

EDWARD A. BIRGE

# Bacterial and Bacteriophage Genetics

An Introduction  
SECOND EDITION



Springer-Verlag

Edward A. Birge

# **Bacterial and Bacteriophage Genetics**

An Introduction

Second Edition

With 150 Figures



Springer-Verlag  
New York Berlin Heidelberg  
London Paris Tokyo

Edward A. Birge  
Department of Microbiology  
Arizona State University  
Tempe, Arizona 85287

LIBRARY OF CONGRESS

Library of Congress Cataloging-in-Publication Data

Birge, Edward A. (Edward Asahel) .

Bacterial and bacteriophage genetics : an introduction / Edward A.  
Birge.—2nd ed.

p. cm.—(Springer series in microbiology)

Includes bibliographies and index.

ISBN 0-387-96644-7

1. Bacterial genetics. 2. Bacteriophage—Genetics. I. Title.

II. Series.

[DNLM: 1. Bacteria—genetics. 2. Bacteriophages—genetics. QW

51 B617b]

QH434.B57 1988

589.9'015—dc19

DNLM/DLC

for Library of Congress

88-12241

CIP

© 1981, 1988 by Springer-Verlag New York Inc.

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer-Verlag, 175 Fifth Avenue, New York, NY 10010, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use of general descriptive names, trade names, trademarks, etc. in this publication, even if the former are not especially identified, is not to be taken as a sign that such names, as understood by the Trade Marks and Merchandise Marks Act, may accordingly be used freely by anyone.

Typeset by David E. Seham Associates/Metuchen, New Jersey.

Printed and bound by Arcata Graphics/Halliday, West Hanover, Massachusetts.

Printed in the United States of America.

9 8 7 6 5 4 3 2 1

ISBN 0-387-96696-X

ISBN 3-540-96696-X

Springer-Verlag New York Berlin Heidelberg

Springer-Verlag Berlin Heidelberg New York

# Springer Series in Microbiology

**For Lori, Anna, and Colin**

# Preface to the Second Edition

It is with real pleasure that I offer some introductory remarks to this second edition of *Bacterial and Bacteriophage Genetics*. The reception of the first edition was very good, and most of the criticisms offered have only served to strengthen this new edition. The majority of the suggestions for revision of material included requests for more molecular detail. I have tried to provide this throughout the text but especially in the heavily revised Chapter 1 and completely rewritten Chapters 2 and 10. The former Chapter 2 has been repositioned as an appendix so as to offer an uninterrupted flow of material in the main body of the text.

The tremendous increase in our knowledge of the genetics of eukaryotic microorganisms has permitted another sort of change in coverage. Instead of general discussions about eukaryotic organisms, wherever possible I have tried to offer specific details about *Saccharomyces cerevisiae*, whose wealth of genetic detail will soon rival that of *Escherichia coli*. Although this material is not intended as a substitute for a course in yeast genetics, it is my hope that it will enable the beginning student to comprehend some of the similarities and differences between these two popular microorganisms.

As before this book is intended for the student who is taking a first course in bacterial and bacteriophage genetics and who brings to it some background in genetics. The best background would be an introductory course in general genetics, but extensive coverage of genetics in an introductory biology course might well prove sufficient. As an example, it is assumed that the student is familiar with the standard Watson and Crick model for DNA structure. A broad knowledge of microorganisms is helpful but not required to understand the material presented. In general the material from a good introductory biology course should be adequate.

Several books can be noted as being particularly useful sources of detailed supplementary information. David Freifelder's *Physical Biochemistry*, Second Edition, is particularly valuable as a resource for methods used in analyzing macromolecules. Additional information about *Saccharomyces* can be found in *Yeast Genetics: Fundamental and Applied Aspects* edited by J.F.T. Spencer, D.M. Spencer, and A.R.W. Smith. Cold Spring Harbor Laboratory constantly publishes many monographs dealing with various aspects of bacterial and viral genetics. Their current book list should be consulted for details. Probably the most important one for this book is *Genetic Maps*, Volume 4, edited by S.J. O'Brien. The American Society for Microbiology also is a good source for monographic literature. Their recent publications include *Escherichia coli and Salmonella typhimurium: Cell and Molecular Biology*, edited by F.W. Neidhardt. Most references to classic papers have been omitted, and the reader is referred to collections of papers that have been reprinted such as the volume edited by Abou-Sabe.

# Acknowledgments

I am indebted to many people for helpful suggestions and comments about the first edition. I would particularly like to acknowledge the following individuals who assisted in correcting errors in the first edition: Dr. Paul A. Lemke, Auburn University; Dr. Margarita Salas, Universidad Autonoma de Madrid; and Dr. T.A. Trautner, Max Planck Institut, Berlin. Dr. W. Scott Champney's suggestions on reorganizing the material were most helpful. Dr. Martha Howe graciously volunteered some assistance with the material on phage Mu. However, any errors that remain must be attributed to me.

It has once again been a pleasure to work with the people at Springer-Verlag who have been very helpful during all phases of this revision.



# Linkage Maps

*Inside the front cover* The illustrations on the front inside cover are linear scale drawings representing the circular linkage map of *E. coli* K-12. The time scale of 100 minutes, beginning arbitrarily with zero at the *thr* locus, is based on the results of interrupted-conjugation experiments. Major genetic symbols used in this figure are defined in Table 2-2. Parentheses around a gene symbol indicate that the position of that marker is not well known and may have been determined only within 5 to 10 minutes. An asterisk indicates that a marker has been mapped more precisely but that its position with respect to nearby markers is not known. The small vertical arrows indicate the directions of transcription of certain well-studied loci. Parentheses around an operon indicate that, although the direction of transcription of the genes in the operon is known, the orientation of the operon on the chromosome is not known. A similar map for *Salmonella typhimurium* may be found inside the back cover. From Bachmann, B.J. (1983) Linkage map of *E. coli* K-12, edition VII. Microbiological Reviews 47:180–230.

*Inside the back cover* The illustrations on the back inside cover are linear scale drawings representing the circular linkage map of *Salmonella typhimurium*. The scale of 100 units has been chosen to emphasize the similarities to the *E. coli* map (inside the front cover). A length of one unit represents the amount of DNA carried by P22, KB1, or ES18 transducing phage particles, while a length of two units represents the amount of DNA carried by a P1 transducing phage particle (see Chapter 7). The segmented lines to the right of the gene symbols indicate genes that are jointly transduced and the linear distances determined from these data. Major genetic

symbols used in this figure are defined in Table 2-2. Parentheses around a gene symbol indicate that the location of the gene is known only approximately, usually from conjugation studies. An asterisk indicates that a marker has been mapped more precisely, usually by phage-mediated transduction, but that its position relative to adjacent markers is not known. Arrows to the extreme right of operons indicate the direction of mRNA transcription at these loci. From Sanderson, K.E., Roth, J.R. (1983). Linkage map of *Salmonella typhimurium*, edition VI. Microbiological Reviews 47:410–453.

# Contents

Preface to the Second Edition	vii
Linkage Maps	xi
CHAPTER 1	
Prokaryote Molecular Biology	
Prokaryotic Cells and Eukaryotic Cells	1
Chromosome Structure	7
Nucleic Acids	13
Translation of the Genetic Message	27
Summary	29
References	30
CHAPTER 2	
Genetic Processes and Procedures	
Selection: An Essential Element of Microbial Genetics	32
Major Genetic Transfer Processes Observed in Microorganisms and Their Viruses	33
Nomenclature	36
Analytic Techniques for DNA Molecules	39
DNA Splicing	48
Summary	54
References	57
CHAPTER 3	
Mutations and Mutagenesis	
Bacterial Variation	58
Expression and Selection of Mutant Cell Phenotypes	67

Genetic Code	72
Kinds of Mutation	73
Mutagens	81
Summary	86
References	87

#### CHAPTER 4

##### T4 Bacteriophage as a Model Genetic System

Morphology and Composition	89
Experimental Methods Used to Study Phage Infection	92
Genetic Organization of T4	97
Molecular Biology of the Phage Infection	112
DNA Replication and Maturation	117
Summary	122
References	122

#### CHAPTER 5

##### The Genetics of Other Intemperate Bacteriophages

Other Members of the T Series	124
Bacteriophages Containing Single-Stranded DNA	135
RNA-Containing Bacteriophages	143
Bacteriophages Infecting <i>Bacillus subtilis</i>	148
Summary	150
References	152

#### CHAPTER 6

##### Genetics of Temperate Bacteriophages

General Nature of the Temperate Response	154
Bacteriophage Lambda as the Archetypal Temperate Phage	157
Bacteriophage P22	169
Bacteriophages P2 and P4	170
Bacteriophage P1	173
Bacteriophage Mu	176
Bacteriophage PBS1: Example of a Pseudotemperate Phage	178
Summary	179
References	180

#### CHAPTER 7

##### Transduction

Bacteriophage Lambda: A Specialized Transducing Phage	183
Specialized Transducing Phages Other than Lambda	186
Generalized Transduction	189
Analysis of Transductional Data	193
Summary	197
References	197

## CHAPTER 8

## Genetic Transformation

The Pneumococcus– <i>Bacillus</i> Transformation System	200
Other Transformation Systems	205
Transfection	209
Genetic Mapping Using Transformation	213
Summary	217
References	218

## CHAPTER 9

Conjugation in *Escherichia coli*

Basic Properties of the <i>E. coli</i> Conjugative System	220
Interactions of the F Plasmid with the Bacterial Chromosome	229
Physiology of Conjugation	233
Analysis of the F Plasmid	239
Summary	245
References	245

## CHAPTER 10

## Other Plasmids and Other Conjugation Systems

Major Chromosome-Mobilizing Plasmids	248
Bacteriocins	256
Resistance Plasmids	262
Conjugal Plasmid Interactions	269
Summary	271
References	271

## CHAPTER 11

## Plasmid Molecular Biology

Plasmid DNA Replication	274
Partitioning	280
Incompatibility	282
Conjugal Functions	283
Summary	288
References	290

## CHAPTER 12

## Regulation

Regulation of Simple Functional Units	293
Regulation of Complex Operon Systems	309
Other Regulatory Systems	320
Summary	323
References	323

## CHAPTER 13

## Repair and Recombination of DNA Molecules

Possible Types of Damage to DNA Structure	327
DNA Repair in <i>E. coli</i>	328
Genetic and Functional Analysis of Recombination	334
Double Site-Specific Recombination	348
Single Site-Specific Recombination	351
Summary	357
References	358

## CHAPTER 14

## Applied Bacterial Genetic Principles

More Information About DNA Splicing Technology	360
Cloning Vectors	368
Commercial Successes of DNA Cloning	374
Other Types of Applied Bacterial Genetics	376
Basic Research	380
Summary	383
References	383

## Appendix

## The Laws of Probability and Their Application to Prokaryote Cultures

Definition of Probability	386
Dependent versus Independent Events	388
Application of the Binomial Expansion to Probability Theory	390
Poisson Approximation	393
Summary	396
Problems	397

## INDEX

## Chapter 1

# Prokaryote Molecular Biology

When beginning a study of the genetics of bacteria and bacteriophages, it is important to have clearly in mind the ways in which these prokaryotes and their viruses organize their genetic and molecular processes, and the ways in which these processes differ from those used by eukaryotic organisms. This chapter provides a brief overview of major cell activities with comparisons specifically between the eubacteria, primarily represented by *Escherichia coli*, and the “lower” eukaryotes, primarily represented by *Saccharomyces cerevisiae*. The focus in this chapter is on the major molecular biologic processes of DNA replication, transcription, and translation. Specific genetic considerations are the subject of the next chapter. Some of the material in these chapters may be familiar from introductory biology classes, but all of it forms a necessary foundation for the topics to be presented later. The many varied replication and transcription mechanisms found among the bacteria, their plasmids, and their viruses refer back to this material.

## Prokaryotic Cells and Eukaryotic Cells

### Structure

The key feature that distinguishes prokaryotic organisms from eukaryotic organisms is their lack of an organized nucleus. They are also typically smaller than eukaryotic cells. For example, an average, rapidly growing *E. coli* cell is a cylinder about  $1 \times 0.5 \mu\text{m}$ , whereas a typical *S. cerevisiae* cell is round to ovoid and about 3 to 5  $\mu\text{m}$  in diameter. The approximately 64-fold difference in cell volume is reflected in their internal cytoplasmic

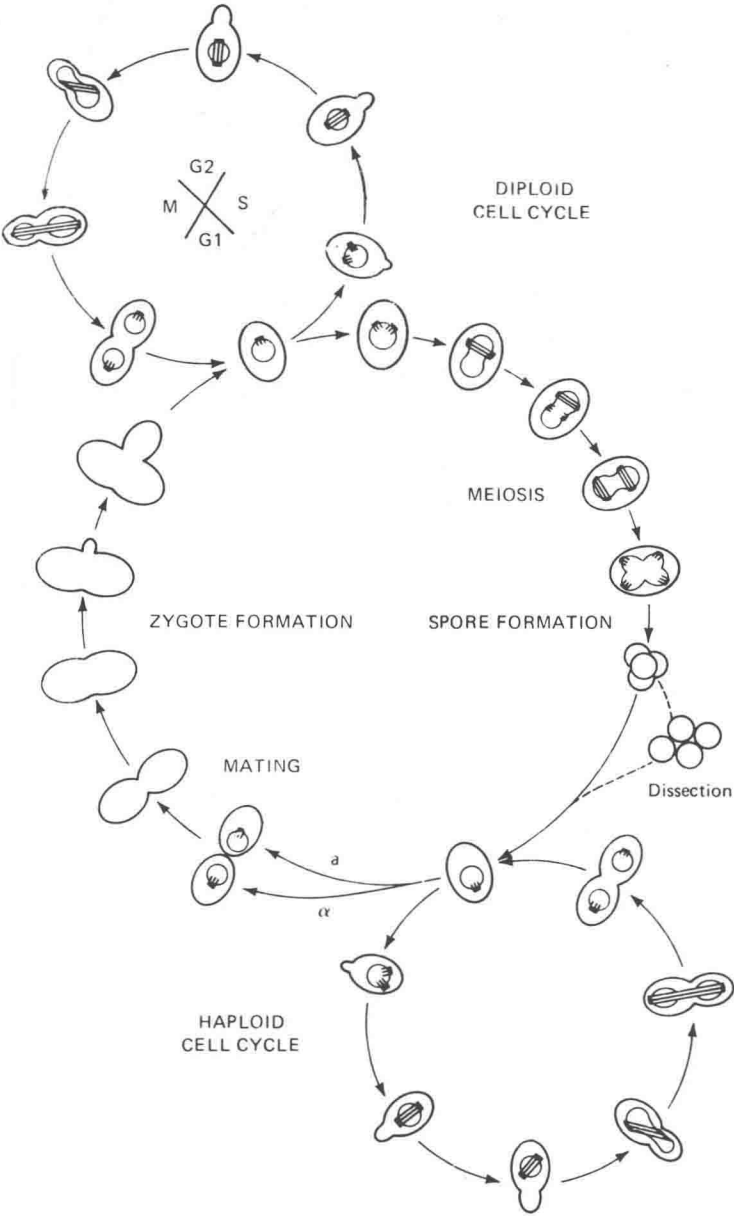
complexity, with *S. cerevisiae* containing the usual intracellular organelles such as mitochondria and endoplasmic reticulum, and prokaryotic cells having no real compartmentalization of function. In all the comparisons that follow, only the nuclear activity in the eukaryotic cells is considered, as modern evolutionary theory assumes that mitochondria and chloroplasts are descended from prokaryotic ancestors.

Another difference between the cell types is found in the way in which the cells carry out the processes of cell division and segregation of DNA. In both cases the cell volume increases during metabolism until a particular size is attained. At that point a complex series of events begins that culminates in the production of two daughter cells, each containing an exact copy of the DNA found in the parent cell.

Eukaryotic cells in general divide by a simple fission process coupled to mitosis that yields two equal-sized cells. However, in the case of many of the yeasts and *Saccharomyces* in particular, the process of cell division is called **budding**, because the new cell is produced as a small protrusion from the surface of the parent cell that rapidly enlarges and eventually pinches off (Fig. 1-1). During formation of the bud, the process of mitosis occurs. Spindle fibers form and attach to the centromeres on the already duplicated yeast chromosomes. The pairs of chromosomes line up and then are separated, moving along the spindle toward the poles of the elongating nucleus. The nuclear membrane persists at all times, unlike the situation in animals and plants. The nucleus continues to elongate, eventually entering the already large bud. When the nucleus splits in two, cytokinesis can occur to form the new cells.

The eubacteria reproduce by binary fission (Fig. 1-2), in which cell mass and volume enlarge linearly until the cell undergoes cytokinesis to yield two equal-sized daughters. The cell density remains roughly constant throughout the cycle. This mechanism is grossly similar to that in eukaryotic cells, but the process of mitosis is unknown in prokaryotic organisms. Moreover, there is no prokaryotic structure that is physically analogous to a centromere, and no microtubules have been seen. Therefore it is obvious that the cells must employ some other means to ensure proper segregation of their DNA molecules. The generally accepted theory, formulated by Jacob and co-workers, is that the replicating DNA molecules are attached to the plasma membrane, presumably at a site near the origin of replication. As each new round of replication begins, a new attachment site is formed on the membrane. The plasma membrane of a bacterial cell appears to grow primarily at the region along which the new septum will form. The insertion of new membrane material into this preexisting structure implies that two points lying astride the center line of the cell membrane and that are therefore initially close together gradually separate as the membrane grows (Fig. 1-3). Electron micrographic evidence indicates that the points of attachment of the replicating DNA molecules do lie on the plane of the future cell cleavage, and this mechanism apparently does





**Figure 1-1.** Possible life cycles of heterothallic strains of *S. cerevisiae*. In the diploid cycle there are two growth phases separated by DNA synthesis (S) and mitosis (M). The nuclear configuration is indicated for all dividing cells. From Dawes, I.W. (1983). Genetic control and gene expression during meiosis and sporulation in *Saccharomyces cerevisiae*, pp. 29–64. In: Spencer, J.F.T., Spencer, D.M., Smith, A.R.W. (eds.) *Yeast Genetics*. New York: Springer-Verlag.