

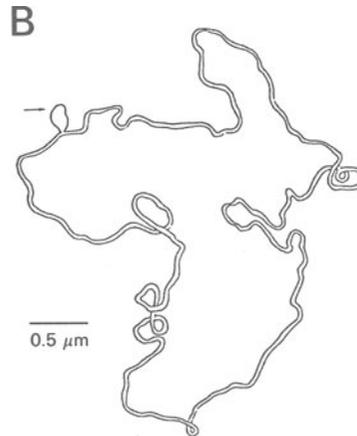
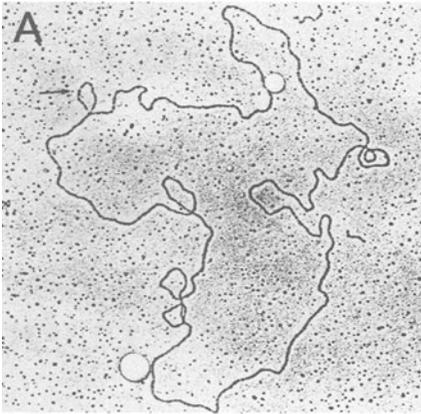
Genetic Elements
in Escherichia coli

Peter Smith-Keary



MACMILLAN MOLECULAR BIOLOGY

Genetic Elements in *Escherichia coli*



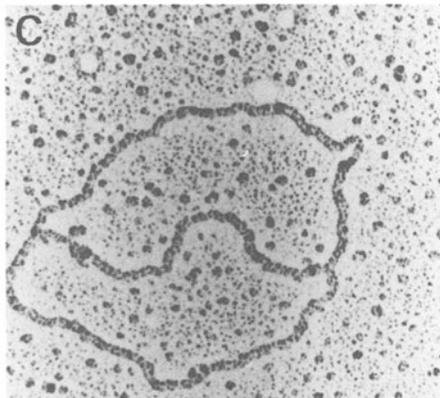
Frontispiece

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 (\approx 1.8 kb) insertion mutation.

Electron micrograph kindly provided by Dr Hans-Joachim Burkardt.

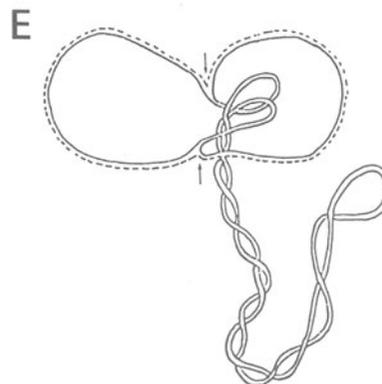
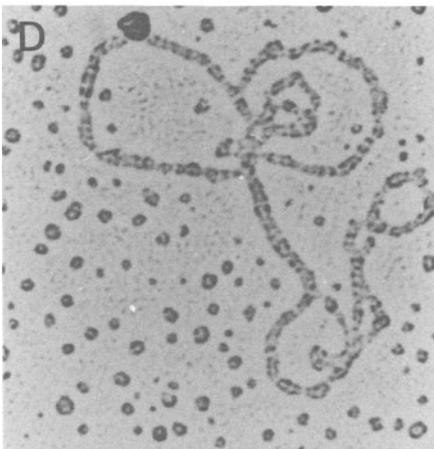


LOWER: Replicating molecules of ColE1 DNA

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.



MACMILLAN MOLECULAR BIOLOGY SERIES

Series Editor: Dr C. J. Skidmore

Genetic Elements in *Escherichia coli*

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MACMILLAN
EDUCATION

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To Jean

By the same author
Genetic Structure and Function

CONTENTS

<i>Series Editor's Preface</i>	xi
<i>Preface</i>	xii
<i>Acknowledgements</i>	xiv
<i>Abbreviations and Symbols</i>	xv
1 Structure and Replication of Genetic Elements in <i>E. coli</i>	1
1.1 Introduction	1
1.2 The structure of <i>E. coli</i>	1
1.3 The chromosome of <i>E. coli</i>	3
1.3.1 Replication of <i>E. coli</i> chromosome	4
1.3.2 The replication fork	6
1.4 Insertion sequences, transposons and plasmids	10
1.4.1 Plasmid replication and the control of copy number	12
1.5 Bacteriophage λ	15
1.5.1 The λ chromosome and its replication	18
1.5.2 Prophage integration and site-specific recombination	23
Exercises	26
References and related reading	26
2 Transduction and Transformation	27
2.1 Introduction	27
2.2 Generalised transduction	27
2.3 Mapping by transduction	32
2.3.1 The co-transduction method	33
2.3.2 The three-point transduction method	35
2.3.3 Deletion mapping	37
2.4 Specialised transduction	39
2.4.1 Low frequency transduction	41
2.4.2 High frequency transduction	43

2.5	Transformation	45
2.6	The transformation process	47
2.7	Mapping by transformation	49
2.8	Plasmid transformation	50
	Exercises	51
	References and related reading	53
3	Plasmids and Conjugation	54
3.1	Introduction	54
3.2	The F plasmid	55
3.2.1	The structure of F	55
3.2.2	The F-pilus	56
3.2.3	The transfer of F DNA	57
3.2.4	The formation of Hfr strains	58
3.2.5	The origin of F' strains	60
3.3	R plasmids	62
3.3.1	The relationships between F and R plasmids	63
3.3.2	The structure of R plasmids	66
3.4	The Col plasmids	67
3.5	Recombinant plasmids	68
3.5.1	Restriction endonucleases	70
3.5.2	Gene cloning using pBR322	72
	Exercises	74
	References and relating reading	75
4	Genetic Analysis using Hfr and F' Strains of <i>E. coli</i>	76
4.1	Introduction	76
4.2	Chromosome transfer by Hfr cells	77
4.3	Mapping by gradient of transmission	79
4.4	Mapping by interrupted mating	81
4.5	The linkage map of <i>E. coli</i>	83
4.6	Genetic analysis using F' strains	86
4.6.1	The fate of F' plasmids after transfer	88
	Exercises	88
	References and related reading	90
5	Recombination	91
5.1	Introduction	91
5.2	Aberrant segregations	93
5.3	The molecular basis of genetic recombination	94
5.4	The Holliday model for genetic recombination	95
5.4.1	Post-meiotic segregation and error correction	97
5.4.2	Reciprocal and non-reciprocal heteroduplex segments	99
5.5	The Meselson and Radding model for genetic recombination	99
5.6	Recombination in bacteria and bacteriophage	101
5.6.1	The RecA protein of <i>E. coli</i>	104
5.6.2	The resolution of chi-form molecules	106
5.7	Chi sites in λ	106

5.8	Recombinational pathways in <i>E. coli</i>	108
5.8.1	The <i>RecE</i> pathway	109
5.8.2	The <i>RecF</i> pathway	110
	Exercises	111
	References and related reading	111
6	Repair and Mutation	113
6.1	DNA repair	113
6.2	Types of DNA damage	113
6.3	DNA repair systems	115
6.4	The reversal of damage to DNA – photoreactivation	115
6.5	Excision repair	116
6.5.1	Mismatch repair	118
6.5.2	N-glycosylase excision repair systems	118
6.6	Post-replicative repair (SOS)	120
6.6.1	By-passing unexcised dimers	122
6.6.2	A model for post-replication repair	122
6.7	The SOS hypothesis	123
6.7.1	A model for SOS induction	124
6.8	Mutation	126
6.8.1	The isolation of mutants	127
6.8.2	Types of mutation	128
6.8.3	The effect of point mutations on protein structure	129
6.9	Mutagenesis	131
6.10	The molecular basis of mutagen activity	132
6.10.1	Base analogue mutagens	132
6.10.2	Mutagens that modify bases in DNA	135
6.10.3	Mutagens that bind to DNA	136
6.10.4	Electromagnetic irradiation	136
6.11	Hot-spots	137
	Exercises	138
	References and related reading	139
7	Transposable Genetic Elements	140
7.1	Introduction	140
7.2	Insertion sequences	141
7.3	Transposons	144
7.3.1	Composite transposons	144
7.3.2	Complex transposons	147
7.4	Transposition	147
7.4.1	The transposition of Tn3	149
7.4.2	The mechanism of transposition	150
7.5	Chromosome aberrations induced by transposable elements	155
7.6	Factors affecting transposition	157
7.6.1	The regulation of transpositional activity by DNA methylation	157

7.7	The uses of transposable elements in molecular biology	159
	Exercises	160
	References and related reading	161
8	Bacteriophages	163
8.1	Introduction	163
8.2	Bacteriophage Mu	163
8.2.1	The Mu genome	163
8.2.2	Lysogenisation by Mu	165
8.2.3	Mu – the lytic cycle	165
8.2.4	The invertible G-segment of Mu	166
8.2.5	The genetic uses of Mu	167
8.3	Site-specific recombination in λ	169
8.3.1	The structure of the attachment sites	169
8.3.2	<i>attP</i> \times <i>attB</i> site-specific recombination	170
8.4	Phages with single-stranded DNA	171
8.4.1	Phage X174, an icosahedral phage with single-stranded DNA	172
8.4.2	M13, a filamentous phage with single-stranded DNA	172
	Exercises	176
	References and related reading	177
	<i>Glossary</i>	178
	<i>Further Reading</i>	188
	<i>Index</i>	190

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