Genetic Elements in *Escherichia coli*

Frontispiece

UPPER: A heteroduplex molecule

Micrograph (A) and tracing (B) of a heteroduplex between plasmids RP4 and R68.45. These conjugal plasmids, isolated from *Pseudomonas aeruginosa*, carry genes conferring resistance to ampicillin, Kanamycin and tetracycline and have contour lengths of 19.1 μm and 19.7 μm respectively.

Note that the heteroduplex molecule is completely double stranded except for a single-stranded loop 0.6 μm long (arrowed). Thus R68.45 is probably an RP4 plasmid with a 0.6 μm (≈ 1.8 kb) insertion mutation.

Electron micrograph kindly provided by Dr Hans-Joachim Burkardt.

LOWER: Replicating molecules of ColE1 DNA

ColE1 DNA replicates unidirectionally in both the open circular and covalently closed (supercoiled) forms. The partially replicated intermediates produced by replication of the open circular form are typical θ -type molecules (C) but when the covalently closed form replicates only the newly replicated DNA is untwisted and the unreplicated part of the molecule remains supercoiled (D).

At the right (E) is an interpretation of the 'butterfly' intermediate shown in C. The newly synthesised strand of DNA is represented by the dotted line; one of the forks (arrowed) is the replication origin.

Micrographs by the courtesy of Dr Ronald R. Helinski.

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Series Editor: Dr C. J. Skidmore

Genetic Elements in *Escherichia coli*

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To Jean

By the same author
Genetic Structure and Function

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SERIES EDITOR'S PREFACE

The aim of this Series is to provide authoritative texts of a manageable size suitable for advanced undergraduate and postgraduate courses. Each volume will interpret a defined area of biology, as might be dealt with as a course unit, in the light of molecular research.

The growth of molecular biology in the ten years since the advent of recombinant DNA techniques has left few areas of biology unaffected. The information explosion that this has caused has made it difficult for large texts to keep up with the latest advances while retaining a proper treatment of the basics.

These books are thus a timely contribution to the resources of the student of biology. The molecular details are presented, clearly and concisely, in the context of the biological system. For, while there is no biology without molecules, there is more to biology than molecular biology. We do not intend to reduce biological phenomena to no more than molecular phenomena, but to point towards the synthesis of the biological and the molecular which marks the way forward in the life sciences.

C. J. Skidmore

PREFACE

In recent years molecular biology has acquired new dimensions largely as a result of the outstanding advances in molecular genetics, particularly the development of methods of analysis at the molecular level, culminating in our ability to isolate almost any gene from any organism, to purify it, sequence it, mutate it at will and then re-introduce it into another cell (often the bacterium *Escherichia coli*) where it can be expressed and produce protein. This book is intended to familiarise students of molecular biology with some of the fundamentals of bacterial and phage genetics and so to build a foundation for more advanced molecular genetic studies such as gene cloning, the use of restriction enzymes and recombinant DNA technology. As indicated by the title, I have (very largely) confined myself to considering *Escherichia coli* and its phages; *E. coli* is still the most widely used bacterium in molecular genetic studies and most of the concepts applied to *E. coli* apply equally to other bacterial species, particularly to the closely related species *Salmonella typhimurium*. Once, and only once, I have strayed into the field of fungal genetics as some of the most revealing information on the molecular mechanism of recombination — something fundamental to all genetic studies — has come from studies on gene conversion in the Ascomycetes.

I have assumed that the reader has taken introductory courses in biology and in genetics and is familiar with the basic principles of genetic structure and function in eucaryotes, including the construction of simple linkage maps in a diploid organism; the reader who has forgotten any of the jargon of genetics will find explanations of most of the technical terms in the Glossary.

In a book of limited size, the choice of topics is never simple and I have necessarily restricted myself to the particular aspects of bacterial and phage genetics that are likely to be the most useful to the intending molecular geneticist or molecular biologist; consequently, I have described the structure and mechanistic properties (replication,

recombination, transposition and transmission) of the most important genetic elements of *E. coli* but have almost completely omitted the important fields of gene expression, recombinant DNA technology and the applications of molecular genetics in the present day society. For the same reasons, some topics are considered in more depth than others. For example, insertion sequences and transposons are described in some detail as they are of considerable importance to molecular biologists while the mutational process is only briefly considered; although mutational theory is of great interest and importance, the principal use of mutation to the molecular biologist is as a tool to produce particular mutant phenotypes.

I have tried to arrange the material in a reasonably logical sequence, explaining new terms and developing new concepts as they arise. However, many readers will follow their own sequence of reading and to assist them the text is extensively cross-referenced and most of the terms and concepts are also explained in the Glossary. Some further material, which though relevant lies outside the mainstream of the text, is presented in the form of 'boxes'.

Instead of summaries at the end of each chapter, there is a set of self-assessing questions and problems; for the most part these relate to the most important material presented in that chapter, but a few of the questions require an understanding of previous chapters and these serve to link together closely related aspects of microbial and molecular genetics.

Since this book is primarily intended for molecular biologists rather than for geneticists, very few original references are cited. Instead, at the end of each chapter, there are selected references to review-type articles and monographs; these not only provide more advanced, but comparatively easy-to-understand, further reading but also cite many of the most important original research papers referred to in the text. Some further suggestions for more general reading are given at the end of the book. I firmly believe that a student can only appreciate the complexities of present day molecular genetics if he is familiar with some of the key experiments from past years. These were sometimes very simple but always very elegant and intellectually satisfying, and they not only illustrate the development of new analytical methods but, more importantly, the conclusions made from them have led to the development of what we now regard as established concepts. Space has prevented any detailed consideration of this important earlier work but many of the original key papers are included in the collections of reprints also listed at the end of the book.

I hope the reader will get as much pleasure and benefit from reading this book as I have from writing it.

P. F. Smith-Keary

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This book would not have been written but for the encouragement given to me by the Members of the Department of Genetics and I am most grateful for the advice and criticism they have given during its writing; in particular I would thank Dr Paul Sharp for his valuable assistance in correcting the proofs.

I extend my most sincere thanks to Dr H.-J. Burkhardt and Dr D. R. Helinski for providing the electron micrographs reproduced in the frontispiece.

ABBREVIATIONS AND SYMBOLS

(Note: *E. coli* gene symbols are explained in table 4.3; other gene symbols are explained in the text.)

A	adenosine	μm	micrometre (10^{-6} metres)
AP	apurinic or apyrimidinic site	Met	methionine
2-AP or AP	2-aminopurine	mRNA	messenger RNA
Asp	aspartic acid		
bp	base pair	nm	nanometre (10^{-9} metres)
5-BU or BU	5-bromouracil	<i>O</i>	operator region
C	cytidine	<i>ori</i>	replication origin
Col	a colicin plasmid	<i>P</i>	promoter region
<i>cos</i>	cohesive and sequence of lambda	PRR	post-replicative repair
Δ	deletion	R	a resistance plasmid
DNA	deoxyribonucleic acid	RNA	ribonucleic acid
DNase	deoxyribonuclease	RNase	ribonuclease
DP	DNA polymerase	RTF	resistance transfer factor
F	the <i>E. coli</i> fertility plasmid	SSR	site-specific recombination
G	guanosine	T	thymidine
Gly	glycine	Tn	transposon
HA	hydroxylamine	<i>tra</i>	transfer operon
Hfr	high-frequency recombination	tRNA	transfer RNA
IR	inverted repeat sequence	Trp	tryptophan
IS	insertion sequence	Tyr	tyrosine
kb	kilobases or kilobase pairs	U	uridine
Lac	lactose	UV	ultraviolet irradiation
Leu	leucine		