The Genetics of Bacteria and their Viruses

STUDIES IN BASIC GENETICS

AND MOLECULAR BIOLOGY

BY

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Preface to Second Edition

This second edition is presented in the hope that it will prove as useful to advanced students of molecular genetics as the first edition seems to have done. I stress the word 'advanced' because I am aware that many of the recent ideas in this field, such as the polarity of the operon, or the possible mechanisms of prophage induction or chromosome transfer, although simple to state dogmatically are, in fact, derived from complex lines of reasoning based on data from many different types of experiment and even disciplines. They are sometimes hard enough to grasp thoroughly even when one has grown up with them! The book, then, is not for beginners for whom many excellent, but cheaper and more elementary, volumes are now available.

The four years which have elapsed since this book was first published have witnessed great advances in our knowledge of the molecular basis of life. The most striking has been the elucidation of the genetic code. In the first edition, the summary of the chapter on this subject states that, although we understood the general nature of the code, 'we do not yet know the code which any organism employs to specify even a single amino acid'. Now, just fifteen years after the beginning of what is certainly one of the most exciting periods of discovery and advance in the whole history of biology, the genetic code has been solved in detail and in its entirety, mainly by the development and use of ingenious new chemical techniques. Other fields which have seen striking and important, if not such dramatic, progress are those concerned with the conformation of protein and RNA molecules, the repair of radiation damage, the mechanism of recombination, the mode of replication of viral single-stranded DNA and RNA molecules, the provirus state and its control, the genetic regulation of biosynthesis, and the nature and behaviour of bacterial plasmids. All these recent advances have been treated in considerable detail, and most of the chapters, except some of the introductory ones, have been extensively re-written. Nevertheless I have to admit that the pace of the advance and the sheer volume of emerging information is now so great that a number of new and important findings have been published too late for inclusion. However this book is intended to be a textbook and a work of reference, and not a compendium of recent advances to which access may be had through other sources.

The increased scope of the edition may be judged from the fact that the bibliography has been nearly doubled by the addition of more than 700 new references, there are 18 new figures and ten new plates, while the text has been increased by about 140 pages. It is hoped that the considerably expanded index, together with extensive cross-referencing and, as in the previous edition, the citation in the bibliography of the pages on which each paper is quoted, will enable the reader to collate the maximum information on any particular topic. However I must stress the fact that the list of references is far from complete (it could hardly be otherwise) and that there is inevitably a strong personal (though, I hope, not too biassed) factor in the selection of experiments recorded here. To any who might justifiably feel a little aggrieved that their work has not been mentioned or, even worse, has been misinterpreted, I would like to offer my regrets, and the excuse of my ignorance and the limitations naturally imposed on a book of this scope and pretension when it is written by a single individual.

In fact this edition, like the first, is far from being the product of my own unaided efforts. I owe a debt to many people for the help and advice they have generously given me. Among these is the late Professor J.B.S.Haldane who read the first edition while recuperating from his operation some months before his death, and who wrote me many long letters of invaluable comment and criticism, one of which opened characteristically with the words, "Dear Hayes, In this letter I am going to give you hell"—and did! Most of the alterations in the early chapters stem from his kindly suggestions and I am indeed grateful. I must also acknowledge my indebtedness to many colleagues and friends who have helped me greatly by advice and discussions about those topics on which they are expert, and/or by reading and criticising what I subsequently wrote about them. Foremost among these are Dr O.Darlington, Dr K.W.Fisher, Dr S.W.Glover, Dr J.D.Gross, Professor D.A.Hopwood, Dr D.Karamata, Dr Elinor Meynell, Professor M.H.Richmond, Dr D.A.Ritchie, Dr K.A. Stacey and Professor N.Symonds. I am particularly obliged to Dr.

J.A.Shapiro for the generous and incisive help he gave me in preparing the sections on lysogeny and on the genetic regulation of biosynthesis. To all these, and to others who have assisted in lesser but useful ways, I offer my sincere thanks, for without their support the task of writing this edition would have been much more burdensome.

In addition, I am again most grateful to those who have embellished this book by permitting me to reproduce their photographs, and particularly to those who provided me with originals. Those who have thus given new material for this second edition are Dr D.P. Allison, Dr J.Cairns, Dr L.Caro, Dr R.H.Epstein, Dr D.T.Hughes, Professor F.Jacob, Dr E.Kellenberger, Dr A.Lawn, Dr D.A.Ritchie and Dr A.Ryter. Finally I wish to pay tribute to my publishers, and especially to Mr Per Saugman and Mr J.L.Robson, for the kindly, tolerant and efficient manner with which they have dealt with the many delays in the production of this book which I have inadvertently inflicted on them.

Edinburgh, July 1968 William Hayes

POSTSCRIPT

Since the publication of the first edition of this book, two geneticists of outstanding distinction, who helped to lay the foundations of molecular genetics by their research and whom I am honoured to have known personally, have died—Milislav Demerec in the fullness of his years, and Harriet Ephrussi-Taylor with tragic prematurity. I felt I could not allow this edition to go to press without paying tribute not only to their key contributions to molecular biology, but also to their endearing qualities of personality and their many acts of kindness for which they will long be remembered by their friends. Their published work will ensure that they are not forgotten by posterity, as I hope the pages of this book make manifest.

W. H.

Preface to First Edition

This is intended to be a rather advanced text book on the genetics of bacteria and bacteriophages. It covers the major advances which have been made in the field during the last few years, and their interpretation at the molecular level. The structure of the book is based on what I conceive to be the requirements of an increasing number of postgraduate workers in related fields, who desire to master the concepts of microbial genetics and molecular biology well enough to be able to apply them to their own problems, to comprehend original papers and to assess future developments with understanding.

My experience of teaching postgraduate courses in microbial genetics, in which biochemists, bacteriologists, virologists and physicists predominate, is that the main barrier to perception is lack of knowledge of classical genetics and of the language (often misconstrued as jargon!) through which genetical principles are expressed. I have therefore devoted the early part of the book to a simple account of the facts and ideas upon which the theory of genetic analysis rests. There are two other reasons for adopting this approach instead of using the physico-chemical structure of the genetic material (DNA) as a starting point. Firstly, this book is primarily about what genes are and how they are inherited and expressed, and it is informative to trace the evolution of our concepts about them as an historical sequence. For example, the fact is insufficiently appreciated that all the essential features of the fine structure of the gene, as well as the idea that genes specify the structure of polypeptide chains rather than of proteins, were deduced from the results of genetic analysis alone and in no way depended on a knowledge of DNA architecture. Secondly, scientific truth is never absolute but only relative to what has gone before, so that the present state of our knowledge can only properly be assessed against its ideological background. The apex of a pyramid is significant only when it crowns the completed structure.

Much of this book is concerned with presenting the various novel genetic systems which have been found in microorganisms, and especially in bacteria and their viruses, against the background of more classical behaviour. However, the most exciting and revolutionary aspect of microbial genetics has come from the integration of genetic studies with biochemical and physical analyses of the synthesis, structure and function of nucleic acids and proteins, which are the main macromolecular constituents of cells. This collaboration has produced an explosion of new knowledge about the fundamental nature of genetic and other vital phenomena, in terms of the structure and behaviour of molecules. The molecular basis of the genetic code, its alteration by mutation, and its transcription and translation into specific protein synthesis are now well understood, not only in principle but also in considerable detail. Only the mechanism of genetic recombination has so far eluded analysis. The book describes and discusses these advances in molecular biology, although my limited knowledge and experience of the methods of chemistry and physics has necessarily imposed a presentation which may appear unsophisticated to the general reader and naïve (though not, I hope, misleading) to the expert. I trust that at least the fundamental concepts of molecular biology have been set forth in a clear and understandable way.

This book should have been completed three years ago. In many ways the delay has been fortunate, for otherwise large and important sections of it would now be out of date while I, instead of enjoying my new-won freedom, might well be back in prison rewriting it. As it is, the last three years have witnessed so many fundamental advances that the outlines of a rather clear picture are now beginning to take shape, while the book has grown to three times the size originally projected for it. Although there are still many problems to be solved and many details to be filled in, and although many of our current ideas may have to be modified as exceptions are discovered to the rules we now envisage, it seems probable that most of our present concepts will remain fruitful for some time to come. Apart from the mystery of recombination, I would hazard a guess that the fundamental advances of the future will probably spring from the application of these existing notions to the complex problems of the regulation and coordination of biochemical processes, of differentiation, and of development.

To keep the book within reasonable bounds, a high degree of selection has been necessary with respect to the experiments chosen to illustrate the development of various principles, and this selection is, of course, reflected in the bibliography. I make no claim that my choices are the best that could have been made. They were chosen mainly on the basis of personal familiarity or appeal, and because I considered that they illustrated well the points I wished to make. The degree of engagement involved in writing a book of this scope has precluded the simultaneous, critical reading of the deluge of papers, not to mention pre-prints, which now threaten to engulf us. The easiest way to escape drowning is to float on the surface of the flood and grasp what drifts within one's reach. In this process I have been greatly helped by my friends and colleagues, who have cast their nets more deeply and have presented me with the best of their catch.

William Hayes

London, July 1963

Acknowledgements to First Edition

It gives me great pleasure to acknowledge my indebtedness to all my friends and colleagues who have given me invaluable help, in one way or another, in the preparation of this book. Foremost among these are Dr R.C.Clowes, Dr K.W.Fisher, Professor R.H.Pritchard and Dr N.Symonds, who not only read and criticised large parts of the book in manuscript and proof but who, in many discussions, generously provided me with expert advice on the presentation of their own specialities. I would particularly like to express my gratitude to Professor Pritchard who patiently guided me past many pitfalls in the field of classical genetics into which I (and my readers!) would otherwise have fallen, and to Dr Symonds who, with equal patience and lucidity, initiated me into the mysteries of bacteriophage genetics. I hope that what they taught me will seem as clear at second hand. In addition, I must thank Dr J.Beckwith, Dr S.W.Glover, Dr J.D. Gross, Mr D.A.Ritchie, Dr S.D.Silver and Mr J.G.Scaife, each of whom provided me with frank and useful comments on one or more chapters.

I regard the photographic plates as a valuable adjunct to the book, since they help to link my rather theoretical approach with actuality. I am most grateful to the many authors, and to the editors of the journals concerned, who have permitted me to reproduce previously published photographs, the source of which is acknowledged in the respective legends. However, I would like to express my special gratitude to Dr T.F.Anderson, Dr J.Cairns, Mrs Maureen de Saxe, Dr G.W.Fuhs, Dr R.W.Horne, Dr E.Kellenberger, Dr J.C.Kendrew, Dr A.K.Kleinschmidt, Miss Janet Mitchell, Dr G.E.Palade, Dr H. Slizynska and Dr D.R.Stadler, all of whom kindly provided me with original photographs or negatives.

In writing the introductory chapters on classical genetics I have leaned heavily on *Principles of Genetics* by Sinnot, Dunn and Dobzhansky (McGraw-Hill), while my task has been much alleviated by the many excellent reviews which are referred to in the text and to whose authors I am indebted. Dr Alice Orgel and Dr J.Gordon, whose Ph.D.Theses I had the opportunity to read, helped me to clarify my views about the molecular basis of mutagenesis.

I also wish to thank my secretary, Miss Deirdre Nadal, for relieving me, in the most competent and helpful way, of so much administrative work which would otherwise have seriously interfered with the writing of this book. Finally I must pay tribute to my publishers, and in particular to Mr Per Saugman and Mr J.L.Robson, for the patient, efficient and charming manner in which they have cooperated with me in this enterprise.

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Part I
An Introduction to Genetics

CHAPTER 1

The Anatomy of Inheritance

Heredity means the process whereby men, mice, flies, plants, fungi, bacteria, viruses and all forms of living things reproduce themselves, or at least something unmistakably like themselves. If we had to define the phenomenon of life by a single one of its manifestations, this capacity for self-reproduction of an organised system would undoubtedly be the one that we would choose, for although we know of many processes of recreation and adaptation at the molecular level, the word 'life' suggests to us much more than this. It involves the idea not only of growth, organisation and continuity of existence over long periods of time, but also of change, of the capacity for modification of form in response to environmental changes which has expressed itself in evolution. Only heredity encompasses and unites all these ideas within a single category. Thus the science of *genetics*, which may be defined as the study and analysis of heredity in all its aspects, is the fundamental biological science, the focal point upon which all other aspects of biology necessarily converge.

The scientific method consists essentially in the observation and comparison (which is usually quantitative) of differences between things or phenomena which are otherwise equivalent; then follows the formulation of an hypothesis to correlate and explain the differences and, finally, the testing, by experiment, of predictions based on the hypothesis. If all the predictions prove correct, the hypothesis is elevated to the status of a theory which offers a new perspective from which to make further observations. The difficulty about science is to know what to observe and whether the differences we do observe are, in fact, between comparable things. The more complicated is the system we wish to study, the harder it is to make correct decisions on these matters, which is one of the reasons why knowledge of the mechanisms of evolution and heredity made little progress during the twenty-one centuries that elapsed between the time of Aristotle and that of Darwin and Mendel.

To study heredity we must begin with those differences that we can observe between organisms and their progeny. These differences are called *character* differences and they arise through *variation* which we will talk about later. The nature of the characters used in genetic analysis differs widely and may range from such obvious features as the colour of the eyes or skin, or the length and arrangement of limbs, wings, bristles or hair in vertebrates, to the ability or inability of fungi or bacteria to perform a single biochemical step in the synthesis of a particular amino acid or vitamin, or in the fermentation of a particular carbohydrate. We are more likely to understand correctly how a motor car works and is made if we are allowed to watch the individual parts being constructed and assembled, than if our observation is restricted to the completed product as it leaves the factory. For the same reason, one of the major advantages of microbial genetics is, as we shall see, the opportunity that microorganisms offer for the study of the inheritance of characters that we know to be simple and unitary. Confronted with gross morphological features in complex organisms, such as wing shape or bristle distribution in the fruit fly, *Drosophila*, which emerge as the end product of a long series of developmental events, we have no *a priori* way of telling just how complex the character really is and whether or not the same final difference results from alterations at the same or different points in the production line. To extend the analogy of the motor factory, if we have watched every step of manufacture and assembly and then observe that one of the cars leaving the factory will not go, we may know that the defect is a faulty carburettor. On the other hand, if we have only been permitted to look at the final products in a shop window, we cannot tell whether the fault lies in the carburettor, the ignition, the clutch or elsewhere; we only know that the car will not go and are unable to distinguish it from any other new car of the same make that will not go. Having made this point we must go on to state that the fundamental laws of genetics, and the greater part of our present detailed knowledge of inheritance, have come from the observation of overt characters in complex organisms, beginning with Mendel's study of such characters in peas as the surface texture and colour of the seeds, and the colour and position of the flowers. Conversely, many of the character differences employed in bacterial genetics may be imprecise. For example, the inheritance of a genetic alteration resulting in inability to ferment a carbohydrate such as lactose, or in resistance to an antibiotic, may be studied without knowing whether the alteration involves a change in the permeability of the cell wall or in one of several possible metabolic steps.

GENOTYPE AND PHENOTYPE

We have seen that genetics involves the study of character differences between parents and offspring, and the manner in which such differences are inherited. It is important to realise that what is inherited is not the character itself but *the potentiality to express it*, and that this potentiality may be profoundly affected by the environment to which the organism is exposed. The analogy between the mechanical operations of a factory and the biochemical activities of a cell is usually such an apt one that we shall often invoke it, though we will try not to give undue publicity to the motor industry. In the present instance, what we inherit are the blueprints which determine the specifications of the products, and the tools and jigs to make them. Before anything can be made, however, raw materials, a source of energy and suitable conditions for the workers are required and these come from the environment; variations in the environment may greatly alter the output and quality of the products.

There are many examples in nature of the influence of environment on the expression of the hereditary constitution. A striking example is the effect of temperature on hair pigmentation in Himalayan rabbits. The fur of the feet, ears and tails of these animals is black while that of the body is white, and this characteristic is faithfully inherited. If, however, the white fur is plucked from a part of the body and the animal is kept in the cold while new fur is growing, this new fur is black instead of white; conversely, if the black fur is removed and the depilated extremity is kept warm during regeneration, then the new fur is white instead of black. This experiment shows that what is inherited is not the pattern of distribution of black and white fur, but a dependence upon temperature of the ability to synthesise black pigment; the temperature of the feet, ears and tail is normally sufficiently lower than that of the rest of the body to permit pigment synthesis to occur.

Microbial genetics offers numerous demonstrations of this kind of effect, some obvious and others not so obvious. Among the more obvious effects is that of environment on certain morphological features of bacteria. Addition to the culture medium of phenol or ethanol in concentrations too low to affect growth, or even cultivation on a rather dry solid medium, may suppress completely the synthesis of flagella which are the whip-like organs of locomotion, while excess of calcium salts inhibits spore formation, but these features rapidly reappear in all the cells when they are transferred to a normal medium.

A great variety of variant strains of fungi and bacteria have been isolated which, unlike the original or *wild type* strain from which they were derived, are unable to synthesise one or more amino acids, or other factors required for growth, from elementary precursors; such strains are known as *auxotrophs* (Latin: *auxilium*=help; Greek: *trophē*=nourishment) while the nutritionally independent, wild type strains are called *prototrophs* (Greek: *protos*=first). An auxotrophic strain may be identified by its inability to grow and multiply in a synthetic medium lacking its specific growth requirements, in which the wild type, prototrophic strain flourishes. If, however, the synthetic medium is supplemented with the growth factor which the auxotroph cannot synthesise for itself, then auxotroph and prototroph may grow equally well in it and be indistinguishable. Similarly, strains of bacteria which have become resistant to the lethal action of antibiotics or bacterial viruses may appear identical to the sensitive, wild type strains unless the drug or virus is present in the environment to reveal the difference between them. In the fungus *Neurospora crassa* several types of variant have been described which react differently from the wild type with respect to temperature. One of these requires the amino acid tryptophan for growth at 25°C, but is prototrophic within the temperature range 30° to 35° when it produces the enzyme tryptophan synthetase which catalyses the condensation of indole and L-serine to form L-tryptophan. No active enzyme is made when the cells are grown at 25° in the presence of tryptophan, but a protein can be extracted from them which, although enzymically inactive, is antigenically related to the active enzyme; further purification of this protein restores some of its enzymic properties, apparently due to removal of zinc which acts as an inhibitor.

Another more complicated example of environmental influence, is the case of an inducible enzyme system in the bacterium, *Escherichia coli*. Strains of this organism may fail to ferment lactose because of one or the other of two inheritable defects: one defect involves the capacity to synthesise the enzyme β-galactosidase which initiates the fermentation of lactose by splitting it into glucose and galactose; the other prevents synthesis of a second enzyme, called permease, which mediates the penetration of lactose into the cell. If, now, we examine lactose-fermenting strains of *E. coli* for their capacity to produce β-galactosidase,