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MICROBIAL GENETICS

Edited by MORAD A. ABOU-SABÉ

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National Academy of Sciences—*Proceedings of the National Academy of Sciences*
- "A New Type of Bacterial Pilus Genetically Controlled by the Fertility Factor of *Escherichia coli* K_{12} and Its Role in Chromosome Transfer"
- "On the Topography of the Genetic Fine Structure"
- "Chromosome Breakage Accompanying Genetic Recombination in Bacteriophage"
- "On the Colinearity of Gene Structure and Protein Structure"

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Academic Press, Inc. (London) Limited

Journal of Molecular Biology
- "The Bacterial Chromosome and Its Manner of Replication as Seen by Autoradiography"
- "Fate of Transforming DNA Following Uptake by Competent *Bacillus subtilis:* IV. The Endwise Attachment and Uptake of Transforming DNA"
- "Identification of Individual Sex-Factor DNA Strands and Their Replication During Conjugation in Thermosensitive DNA Mutants of *Escherichia coli*"
- "Mapping of the Lactose Operator"
- "Codon–Anticodon Pairing: The Wobble Hypothesis"
- "The Nature of the Polypeptide Chain Termination Signal"
- "Orientation of Transcription of the *lac* Operon and Its Repressor Gene in *Escherichia coli*"

Journal of Theoretical Biology
- "A Theory of DNA Replication"

American Society for Microbiology

Bacteriological Reviews
- "Chromosome Transfer in Bacterial Conjugation"

Journal of Bacteriology
- "Cellular Metabolism in Genetic Transformation of Pneumococci: Requirement for Protein Synthesis During Induction of Competence"
- "Mode of Action of the Competence-Inducing Factor of *Bacillus stearothermophilus*"

Annual Reviews, Inc.—*Annual Review of Genetics*
- "Transduction Mechanisms"

Australian Acadmey of Science—*Australian Academy of Science, International Conference on Replication and Recombination of Genetic Material*
- "Molecular Mechanisms of Recombination in *Bacillus subtilis* Transformation"

Brookhaven National Laboratories—*Brookhaven Symposia in Biology*
- "On the Molecular Explanation of Spontaneous and Induced Mutations"

Cold Spring Harbor Laboratory—*Cold Spring Harbor Symposia on Quantitative Biology*
- "The Structure of DNA"
- "On the Regulation of DNA Replication in Bacteria"

"Frameshift Mutations and the Genetic Code"
"Transposition of the *Lac* Region of *E. coli*"
"On the Fundamental Nature and Evolution of the Genetic Code"
"Transcription Initiation and Degradation of *trp* mRNA"
"Electron Microscopic Visualization of Transcription"

Elsevier Publishing Company—*Biochimica et Biophysica Acta*
"Sequential Transcription and Translation in the Lactose Operon of *Escherichia coli*"

Macmillan (Journals) Ltd.
Nature
"Infectivity of Ribonucleic Acid from Tobacco Mosaic Virus"
Nature New Biology
"*Lac* DNA, RNA Polymerase and Cyclic AMP Receptor Protein, Cyclic AMP, *Lac* Repressor and Inducer Are the Essential Elements for Controlled *Lac* Transcription"

Rockefeller University Press
Journal of Experimental Medicine
"Studies of the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III"
Journal of General Physiology
"Independent Functions of Viral Proteins and Nucleic Acid in Growth of Bacteriophage"

Series Editor's Preface

There is perhaps no area of microbiology in which the advancement of knowledge has been as rapid as in microbial genetics; indeed, no area (except its sister science, molecular biology) has attracted more workers. Therefore, selecting the "classic" papers becomes somewhat difficult. Further, the information has been of such interest that its fundamental contributions are already widely known and have become part of modern scientific training. This volume is not, therefore, an attempt to preserve classic papers buried in the past or lost in the jungle of journals; many have already been collected. Rather it is an attempt to bring together those contemporary papers upon which, on the basis of considered judgment, one feels that future development will be based.

This volume assumes that the reader is familiar with microbial genetics at the elementary level. This is not an introduction; it is a selection of the really significant papers that a professional microbial geneticist would want to have at hand. The advances of the past decade have clarified much that was obscure and have built a base upon which sound progress is possible. In the biblical concept, "Then we saw as through a glass, darkly, but now we see in the light."

Wayne W. Umbreit

Dedicated to A. H. Abou-Sabé

and M. M. Moukhtar

Preface

Microbial genetics is a relatively young field in which active research started three decades ago; however, advancement of our knowledge in this area of science has been immense. The impact of the collaborative efforts of biochemists, physicists, mathematicians, and geneticists on this advancement has certainly been great; indeed, it has been felt in basic, applied, and industrial research. To publish a collection of the "classical" papers in the field would, therefore, require many volumes the size of this one. In consideration of the limited space available, I have chosen to provide a developmental approach to the subject, covering some of the basic concepts and areas of microbial genetics as would be taught in a beginning graduate course. I hope that this book will be useful not only as a textbook but as an introduction to the field for scientists and investigators.

The book is divided into four sections, covering the major areas of microbial genetics, and each section is subdivided into several parts for a detailed discussion of the various aspects of each area. The individual papers were chosen on the basis of their influence on the development of the field. In some cases, however, current papers, which in my opinion provide basic information furthering our knowledge in a specific area, were also included.

For the sake of continuity, each section is prefaced with a historical introduction. These introductions are in no way meant to be reviews of the literature, rather a brief tracing of the development of information in the section.

I would like to express my appreciation to V. Bryson, W. Umbreit (Series Editor), and D. Underhill for reviewing the manuscript and for their valuable advice in the course of this work.

M. A. Abou-Sabé

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II. GENETIC TRANSFER AND RECOMBINATION ANALYSIS IN BACTERIA

III. THE GENETIC CODE

IV. REGULATION OF INTRACELLULAR TRANSCRIPTION AND TRANSLATION

Introduction

The development of microbial genetics started in an atmosphere characterized by old concepts in which microorganisms were not considered in genetic terms. The fact that bacteria had no nucleus in the true sense of eucaryotic cells, nor any cytologically recognizable chromosomal structure, made it difficult to justify a genetic organization in such creatures. The view generally held at the time, that bacteria were capable of adapting with tremendous plasticity to variable environmental conditions, made it difficult to determine whether bacterial variation in nature was caused by mutation. Investigating the origin of bacteriophage-resistant variants in *Escherichia coli*, Luria and Delbruck (1943) formulated two basic hypotheses as possible causes for the production of these bacterial variants. In one hypothesis, they postulated that bacteriophage-resistant variants could arise as a result of a finite probability for a bacterium to mutate from bacteriophage-sensitive to -resistant, thus producing resistant progeny (mutation hypothesis). In the second hypothesis, it was postulated that the bacteriophage-resistant bacteria arise as a result of a finite probability for any bacterium to survive a virus attack and that survival of an infection confers immunity on its offspring as well.

On the basis of the mutation hypothesis, one would expect that mutation to resistance may occur at any time during the growth of the culture prior to exposure to bacteriophage; whereas on the basis of the hypothesis of acquired immunity, survivors of the bacteriophage attack will be a random sample of the culture. Similarly, if mutation occurs, the proportion of resistant bacteria will be dependent upon the time at which the mutation event takes place during culture growth, thus increasing with time; whereas a constant proportion would be expected on the basis of the second hypothesis.

To differentiate between these two hypotheses, Luria and Delbruck designed a quantitative test (the fluctuation test) for experimental verification of the mutation hypothesis and an elegant theoretical analysis of the probability distribution of the

virus-resistant mutants expected from each hypothesis. In the fluctuation test, a bacteriophage-sensitive liquid culture was divided into two equal portions. A series of 100 cultures was set up from one portion while the other was maintained as a bulk culture; both were grown until maximum growth was reached. Equal samples from both cultures were tested for bacteriophage-resistant variants and the data were statistically analyzed. The results of the fluctuation test showed a tremendous variation in the number of resistant colonies in the tubes series; only sampling error variations were found in the samples of the bulk cultures, thus confirming the mutation hypothesis.

Although the development of the fluctuation test by Luria and Delbruck provided the information required to substantiate the mutation hypothesis, questions remained in the minds of some who were not about to yield to the new avenues which were to lead to a new kind of genetics, microbial genetics. Since the fluctuation test did involve the exposure of the bacterial population to bacteriophage, proponents of the adaptation hypothesis regarded this test as nonconclusive and Luria and Delbruck's demonstration of the occurrence of spontaneous mutation was disregarded for six years until the question was put to more tests. Finally, Newcombe in 1949, then Lederberg and Lederberg in 1952, were able to confirm that the origin of the bacteriophage-resistant variants was indeed due to mutation, especially after the Lederbergs' elegant demonstration of the indirect selection (using replica plating) of bacteriophage-resistant mutants, performed without ever exposing the bacterial population to the phage itself. Since then, mutation in bacteria has been acknowledged, and the replica plating technique has been widely used in much of bacterial genetics research.

The Chemical Basis of Heredity

I

Editor's Comments on Papers 1, 2, and 3

1 **Avery, MacLeod, and McCarty:** *Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococal Types; Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III*

2 **Hershey and Chase:** *Independent Functions of Viral Proteins and Nucleic Acid in Growth of Bacteriophage*

3 **Gierer and Schramm:** *Infectivity of Ribonucleic Acid from Tobacco Mosaic Virus*

Identification of the Hereditary Material

Once the question of the occurrence of mutation in bacteria was settled for the most part, it was of importance to test the effect of culture growth rate and the number of generations on the mutation rate in a bacterial population. The development of the chemostat, allowing continuous culture growth under specified conditions (Novick and Szilard, 1950 and 1951) was significant for this purpose. If bacterial mutations occur as a result of a copy error in the replication of the genetic material, one would expect an inverse relationship between the generation time of the bacterial culture and the mutation rate. This prediction, however, has been found experimentally not to be true (Novick and Szilard, 1950), as in the case of the mutation to T5 resistance. Using tryptophan as the controlling growth factor, it was found that in cultures with generation times varying from 2 to 12 hours, mutation to T5 resistance occurred at a rate unaffected by the different growth rates (Novick and Szilard, 1950). Mutation rate per generation was also found to be constant under conditions of high growth rates, as shown by Fox (1955). It is indicated, therefore, that mutation rate is dependent upon gene generation rather than bacterial generation.

Much work on the nature of mutation and the molecular basis of the various types of mutations was to come later, analyzing in depth the biochemical consequences of mutation, an aspect that was more difficult where higher forms of life were used.

In this same period of development, serious efforts were underway to seek answers to the very fundamental problem of the nature of the carrier of the hereditary information. Since the discovery, made by Griffith (1928), that a genetic change may be induced in a nonpathogenic strain of pneumococcus (R) by a preparation of heat-killed S cells of a pathogenic strain (S) of the same organism, causing the trans-

formation of the R cells into S cells that are pathogenic to mice, attention had been focused on the kind of molecules in this preparation that were responsible for such transformation. Since proteins and polysaccharides were major components of the heat-killed S cells, with proportionally very little nucleic acid, attention was directed to the first two components, especially to proteins in the search for a carrier of genetic information. Knowledge of the properties of protein molecules and the great numbers of heterologous proteins in the same organism, as well as the known differences between the "same" proteins isolated from different organisms, made it appear more likely that proteins had the informational role of the genetic material, a property presumably not shared by DNA, then thought of as a monotonous polymer of tetranucleotides. The systematic approach given by Avery and his colleagues (1944, Paper 1) in analyzing the chemical nature of the transforming principle, unprejudiced with respect to one component or another, was the basis for the identification of DNA as the transforming principle and thus the carrier of the hereditary information. This evidence was not accepted at face value, and several years of arguments passed until Hershey and Chase (1952, Paper 2) provided independent evidence, from the study of bacteriophage infection, that, in bacteriophages too, DNA is the sole component that carries the information for the reproduction of the bacteriophage in the host cell and that it is the only component that is injected into the bacterial host in the infection cycle. With this discovery more and more faith in the role of DNA was evidenced, and a great deal of work was started scrutinizing the fitness of DNA as a molecule capable of fulfilling all the requirements of a genetic carrier in terms of structure, replication, recombination and coding.

There are, however, organisms, such as RNA viruses and bacteriophages, that do not contain DNA. This problem, too, was soon to be solved by Gierer and Schramm's discovery (1956, Paper 3) of the genetic role of the RNA of the tobacco mosaic virus (TMV) which was shown to be as infective as the DNA of DNA bacteriophages. With this discovery, Frankel-Conrat and his colleagues (1957) were able to design a number of elegant experiments showing (1) the possible reconstitution of RNAs of different TMV mutants with their proteins in various combinations, and (2) that the progeny of an infective TMV particle carry the genetic properties of the infective RNA, regardless of the origin or properties of the protein component of the infective particle. Thus, RNA is also a carrier of genetic information in certain viruses, where it acts as its own messenger and template for replication *via* an RNA replicase (Spiegelman et al., 1965).