

A dark, textured background with a pattern of irregular, interconnected lines and spots, resembling a microscopic view of DNA or a similar biological structure. The lines are dark brown or black, and the spots are lighter, creating a complex, organic pattern.

# DNA

Insertion  
Elements,  
Plasmids,  
and Episomes

# **DNA**

## **Insertion Elements, Plasmids, and Episomes**

Edited by

**A.I. Bukhari  
J.A. Shapiro  
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**Cold Spring Harbor Laboratory 1977**

**DNA  
Insertion  
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and Episomes**

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**Cover:** An example of stem-loop structures seen when DNA from *E. coli* is denatured and reannealed. Inverted duplications, separated by unique sequences, snapped back to form these structures. In the cover picture, the stem has a length equal to that of IS1, 0.8 kb, and the loop consists of 13.7 kb. (*Photograph by L.T. Chow and T.R. Broker, Cold Spring Harbor Laboratory*)

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# Preface

The Cold Spring Harbor Laboratory was the scene of an exciting meeting on DNA Insertions from May 18 to May 21, 1976. The meeting brought together biologists whose interests ranged from *Escherichia coli* to maize and from bacteriophage  $\lambda$  to herpesviruses. It is not possible in a scientific monograph to capture entirely the spirit of the meeting—the excitement, the inspired discussions. This book is an experiment in publishing the proceedings of a meeting. The book presents not only papers that were discussed at the meeting but also other articles and information that we hope readers will find useful. We have made an effort to scan a broad spectrum of studies and to focus on many facets of the insertion phenomena. The introduction outlines the topics covered and explains the organization of the book.

The DNA Insertions meeting and this monograph would not have been possible without the encouragement and support of J.D. Watson, Director of the Cold Spring Harbor Laboratory. The meeting was supported by funds from the National Science Foundation (PCM 76-06478), the National Cancer Institute (CA-13106), and the Cold Spring Harbor Laboratory.

We are indebted to all of our colleagues who participated in the meeting: to D. Botstein, A. Campbell, H. Lewis, M. Malamy, M. Meselson, R. Novick, J. Sambrook, A.L. Taylor, and R. Weisberg for chairing the sessions, and to S. Brenner for ending the meeting with his spirited summary. We are also grateful to B. McClintock, W. Szybalski, S. Cohen, and other colleagues for their help and advice. We are especially indebted to the authors who contributed their papers and who patiently listened to the various editorial demands.

We wish to express our appreciation to Gladys Kist of the meetings office for the meeting arrangements, to Bob Yaffee for artwork, and to Roberta Salant, Annette Zaninovic, and Judith Atkin of the publications office for help with the preparation of the book. Special thanks are due to Nancy Ford, Director of Publications, who guided the development of this book.

**A.I. Bukhari**  
**J.A. Shapiro**  
**S.L. Adhya**

# Contents

Preface	xiii
Introduction: New Pathways in the Evolution of Chromosome Structure <i>J.A. Shapiro, S.L. Adhya and A.I. Bukhari</i>	3
The Nomenclature Problem	13
Nomenclature of Transposable Elements in Prokaryotes <i>A. Campbell, D. Berg, D. Botstein, E. Lederberg, R. Novick, P. Starlinger and W. Szybalski</i>	15
<b>SECTION I IS Elements</b>	
Mutations Caused by the Integration of IS1 and IS2 into the <i>gal</i> Operon <i>P. Starlinger</i>	25
Specific Sites for Integration of IS Elements within the Transferase Gene of the <i>gal</i> Operon of <i>E. coli</i> K12 <i>D. Pfeifer, P. Habermann and D. Kubai-Maroni</i>	31
The <i>gal3</i> Mutation of <i>E. coli</i> <i>A. Ahmed</i>	37
Repeated DNA Sequences in Plasmids, Phages, and Bacterial Chromosomes <i>H. Ohtsubo and E. Ohtsubo</i>	49
IS1 and IS2 in <i>E. coli</i> : Implications for the Evolution of the Chromosome and Some Plasmids <i>H. Saedler</i>	65

The Organization of Putative Insertion Sequences on the <i>E. coli</i> Chromosome <i>L.T. Chow</i>	73
Chromosomal Rearrangements in the <i>gal</i> Region of <i>E. coli</i> K12 after Integration of IS1 <i>H.-J. Reif and H. Saedler</i>	81
Polarity of Insertion Mutations Is Caused by Rho-mediated Termination of Transcription <i>A. Das, D. Court, M. Gottesman and S. Adhya</i>	93
Isolation of Mutations in Insertion Sequences That Relieve IS-induced Polarity <i>P.K. Tomich and D.I. Friedman</i>	99
The Isolation of IS1 and IS2 DNA <i>F. Schmidt, J. Besemer and P. Starlinger</i>	109
Physical Mapping of IS1 by Restriction Endonucleases <i>N.D.F. Grindley</i>	115
Preliminary Observations	
A. On Mutations Affecting IS1-mediated Deletion Formation in <i>E. coli</i> <i>P. Nevers, H.-J. Reif and H. Saedler</i>	125
B. On the Presence of a Promoter in IS2 <i>B. Rak</i>	129
C. On the Polarity of Insertion Mutations <i>J. Besemer</i>	133
 <b>SECTION II Transposons and Plasmids</b>	
Formation of Conjugative Drug Resistance (R) Plasmids <i>S. Mitsuhashi, H. Hashimoto, S. Iyobe and M. Inoué</i>	139
Recombination between <i>Pseudomonas aeruginosa</i> Plasmids of Incompatibility Groups P-1 and P-2 <i>G.A. Jacoby and A.E. Jacob</i>	147
Transposition of a Plasmid DNA Sequence Which Mediates Ampicillin Resistance: General Description and Epidemiologic Considerations <i>F. Heffron, C. Rubens and S. Falkow</i>	151
Deletions Affecting Transposition of an Antibiotic Resistance Gene <i>F. Heffron, P. Bedinger, J. Champoux and S. Falkow</i>	161
Promotion of Insertions and Deletions by Translocating Segments of DNA Carrying Antibiotic Resistance Genes <i>J. Brevet, D.J. Kopecko, P. Nisen and S.N. Cohen</i>	169

Transposition of Tn1 to a Broad-host-range Drug Resistance Plasmid <i>J.P. Hernalsteens, R. Villarroel-Mandiola, M. Van Montagu and J. Schell</i>	179
Translocation and Illegitimate Recombination by the Tetracycline Resistance Element Tn10 <i>D. Botstein and N. Kleckner</i>	185
Insertion and Excision of the Transposable Kanamycin Resistance Determinant Tn5 <i>D.E. Berg</i>	205
Transposition and Deletion of Tn9: A Transposable Element Carrying the Gene for Chloramphenicol Resistance <i>J.L. Rosner and M.M. Gottesman</i>	213
Physical Structure and Deletion Effects of the Chloramphenicol Resistance Element Tn9 in Phage Lambda <i>L.A. MacHattie and J.B. Jackowski</i>	219
Structure and Location of Antibiotic Resistance Determinants in Bacteriophages P1Cm and P7 ( $\phi$ Amp) <i>T. Yun and D. Vapnek</i>	229
Amplification of the Tetracycline Resistance Determinant on Plasmid pAM $\alpha$ 1 in <i>Streptococcus faecalis</i> <i>D.B. Clewell and Y. Yagi</i>	235
 <b>SECTION III Bacteriophage Mu: A Transposable Element</b>	
The Mechanism of Bacteriophage Mu Integration <i>A.I. Bukhari, E. Ljungquist, F. de Bruijn and H. Khatoon</i>	249
Characterization of Covalently Closed Circular DNA Molecules Isolated after Bacteriophage Mu Induction <i>B.T. Waggoner, M.L. Pato and A.L. Taylor</i>	263
Mu-mediated Illegitimate Recombination as an Integral Part of the Mu Life Cycle <i>A. Toussaint, M. Faelen and A.I. Bukhari</i>	275
On the <i>kil</i> Gene of Bacteriophage Mu <i>P. van de Putte, G. Westmaas, M. Giphart and C. Wijffelman</i>	287
Bacteriophage Mu Genome: Structural Studies on Mu DNA and Mu Mutants Carrying Insertions <i>L.T. Chow and A.I. Bukhari</i>	295
Electron Microscope Studies of Nondefective Bacteriophage Mu Mutants Containing Deletions or Substitutions <i>L.T. Chow, R. Kahmann and D. Kamp</i>	307



Structure and Packaging of Mu DNA <i>E. Bade, H. Delius and B. Allet</i>	315
DNA Partial Denaturation Mapping Studies of Packaging of Bacteriophage Mu DNA <i>M.M. Howe, M. Schnöds and R.B. Inman</i>	319
Asymmetric Hybridization of Mu Strands with Short Fragments Synthesized during Mu DNA Replication <i>C. Wijffelman and P. van de Putte</i>	329
Mapping of Restriction Sites in Mu DNA <i>R. Kahmann, D. Kamp and D. Zipser</i>	335
 <b>SECTION IV Nonhomologous Recombination and the <math>\lambda</math> Paradigm</b>	
Flexibility in Attachment-site Recognition by $\lambda$ Integrase <i>L. Enquist and R. Weisberg</i>	343
Isolation of <i>Escherichia coli</i> Mutants Unable to Support Lambda Integrative Recombination <i>H.I. Miller and D.I. Friedman</i>	349
A Mutant of <i>Escherichia coli</i> Deficient in a Host Function Required for Phage Lambda Integration and Excision <i>J.G.K. Williams, D.L. Wulff and H.A. Nash</i>	357
Integrative Recombination of Bacteriophage $\lambda$ – The Biochemical Approach to DNA Insertions <i>H.A. Nash, K. Mizuuchi, R.A. Weisberg, Y. Kikuchi and M. Gellert</i>	363
The Integrase Promoter of Bacteriophage Lambda <i>A. Campbell, L. Heffernan, S.-L. Hu and W. Szybalski</i>	375
Position Effects of Insertion Sequences IS2 near the Genes for Prophage $\lambda$ Insertion and Excision <i>J. Zissler, E. Mosharrafa, W. Pilacinski, M. Fiandt and W. Szybalski</i>	381
The Phage $\lambda$ Integration Protein (Int) Is Subject to Control by the <i>cII</i> and <i>cIII</i> Gene Products <i>D. Court, S. Adhya, H. Nash and L. Enquist</i>	389
Temperate Coliphage P2 as an Insertion Element <i>R. Calendar, E.W. Six and F. Kahn</i>	395
Recombination Models for the Inverted DNA Sequences of the Gamma-Delta Segment of <i>E. coli</i> and the G Segments of Phages Mu and P1 <i>T.R. Broker</i>	403

An Electron Microscope Study of Actively Recombining Plasmid DNA Molecules	409
<i>H. Potter and D. Dressler</i>	

## **SECTION V Eukaryotic Systems**

An Introductory Note on the Controlling Elements in Maize	425
The Position Hypothesis for Controlling Elements in Maize	429
<i>P.A. Peterson</i>	
The Case for DNA Insertion Mutations in <i>Drosophila</i>	437
<i>M.M. Green</i>	
"Flip-Flop" Control and Transposition of Mating-type Genes in Fission Yeast	447
<i>R. Egel</i>	
The Cassette Model of Mating-type Interconversion	457
<i>J.B. Hicks, J.N. Strathern and I. Herskowitz</i>	
The Origin and Complexity of Inverted Repeat DNA Sequences in <i>Drosophila</i>	463
<i>R.F. Baker and C.A. Thomas, Jr.</i>	
Nucleotide Sequence Arrangements in the Genome of Herpes Simplex Virus and Their Relation to Insertion Elements	471
<i>W.C. Summers and J. Skare</i>	
The Structure of the Adeno-associated Virus Genome	477
<i>B.J. Carter, L.M. de la Maza and F.T. Jay</i>	

## **SECTION VI Genetic Rearrangements; Techniques and Applications**

Mapping Ribosome Protein Genes in <i>E. coli</i> by Means of Insertion Mutations	487
<i>S.R. Jaskunas and M. Nomura</i>	
Chromosomal Rearrangements Resulting from Recombination between Ribosomal RNA Genes	497
<i>C.W. Hill, R.H. Grafstrom and B.S. Hillman</i>	
Potential of RP4::Mu Plasmids for In Vivo Genetic Engineering of Gram-negative Bacteria	507
<i>J. Dénarié, C. Rosenberg, B. Bergeron, C. Boucher, M. Michel and M. Barate de Bertalmio</i>	
In Vivo Genetic Engineering: The Mu-mediated Transposition of Chromosomal DNA Segments onto Transmissible Plasmids	521

<i>M. Faelen, A. Toussaint, M. Van Montagu, S. Van den Elsacker, G. Engler and J. Schell</i>	
Construction and Use of Gene Fusions Directed by Bacteriophage Mu Insertions	531
<i>M.J. Casadaban, T.J. Silhavy, M.L. Berman, H.A. Shuman, A.V. Sarthy and J.R. Beckwith</i>	
In Vivo Genetic Engineering: Exchange of Genes between a Lambda Transducing Phage and ColE1 Factor	537
<i>K. Shimada, Y. Fukumaki and Y. Takagi</i>	
Translocation of Ampicillin Resistance from R Factor onto ColE1 Factor Carrying Genes for Synthesis of Guanine	543
<i>S. Maeda, K. Shimada and Y. Takagi</i>	
Selected Translocation of DNA Segments Containing Antibiotic Resistance Genes	549
<i>P.J. Kretschmer and S.N. Cohen</i>	
Detection of Transposable Antibiotic Resistance Determinants with Phage Lambda	555
<i>D.E. Berg</i>	
Properties of the Plasmid RK2 as a Cloning Vehicle	559
<i>R.J. Meyer, D. Figurski and D.R. Helinski</i>	
Insertion of Mu DNA Fragments into Phage $\lambda$ In Vitro	567
<i>D.D. Moore, J.W. Schumm, M.M. Howe and F.R. Blattner</i>	
The <i>E. coli</i> Gamma-Delta Recombination Sequence Is Flanked by Inverted Duplications	575
<i>T.R. Broker, L.T. Chow and L. Soll</i>	

## SECTION VII Appendices

### APPENDIX A IS Elements

1. IS Elements in <i>Escherichia coli</i> , Plasmids, and Bacteriophages	583
<i>Compiled by W. Szybalski</i>	
2. Sequence of the Ends of IS1 Element	591
<i>Contributed by H. Ohtsubo and E. Ohtsubo</i>	
3. DNA Sequences at the Ends of IS1	595
<i>Contributed by N.D.F. Grindley</i>	
4. Nucleotide Sequences at Two Sites for IS2 DNA Insertion	597
<i>Contributed by R.E. Musso and M. Rosenberg</i>	

## APPENDIX B Bacterial Plasmids

1. Tables	
a. Introduction	601
<i>By J.A. Shapiro</i>	
b. Plasmids Studied in <i>Escherichia coli</i> and Other Enteric Bacteria	607
<i>Compiled by A.E. Jacob, J.A. Shapiro, L. Yamamoto, D.I. Smith, S.N. Cohen and D. Berg</i>	
c. Plasmids Studied in <i>Pseudomonas aeruginosa</i> and Other Pseudomonads	639
<i>Compiled by G.A. Jacoby and J.A. Shapiro</i>	
d. Plasmids of <i>Staphylococcus aureus</i>	657
<i>Compiled by R.P. Novick, S. Cohen, L. Yamamoto and J.A. Shapiro</i>	
e. Plasmids of Other Gram-positive Bacteria	663
<i>Compiled by A.E. Jacob, J.A. Shapiro and L. Yamamoto</i>	
f. Plasmids Constructed In Vitro and In Vivo	665
<i>Compiled by S.N. Cohen</i>	
2. Maps	
a. F, the <i>E. coli</i> Sex Factor	671
<i>Contributed by J.A. Shapiro</i>	
b. Special Sequences in the Structure of Cointegrate Drug Resistance Plasmids Related to F	672
<i>Contributed by S.N. Cohen</i>	
c. <i>EcoRI</i> , <i>HindIII</i> , and <i>BamHI</i> Cleavage Map of R538-1	674
<i>Contributed by D. Vapnek</i>	
d. Tn7 Insertion Map of RP4	675
<i>Contributed by P.T. Barth and N.J. Grinter</i>	
e. Physical Map of RP4	678
<i>Contributed by A. DePicker, M. Van Montagu and J. Schell</i>	
f. Restriction Enzyme Map of RK2	680
<i>Contributed by R. Meyer, D. Figurski and D.R. Helinski</i>	
g. Restriction Map of the ColE1 Derivative pCR1	681
<i>Contributed by K. Armstrong and D.R. Helinski</i>	
h. Restriction Map of ColE1 and pNT1 Plasmids	682
<i>Contributed by H. Ohmori and J.-I. Tomizawa</i>	
i. The Circular Restriction Map of pBR313	684
<i>Contributed by F. Bolivar, R.L. Rodriguez, M.C. Betlach and H.W. Boyer</i>	
j. The Circular Restriction Map of pBR322	686
<i>Contributed by F. Bolivar, R.L. Rodriguez, P.J. Greene, M.C. Betlach, H.L. Heyneker, H.W. Boyer, J.H. Crosa and S. Falkow</i>	

3.	Bibliography	689
	<i>Compiled by J.A. Shapiro, A.E. Jacob, R.P. Novick and L. Yamamoto</i>	

### APPENDIX C Temperate Bacteriophages

1.	The Genomes of Temperate Viruses of Bacteria	705
	<i>Contributed by E. Ljungquist and A.I. Bukhari</i>	
2a.	Genetic, Physical, and Restriction Map of Bacteriophage $\lambda$	713
	<i>Contributed by S. Gottesman and S. Adhya</i>	
b.	Restriction Enzyme Cleavage Maps of Bacteriophage $\lambda$ with a Focus on the Attachment-site Region	719
	<i>Contributed by D. Kamp and R. Kahmann</i>	
3.	Genetic and Physical Structure of Bacteriophage P1 DNA	721
	<i>Contributed by M.B. Yarmolinsky</i>	
4.	Genetic and Physical Map of Bacteriophage P2	733
	<i>Contributed by D.K. Chattoraj</i>	
5.	Genetic, Physical, and Restriction Map of Bacteriophage P22	737
	<i>Contributed by M. Susskind and D. Botstein</i>	
6.	Genetic, Physical, and Restriction Map of Bacteriophage $\phi 80$	741
	<i>Contributed by P. Youderian</i>	
7.	Genetic and Physical Map of Bacteriophage Mu	745
	<i>Contributed by B. Allet, F. Blattner, M. Howe, M. Magazin, D. Moore, K. O'Day, D. Schultz and J. Schumm</i>	
8.	Bacteriophage Mu: Methods for Cultivation and Use	749
	<i>Contributed by A.I. Bukhari and E. Ljungquist</i>	

### APPENDIX D Restriction Endonucleases

1.	Restriction and Modification Enzymes and Their Recognition Sequences	757
	<i>Compiled by R.J. Roberts</i>	

<b>Index</b>		<b>769</b>
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# Introduction: New Pathways in the Evolution of Chromosome Structure

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This book deals with a new class of genetic elements: DNA insertions. The existence of DNA insertion elements has become clear from a decade of research on the expression and structure of bacterial genomes. Recent developments indicate that DNA insertion elements serve a special evolutionary function. They mediate the integration of one segment of genetic information into another. This process is independent of previously recognized mechanisms for the interaction of DNA molecules. There are suggestions that DNA insertion elements exist not only in prokaryotic cells but also in eukaryotic cells. Thus we face the discovery of new pathways for the reassortment of genetic information and the evolution of chromosome structure in both higher and lower organisms.

Because the study of DNA insertion elements is so young, hypotheses and problems far outnumber accepted concepts. The papers in this book constitute a first attempt to define these problems and state working models to resolve them. They represent the state of knowledge about insertion elements as it stood at the time of the DNA Insertions Meeting at Cold Spring Harbor Laboratory in May, 1976. The purpose of this introduction is to explain the developments which prompted that meeting and present our view of how several different lines of research converged to define an exciting new field in genetics.

About a decade ago, the first examples of DNA insertion elements in bacterial systems were recognized in laboratories whose primary interest was the regulation of gene expression, not chromosome structure. Analysis of various operons in *Escherichia coli* revealed an unexpected class of pleiotropic mutations. They



were often located in structural genes for pathway enzymes, were neither base substitution, frameshift, nor deletion mutations, and exerted strong polar effects on the expression of other cistrons distal to the operon promoter. Eventually it was shown that these mutations resulted from the insertion of fairly large segments (700 to 1400 base pairs) of DNA into the structural or regulatory genes of these operons. Such insertions were observed in a number of genetic systems, including the *E. coli gal*, *lac*, and ribosomal protein operons and bacteriophage  $\lambda$ , P1, and P2. Further analysis of independent insertions by electron microscope heteroduplex methods revealed that many of them appear to be identical. Thus a limited number of specific DNA sequences can insert themselves into different sites in the bacterial genome to shut off gene activity. To date, repeated examples of four such insertion sequences (IS) have been documented; these have been labeled IS1, IS2, IS3, and IS4. They contain, respectively, about 800, 1300, 1200, and 1400 base pairs. A complete tabulation of these and other unclassified *E. coli* insertion mutations can be found in Appendix A1.

The discovery that the same sequences<sup>1</sup> can each appear repeatedly at one or more sites in the bacterial genome leads to an important conclusion: insertion mutations are not the result of random noise in the usually accurate mechanisms for replication and segregation of the genetic material. Indeed, further studies turned up many interesting characteristics of insertion sequences. The bacterial chromosome contains multiple copies of at least some IS elements. IS1 and IS4 exert polar effects when linearly inserted in either of the two possible ways (orientations I and II), whereas IS2 and IS3 are known to be polar only in one direction (orientation I). Moreover, IS2 seems to contain a site which acts as a promoter for gene expression when present in the nonpolar orientation II. Insertion can occur at many sites but is not random, and there are "hot spots" for IS-induced mutations. The presence of some IS elements can lead to a high frequency of spontaneous deletions, which begin at the site of insertion. The one aspect of IS physiology that has received the most attention so far is the effect of insertion mutations on gene expression. We know that at least some of them are polar because they contain sites for the action of the transcription termination factor rho. Although no clear picture is yet available for all the phenotypic consequences of insertion mutations, this short list indicates that IS elements have a complex structure. Presumably, this structure is the result of natural selection for some function(s) useful to the bacterial cell. One such function could involve the control of gene expression. The papers in Section I describe the history, physiology, and structure of IS mutations.

It would be unlikely that these complex genetic elements evolved simply as mutator elements. Some clues about the normal function of IS elements have emerged from a different area of research in bacterial genetics—the study of

<sup>1</sup> It should be remembered that sequence identity is established by heteroduplex formation. This does not exclude small differences in nucleotide sequences.