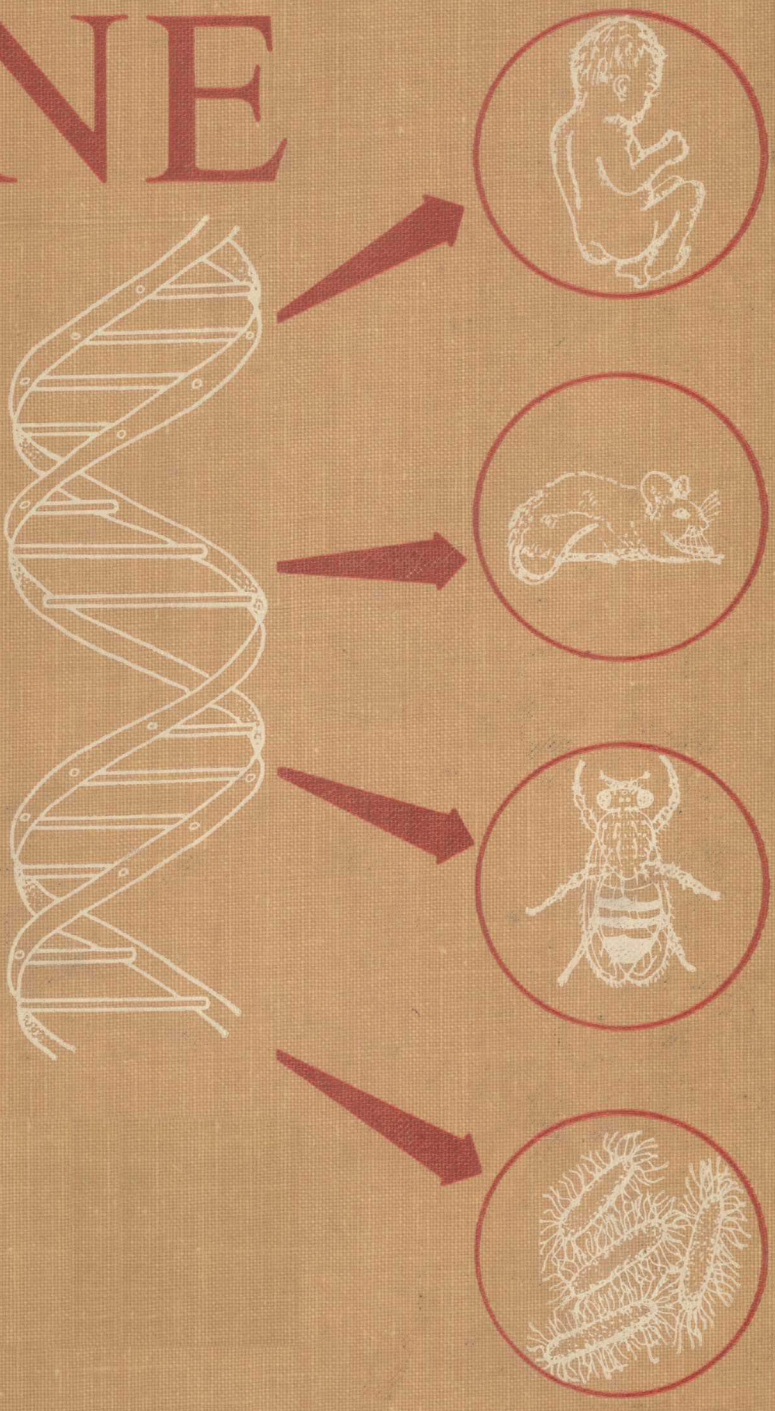
BIOLOGY OF THE GENE



LOUIS LEVINE / Second edition

Biology of the GENE

LOUIS LEVINE

Professor of Biology
City College of New York

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with 199 illustrations

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To my professors of genetics who inspired as they taught

Theodosius Dobzhansky
L. C. Dunn
Howard Levene
Francis J. Ryan

Preface

This book is written for a single-semester undergraduate course in genetics. Two factors influence its content and organization. The first factor is the central and unifying position of genetics in the biological sciences, which requires considering such diverse areas as biochemistry, physiology, cytology, development, behavior, and evolution. The second factor is a desire to present, wherever possible, the experimental procedures and data that have led to our present concepts in genetics. This manner of presentation is an outgrowth of my firm conviction that education should not result solely in the accumulation of a body of information but must also include an understanding of the methodology used in the discipline studied and of the limitations in our knowledge about the subject.

It has been traditional to begin textbooks in genetics with a consideration of the work of Mendel and the principles of inheritance that his findings elucidated. However, recent discoveries on the nature of the genetic material and the manner in which it operates have shed new light on the chemical bases of many previously known genetic phenomena. In order that the student may benefit from this more recently acquired information in his study of inheritance patterns, the first topics considered are the nature and functions of hereditary material, the genetic code, and the physical basis of inheritance. Discussions then follow of gene interactions, multiple-factor inheritance, sex linkage and sex determination, chromosome numbers, chromosome mapping, chromosomal rearrangements, and extrachromosomal inheritance. In all these discussions, the insight provided by recent findings on the nature and functions of the genetic material is stressed. Next follows an analysis of concepts of the gene as a mutable unit, a recombinational unit, and a functional unit. This consideration of the different aspects of the gene leads into a study of the regulation of gene action and the control that genes have over metabolism, development, and behavior. The final topics discussed are the genetic composition of populations of organisms and the fate of genes in succeeding generations of a given population. Other subjects included in these latter discussions are the roles of mutation, selection, migration, and genetic drift in determining the genetic composition of different populations of a species and the permanent establishment of genetically diverse populations through species formation.

Wherever possible, the relationship of genetic phenomena to man has been stressed. This is done not only to increase the student's interest in the material being discussed but also because human genetics has become one of the very active fields in modern research. The present explosive period of genetic research and publication has added a tremendous body of information to an already considerable amount available from the older literature. Because of the necessity of limiting the various discussions in the book to workable dimensions, a list of further readings is provided at the end of each chapter. Some of these references are designed to give the student historical background for topics covered within the chapter, while others are provided

to afford the student a wider acquaintance with modern research efforts in the field.

It has been four years since the first edition of this book was published. In that time, the field of genetics has continued its explosive contribution to virtually every aspect of biology and medicine. The current edition has been revised with this recent material in mind. New topics have been added, and a number of original discussions have been rewritten in the light of new information. In addition, there have been a number of substantial changes in the organization of the book's contents. It is hoped that these additions and rearrangements will help present the material of genetics as the exciting body of information it is.

Since its publication, this book has been used by many genetics classes both in the United States and abroad. I am deeply grateful to those instructors and students who have written either to the publisher or directly to me and have made suggestions for the book's improvement. Their recommendations have received careful study during the preparation of the present edition. I am especially indebted to two of my own students, Miss Harriet Rubenstein and Miss Helena Stuler, who spent many hours giving me the advantage of the student's point of view. The present effort has been guided by and has benefited a great deal from all of the above. I sincerely hope that those who use this book will continue to advise me of their reactions to it.

This book has benefited from the efforts of many persons. I am especially indebted to the following associates, each of whom graciously consented to review parts of the manuscript and also offered valuable suggestions for its improvement: Dr. Leonard C. Norkin, Dr. Rose R. Feiner, Dr. George C. Carmody, Dr. Betty C. Moore, Dr. Donald J. Komma, Dr. Norman M. Schwartz, Dr. Muriel Lederman, Dr. William N. Tavolga, and Dr. Frederick E. Warburton. I also wish to express my gratitude to the reviewer for the thoroughness with which he read the entire manuscript and for the comments he made. Any shortcomings of the book, however, are my responsibility alone. Finally, I wish to express my appreciation to Mr. Joseph T. Fevoli for preparing all the illustrations, Mrs. Rita Berkowitz and Mrs. Anne W. McCartney for performing the arduous task of typing the manuscript, and Mrs. Joan Sobel and Miss Helena Stuler for their aid in preparing the questions and problems. Credits for tables and figures from other publications are given in the legends according to the wishes of the author or publisher. I am indebted to the literary executor of the late Sir Ronald A. Fisher, F.R.S.; to Dr. Frank Yates, F.R.S.; and to Oliver & Boyd, Ltd., Edinburgh, for permission to reprint Tables 3 and 4 from their book *Statistical Tables for Biological, Agricultural and Medical Research.*

Louis Levine

BIOLOGY OF THE GENE

Contents

1 Nature and functions of hereditary material

Genetics is the branch of biology that deals with heredity and variation. This definition would appear to limit the area of study to the transmission of characteristics from parents to offspring and to the study of the variability in traits that may occur from one generation to the next. However, the field of genetics is in reality very broad, for within its scope lie such topics as (1) identification of the hereditary material and the nature of its chemical and structural properties; (2) study of the organization of the genes into chromosomes and the transmission of the chromosomes from parents to progeny either in asexual or sexual reproduction; (3) analysis of the interactions of the different genes and the role of environment in producing the characteristics of the individual; (4) study of the different types of genetic diversity that can occur and the consequences of this diversity to the individual and to the population. Genetic studies may attempt to gather information on such differing subjects as the origin of living material from nonliving matter and the future of mankind.

It is obvious that our knowledge concerning many of these topics is incomplete. In our discussions the limits of our information on a particular subject will be stated, and whenever possible, the areas in which future research might bring meaningful answers to questions will be indicated. Let us begin our study of genetics with a review of our knowledge of the nature and functions of the hereditary material.

IDENTIFICATION OF GENETIC MATERIAL

A prime goal in the study of genetics has been the identification and analysis of the actual genetic material. As it turned out, we had acquired much information about the modes of inheritance and about the relationships of genes to one another before we were able to demonstrate which of the many chemical compounds of the cell contains the genetic material. Furthermore, much of the chemistry of the genetic material was known long before its significance in genetics achieved wide understanding and acceptance.

As long ago as 1807 the distinction between inorganic and organic compounds was made. By 1820 it had become customary to think of the organic compounds as falling into one or another of three broad groups: the carbohydrates, the lipids, and the proteins. By the mid-nineteenth century it seemed clear that, of the three organic compounds, *proteins* were the most complicated in structure and the most important in function. However, in 1871 a chemist named F. Miescher reported that he had isolated from pus cells a substance that turned out not to be carbohydrate, lipid, or protein. Since he had obtained the new substance from nuclei, Miescher named it *nuclein*. Later the substance was discovered

to have acid properties, and it was renamed *nucleic acid*.

Transformation in bacteria

The identification of the genetic material became a point of dispute between investigators who thought the material resided in the protein of the nucleus and those who believed it to be in the nucleic acids. A resolution of the problem did not come until 1944 when Avery, MacLeod, and McCarty reported their work on *transformation* in pneumococci. In man, pneumonia is sometimes caused by the bacterium *Diplococcus pneumoniae*, commonly known as *pneumococcus*. There are two types of pneumococcal cells. In one type of cell a considerable amount of polysaccharide material is secreted by the cell, and a large capsule forms around the cell. The colony produced by these cells has a glistening appearance and is called "smooth" (S). In the other type of cell no polysaccharide slime layer is secreted by the cell. The colony formed by such cells has an irregular appearance and is termed "rough" (R). Smooth (S) cells are virulent and can cause pneumonia, but rough (R) cells are nonvirulent.

Investigations of the S form of *pneumococcus* revealed the existence of many kinds of capsules, each distinguishable on the basis of differences in the chemical composition of its polysaccharide. The S pneumococci were classified as type I-S, type II-S, type III-S, etc. Each type, when it divides, produces cells of the same type as the parental cell. Occasionally an S bacterium will change to an R bacterium (1 per 10^6 or

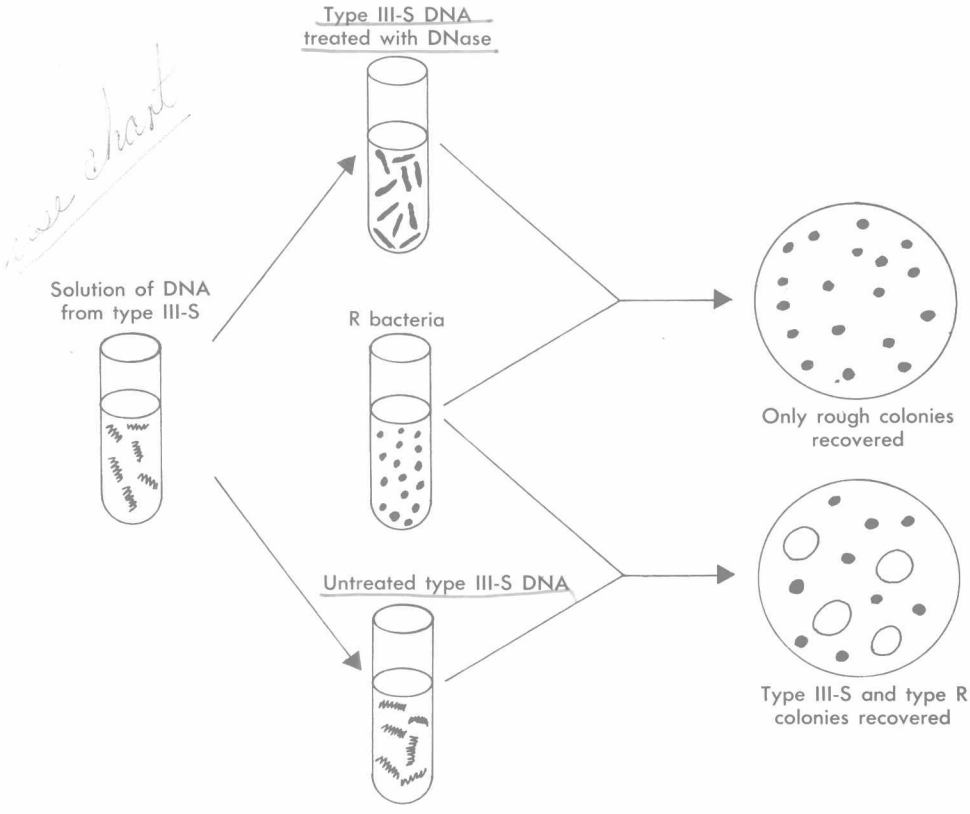

Fig. 1-1. Transformation of pneumococcus, using DNA extracted from heat-killed smooth cells. DNase is an enzyme that breaks down DNA.

10^7 cells). The reverse change, R to S, almost never occurs. When the R cells divide, they always give rise to more R cells. Avery and co-workers disrupted encapsulated cells of type III-S. They then fractionated the debris from the disrupted cells into its various chemical components (carbohydrates, lipids, etc.). After this they took R cells that had been derived from type II-S cells and separately mixed different samples of them with each of the cell components from the type III-S cells. Only the DNA fraction of the type III-S cells was found capable of transforming some of the unencapsulated (R) cells to encapsulated (S) cells. The transformed encapsulated cells were type III-S, the same type as the cell from which the DNA was obtained. This experiment, shown in Fig. 1-1, clearly demonstrated that the genetic property for pneumococcus capsule formation resides only in the DNA of the cell.

In recent years transformation of a large number and variety of hereditary characters has been demonstrated among different species of bacteria. In many cases the character transformed represents the ability to make an enzyme. One such example, in the bacterium *Bacillus subtilis*, is the enzyme β-galactosidase that catalyzes the hydrolytic splitting of the disaccharide lactose into galactose and glucose. Bacteria that either fail to make this protein or make an inactive form of it cannot ferment lactose. They are nutritionally defective and are called *auxotrophic*, whereas those that can utilize lactose are nutritionally competent and are characterized as *prototrophic*. When the DNA from prototrophic *B. subtilis* is mixed in the medium with auxotrophic cells, some of the auxotrophs are transformed into prototrophs.

The role of DNA in bacterial transformation was put to a further test. In a separate experiment the DNA was first exposed to the enzyme deoxyribonuclease (DNase), which selectively breaks down DNA. This treatment resulted in the abolition of the transforming activity. Treatment of the DNA with enzymes that degrade proteins was without effect. Thus the possibility that a protein contaminant might be responsible for the transforming activity can be excluded. The mechanism by which the DNA accomplishes transformation has been shown to be an incorporation of the donor DNA into the chromosome of the recipient cell. This mechanism will be considered further in the discussion of the phenomenon of crossing-over in Chapter 7.

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Returning now to the problem of the identification of the hereditary material, we find that the identification of DNA as the genetic material was further substantiated by experiments involving viruses that multiply inside bacteria (bacteriophage).

Bacteriophage T2

Viruses are small but well-organized entities. They can be seen and photographed through the electron microscope. Their shapes are quite characteristic, and each kind of virus exhibits a specific geometric pattern in its external morphology. Viruses are also specific as to the type of organism and cell that each kind will normally attack. One type of virus attacks the leaves of the tobacco plant (tobacco mosaic virus); another attacks the motor cells in the spinal cord of man (poliomyelitis virus); still others invade bacteria (T2, T4, ΦX174, etc., collectively called *bacteriophage*). All viruses contain at least protein and nucleic acid. The nucleic acid is of either the RNA or the DNA type. Differences in the two types of nucleic acid will be discussed later in this chapter.

There are a number of viruses that will invade the colon bacillus *Escherichia coli*, multiply within it, split (lyse) the bacterium, thereby killing it, and at the same time release more bacteriophage. T2 is such a virus. It is composed of only protein and DNA. The protein is organized into a "coat" that covers a DNA "core." Hershey and Chase in 1952 reported on their studies of the T2 life cycle, made by using radioactive isotopes of sulfur (^{35}S) and phos-

phorus (^{32}P). Protein molecules almost invariably contain sulfur atoms but very few if any phosphorus atoms, whereas nucleic acids always contain phosphorus atoms but never any sulfur atoms. The viruses were allowed to go through a number of life cycles in bacterial hosts in a food medium containing ^{35}S and ^{32}P. As a result the coats of the viruses were labeled with ^{35}S and the DNA with ^{32}P. Hershey and Chase then infected unlabeled bacteria with labeled T2. They found that the ^{35}S remained outside the bacterial cell, in the protein coat. The coat could even be shaken off the bacterial cell shortly after contact without interfering with the subsequent production of new bacteriophage. The ^{32}P, on the other hand, entered the bacterial cell. On lysis, which occurs about 20 to 25 minutes after initial infection, one finds some 100 to 150 new bacterial viruses complete with DNA cores and protein coats. The conclusion, as in the case of bacterial transformation, was simple and clear: the DNA contained the hereditary material (genetic code) that controlled the production of the entire virus. The experimental procedure is shown in Fig. 1-2.

Tobacco mosaic virus

A life cycle similar to that found in T2 has been discovered in the tobacco mosaic virus, which kills the cells of the tobacco leaf and results in a yellow mottling of the leaf (tobacco mosaic disease). This virus also consists of an outer protein coat and an inner core of nucleic acid. The nucleic acid in this case is RNA. In 1956 H. Fraenkel-Conrat reported that preparations of the viral RNA that had been completely

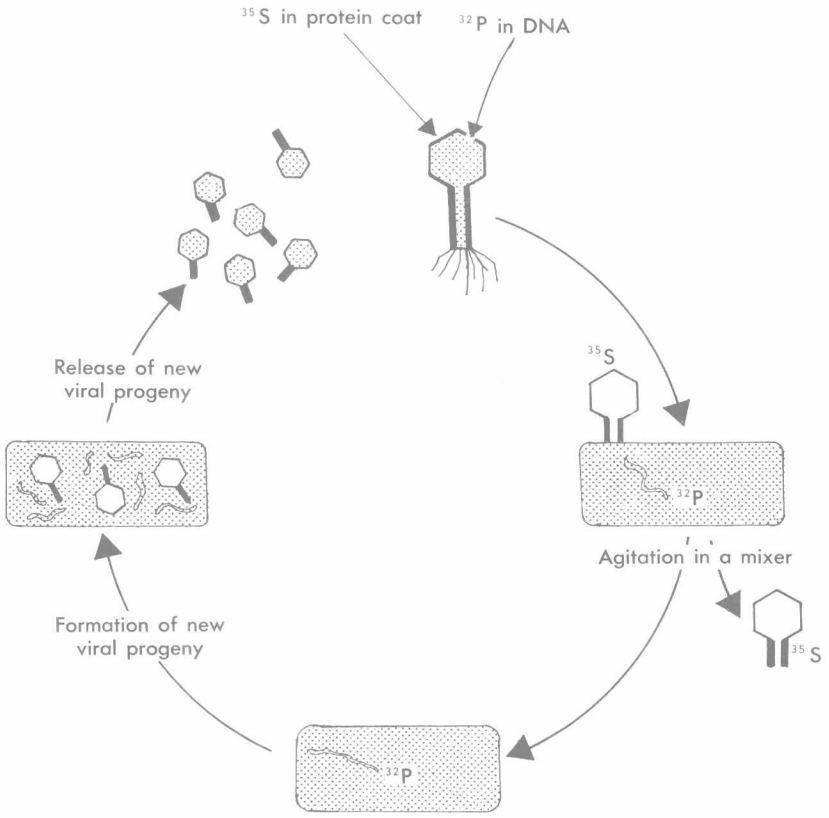

Fig. 1-2. Demonstration that only the DNA component of T2 carries genetic information. (From Watson, J. D. 1965. Molecular biology of the gene. W. A. Benjamin, Inc., New York.)