are metabolized into effective carcinogens in the body, Ames modified his techniques to identify such classes of carcinogens as well. His paper is remarkable for its intellectual quality, its scope and relevance. Perhaps the greatest challenges it develops are in the social sciences; recognizing the seriousness of the problems created by environmental carcinogens marketed and released in industrial society, what, practically, can or should we do about them?

Knudson's, the final paper in this section, illustrates still another kind of Caltech lineage. Like Ames and Temin, Knudson was a graduate student at Caltech in the 1950's. His immediate associations were with the biochemistry of Henry Borsook, and his extensions have been identifiable with the genetics of Morgan, Sturtevant and Lewis. He had received a Bachelor's degree at Caltech in 1944, but then he went to the College of Physicians and Surgeons at Columbia for an M.D. degree, went into pediatrics, and came back to Caltech to take his Ph.D. with Borsook in 1956. He went from Caltech to the City of Hope National Medical Center, where he was Chairman of the Department of Pediatrics and later the Department of Biology; he moved to the New York University system at Stony Brook to become Professor of Pediatrics and Associate Dean of the Health Science Center as the new medical school was developing there, and then to the University of Texas in Houston where he started a biomedical graduate school and served as Professor of Medical Genetics and Dean. Relatively recently, he moved East again to become Director of the Institute for Cancer Research in Philadelphia. His paper deals with heredity and cancer in man. It should serve to clarify an area still subject to considerable confusion among cancer research workers generally--the subject, in fact, of this entire section, Genetics and Cancer. Is cancer "inherited"? We are reminded of the old and, in present light, essentially senseless arguments on nature and nurture. A very large proportion of human cancers are induced by environmental agents--irradiation, chemicals and viruses--and are therefore in principle preventable. But there is good reason to believe that these agents act primarily through their effects on the genetic materials and apparatus, and on hosts that vary in their genetic susceptibility. Knudson's clear delineation of the relationship between genetics and cancer caps this section of the Conference in a most fitting way. And, with the other papers, it reinforces Dulbecco's concluding words: "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 to this Division as its alma mater."

CELL TRANSFORMATION AND CANCER

Renato Dulbecco, Nobel Laureate

The Salk Institute, La Jolla

When I came here about 30 years ago from Bloomington, Indiana, I was greeted by Max Delbrück. I was going to work with him and this, of course, was a very significant event for me because I think Max is the father of all of us who have been virologists at one time or another and we are really very much indebted to him.

Here I started working with animal viruses. Actually, I think I was the first animal virologist at Caltech, and then Marguerite Vogt came to work with me soon afterwards. We spent several years working with cell-killing viruses like polio virus, and then we turned to oncogenic viruses which don't kill the cells but alter them, making them similar to cancer cells. In fact, these viruses do cause cancer in animals.

In my laboratory I had already had some work done in this field, first by Harry Rubin and then by Howard Temin. And they already had made a mark in the field. They were working with a virus with a very famous name, the Rous sarcoma virus. A few years later, around 1960 I would say, I got involved with another oncogenic virus which had just been discovered and that was the polyoma virus; later I also worked with SV40, which is another related virus. I am going to talk to you especially about this type of work and the consequences I can see out of this work.

These viruses, of course, are quite different from the Rous sarcoma virus that Harry and Howard were using. For instance, they have a double-stranded cyclic DNA as genome, whereas the Rous virus has RNA. However, Howard soon found out that within the cells all these viruses do quite the same things, and the outcome in both cases is that cells are changed in a characteristic way which most people call transformation. But Howard always called it conversion.

These names, of course, reveal our background in microbiology--our way of thinking, which was microbiological. So transformation or conversion, if you will, is the emergence of a cell lineage in which all cells are characteristically altered. But let's look a moment at what the alterations are. In the early 60's it was easy to define the transformed cells as cells that grow well with a little serum, that have unusual morphology.

These changes defined transformation operationally and allowed us to work. But in time, the number of characteristics of transformed cells has grown immensely. These changes affect different properties of the cells, in the sense that different cell compartments seem to be involved at the same time. This raised an important question: How is it possible that by introducing a virus we can change the cell in such a fantastically complicated way?

One can introduce a certain kind of order and rationalization by recognizing that some of the changes of transformed cells are probably produced by a common alteration. For instance, we can think that a change in the cytoskeleton will alter the agglutinability; so these two characters go together. Also, a change in the adhesion to the substrate may change the morphology of the cells.

Another kind of clarifying notion in this complexity is that the properties that are present in a given transformed cell are influenced by certain recognizable factors. For instance, one factor will be the cell type we start with before infection. Another factor will be the transforming virus. Another factor will be the procedure by which the transformed cell is isolated, whether it is isolated by looking for different colonial morphology on plastic or by plating in agar. These and other observations suggest another rationalization,

speculative as it may be, i.e., that the multiple differences of transformed cells are produced not only by the action of the virus but also by the state of cellular genes in the particular transformed cell that we examine.

Some differences between the cells may be due to the different states of differentiation of the cells before infection with the virus. Other differences may be introduced subsequently by mutation. Cells with certain changes can be selected by the culture conditions and the media in which we maintain these cells. So it is inevitable that the complex phenotypes of transformed cells result from the summation of events--both viral and cellular.

However, even with the rationalization--that a lot of the complexity of the transformed phenotype is due to the different backgrounds of cellular genes--we must accept the fact that a given transformed cell always has many types of changes that are independent of each other and cannot be easily explained. The action with the virus seems to be pleiotropic. The elucidation of this pleiotropism is actually a major challenge in the study of transformation. As we shall see, it is also a very important clue for understanding the mechanism for transformation.

When we started the work on transformation, we had as a model, or as a hypothesis, that cell transformation must be the counterpart of bacterial lysogeny, namely that it reflects the establishment of a permanent association between a viral and a cellular genome with consequent changes of the cellular phenotype. And our experiments were guided by this model. The immediate success of this approach was the demonstration that the DNA of polyoma virus becomes integrated in the cellular DNA when transformation occurs. This result was very useful because it tended to eliminate one of the hypotheses that were around at the time about transformation, what people used to refer to as the "hit and run" hypothesis. According to this view, the virus hit the cells and then disappeared. This idea was based on the observation that cells transformed by polyoma or SV40 lack the classical evidences of viral infection, or of viral multiplication. However, the DNA persists, and we could also show that a segment of this DNA is transcribed and specific viral proteins are made. These findings then suggested a different hypothesis: that cellular transformation is the consequence of the expression of one or more viral genes superimposed onto the background of cellular genes.

This molecular work was supported and complemented by genetic work which started out in a number of laboratories both with the Rous virus and with the polyoma viruses. The main aim of this work was to find viral mutations affecting transformation. In effect, certain mutants were found. The most striking were temperature-sensitive mutants of the Rous sarcoma virus that affect transformation. The state of the cells transformed by these mutants is entirely dependent on the incubation temperature. At low temperature, the cells have all the characteristics of transformation, but when they are shifted to a higher temperature, they revert to normality. The reversion occurs quite rapidly and affects many cellular characteristics, such as morphology, arrangement of the cytoskeleton, and mobility of surface proteins.

These results allowed the identification of the transforming gene of the Rous virus, which is now called the src gene. With polyoma virus, two types of mutations were found to affect transformation. The first one, in the A gene, was isolated here by Mike Fried. This mutant is temperature-sensitive, and at first sight resembles the src mutants because it transforms cells only at low temperature and not at high temperature. However, the A mutants are unlike the src mutants because the transformed cells, once they are generated at low temperature, tend to remain transformed when they are shifted to high temperatures. Therefore, this A gene of polyoma virus and the src gene of the Rous virus have clearly different roles in transformation. The src gene controls the maintenance of transformation whereas the A gene of polyoma virus mainly controls events that occur once when the permanent lineage of

transformed cells is produced. At least this is the most evident role for this gene. In addition, under certain conditions, perhaps related to the background of functional cellular genes, gene A contributes to the maintenance of transformation, possibly only for certain characteristics.

The mutants in the gene A of polyoma virus can still express transforming function at the nonpermissive temperature because they can induce an abortive transformation. In this type of transformation, the alterations last in the cell lineage for maybe five or six generations and then the cells revert to the normal state. Evidently, the abortive transformation does not require the essential initial event, performed by the A gene, that is required for generating a permanently transformed cell lineage. The event required for permanent, but not for abortive transformation, may be the integration of the viral DNA in the cellular DNA. This is not completely established. It is plausible that the A gene has an integration function because its product interacts with the DNA. However, the main function of this gene is to initiate the autonomous replication of the cyclic viral DNA in the lytic infection.

These studies with the A gene show that the maintenance of transformation by polyoma virus must be due to another gene. Indeed, a second type of mutation was subsequently isolated by Tom Benjamin, who is also a Caltech alumnus. He isolated HRT mutants, which are so called because they have an altered host range and do not transform. These mutations are either small deletions or point mutations. They abolish the ability of the virus to cause either permanent or abortive transformation; therefore, they completely suppress the transforming ability of polyoma virus. Small deletions in the corresponding area of the map of SV40 produced in vitro by beautiful technology in Paul Berg's laboratory at Stanford have similar properties.

These deletions also affect the maintenance of transformation but in a somewhat different way. They cause an incomplete transformation which has fewer and less pronounced changes than regular transformation. It is not very clear why transformation is incomplete. Probably the difference between the polyoma and SV40 mutations can be understood in terms of the interrelation of the expression of viral genes with the cellular genetic background because the cells that are used in the two types of experiments are different.

Once the viral genes involved in transformation were identified, the emphasis shifted to the proteins specified by the genes. With the DNA viruses, this task was facilitated at the beginning by an older discovery of Black and Huebner. They found that the serum of animals carrying a tumor induced by polyoma virus or by SV40 reacts with proteins present in cells infected or transformed by the corresponding virus. These virus-specific proteins are collectively known now as T antigen--tumor antigen. Using this antiserum, the T antigen and the constituent proteins could be purified by immunoprecipitation and gel electrophoresis.

The results show that there is a large T, of molecular weight 90,000 to 100,000. The size of the molecule is affected by temperature-sensitive mutations in gene A and is made smaller by its deletions; therefore, the large T is the product of the A gene. Another protein is the small T (molecular weight ca. 20,000 daltons); this protein is made smaller by deletions in the hr-t gene; therefore, it is the product of that gene. A third protein is the middle T (molecular weight ca. 55,000 daltons), so far only recognized with polyoma virus. This protein is lacking in cells infected by some hr-t mutants.

It is interesting to look at the relationship between these T proteins. They have a common amino acid sequence in the amino end; therefore, they have a common initiation. In fact they are specified by the same DNA segment. How this happens has been shown by the work of several laboratories which have brilliantly revealed the interesting molecular mechanisms. The possibilities of sequencing both the viral DNA and the viral proteins were especially useful in this work. Also useful was the advanced state of the art for translating specific

messenger RNAs in vitro. In brief, it was found that the small T is specified by the primary transcript of the transforming region of the viral genome. The protein is small because translation is interrupted by a termination signal. The large T is obtained from the same transcript after splicing, which leads to excision of the termination signal. The site of the hr-t mutations is eliminated at the same time. The middle T is obtained from the same primary transcript, after a different splicing which again removes the termination signal; but this splicing also changes the reading frame for translation.

As to the functions of the three T proteins, our knowledge is still limited. The large T, which is present in the cell nucleus, is a DNA binding protein; it is required for the replication of the viral DNA, which occurs in the nucleus, and as I already said, possibly also for the integration of the viral DNA in transformation. The middle T is present in the cellular plasma membrane; we may speculate that it affects the function of the membrane in growth regulation. The function of the small T remains not understood. From the study of mutants, it seems that all three T proteins are involved in transformation; but the most important role seems to be that of the small T which expresses the hr-t gene.

These transforming proteins may seem too much; but in fact they help to explain the pleiotropism of transformation because we have three different functions. This works only in part because the changes of the transformed cells cannot be all explained on the basis of three primary effects. A recent discovery of Ray Erikson with the Rous virus offers a new, very interesting possible explanation of the pleiotropism. Erikson first produced the transforming protein of the Rous virus by translating in vitro an appropriate viral messenger RNA. This protein is the expression of the src gene. The same protein is present in the virus-infected cells. But the most interesting thing is that this protein is a protein kinase, or maybe a protein that regulates a cellular kinase.

The experiments were done in the following way. Ray Erikson made an antiserum against the transformed cells with which he precipitated an extract from the transformed cells. After adding [^{32}P]ATP to this immunoprecipitate, he found that the immunoglobulins became highly labeled with the ^{32}P label: a kinase activity in the immunoprecipitate labels the heavy chain of the immunoglobulin.

Of course, experiments of this kind were immediately done with the polyoma virus and SV40, and the results are similar to those obtained with the Rous virus: the immunoprecipitate formed by the anti-T serum with extracts of transformed or lytically infected cells also contains a protein-phosphorylating function. The difference, however, is that the immunoglobulin is not now phosphorylated, but the middle T is. The reasons for the different receptor specificities are not known. Undoubtedly these are very interesting findings; if we want to take these results at face value, we would say that the protein kinases specified by the different viruses are subjected in the cell to some types of restraints, probably different for the various viruses.

On the basis of these facts the pleiotropism of transformation can be explained better than it could at the beginning, although hypothetically. Probably there are at least three different reasons for the pleiotropism. One is that certain groups of cellular changes come from a common origin. Another factor is that in some cases there are several transforming proteins and each one may produce different types of alterations in the cells. The third factor is the virus-specified protein kinase. It is difficult to make predictions at this time, but this may turn out to be the most important factor in pleiotropism. In fact, by phosphorylating a number of cellular proteins, a protein kinase may affect many cellular functions. It is also conceivable that other viruses might specify some other kind of protein-modifying enzyme. Even with kinases, there may be a multitude of different kinds with different specificities.

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