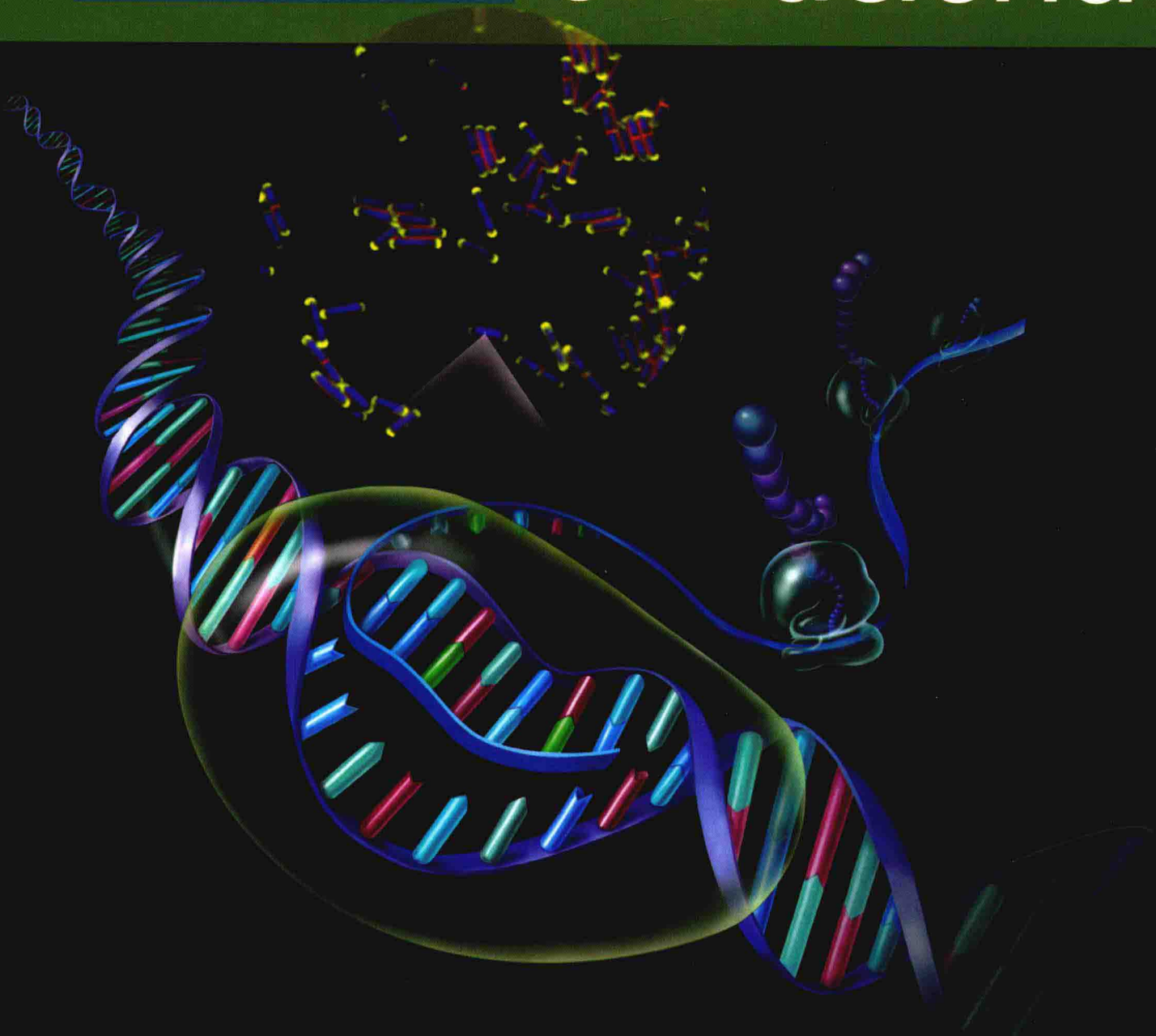


4TH EDITION

Molecular Genetics of Bacteria



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Cover photo: Fluorescence micrograph of *Bacillus subtilis* cells showing the location of the cell membrane (red), DNA (blue), and ConE mating protein fused to green fluorescent protein (yellow-green). The ConE protein is a component of the conjugative DNA translocation channel required for horizontal transfer of the integrating conjugative element ICEBs1. The ConE protein is concentrated at the cell poles, but additional protein is localized around the entire cell periphery. The lateral distribution enables cells to transfer ICEBs1 side to side, and the high concentration at the poles may contribute to the very efficient transfer of ICEBs1 observed in chains of cells from pole to pole. See chapter 5 for details. Photo courtesy of Melanie Berkmen and Alan Grossman. Modified from M. B. Berkmen et al., *J. Bacteriol.*

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4TH EDITION

Molecular Genetics of Bacteria

Preface

WITH THE ADDITION OF TWO NEW COAUTHORS, the fourth edition of the textbook *Molecular Genetics of Bacteria* has been substantially revised and some new sections have been added. We tried to do this without increasing the length of the book, which, at more than 700 pages, was already quite long. While the book retains the same number and order of chapters, many topics have been moved or integrated more completely into the text to reflect a more modern perspective. The purpose was to convey more accurately how one approaches questions in modern bacterial genetics, using the full repertoire of methods now available. Also, to make room for the new material, we made the philosophical decision to condense or eliminate descriptions of methods where they seemed unnecessarily detailed for a textbook.

Chapter 1, on DNA structure, DNA replication, and chromosome segregation, was expanded to include updates in our understanding, including how replication proceeds through obstacles typically found during normal DNA replication in bacteria, while some aspects of repair-associated replication were moved to later chapters. The chapter was also significantly expanded with new information about how numerous cell processes coordinate for the efficient processing and organizing of chromosomes after DNA replication. Scientists now more fully appreciate how sequences “hidden” in the structure guide a variety of systems that aid in repairing, segregating, packaging, and pumping the chromosome for exquisite genome stability in bacteria. In chapter 2, which covers bacterial gene expression, the translation section has been reorganized to follow the same order as the transcription section. It begins with initiation of translation and then discusses elongation followed by termination, rather than following the more historical order with the genetic code coming first. We reasoned that this order makes more sense since most students already have had some exposure to translation and the genetic code. More information on RNA degradation is now included, and the sections on gene regulation have been moved to chapter 12. The protein transport section has been moved from chapter 2 to chapter 14 (see below), where it can be better integrated with other topics of protein export. Chapter 3, on bacterial genetic analysis, also

now takes a less historical approach. Rather than beginning with a review of classical genetic analysis and then contrasting it with bacterial genetic analysis as in previous editions, the chapter now begins with bacterial genetic analysis, again assuming that students have already had some general genetics. Furthermore, rather than putting more recently developed methods such as site-specific mutagenesis, recombineering, etc., into a separate section, we have integrated throughout the text all the methods available nowadays to use in a genetic analysis. The discussion of mapping by Hfr crosses has been sharply condensed, since it is likely that no one will ever again perform the laborious task of constructing the genetic map of a bacterium. The relative ease of DNA sequencing now allows the placing of mutations on the sequenced genomes of bacteria by direct comparison of sequences rather than by Hfr mapping. Transduction and transformation (including electroporation) are used extensively for genetic manipulations, so their use is still covered in some detail. Chapter 4 has been updated with more information about how plasmids are typically used in the laboratory setting in work with model organisms and beyond as well, including updates on our understanding of partitioning systems. Chapter 5 was extensively updated to illustrate the hodgepodge organization of conjugal elements and advances in our understanding of conjugation and to more fully integrate the important role of integrating conjugative elements in bacterial genomes (including a focus on one of these elements from *B. subtilis* on the front cover). Chapter 6 is updated throughout and focuses on similarities and differences between different transformation systems. The bacteriophage chapters (chapters 7 and 8) have been updated, and new material has been added. Some highlights from phage genomics are now covered, as are phage defense mechanisms, including CRISPR. The section on phage lysis is expanded, as is the text box on phage display, whose power is now demonstrated with some current uses. The interaction between lysogenic phages and genetic islands has been updated and moved into the text, as have some more recently developed techniques using lysogenic phages, for example, in detecting protein-protein interactions. Chapter 9, which covers transposable elements and site-specific recombination, has been updated to clarify the basic molecular biology of these elements, and it includes updated sections describing how they are used in the laboratory today. Chapter 10 was significantly reorganized to stress the role of homologous recombination in the repair of DNA double-strand breaks that occur at interruptions in the template DNA during replication. The role of homologous recombination in repair explains the underpinning of the evolution of the process and also clarifies how the process works in concert with DNA replication.

A more comprehensive treatment for how DNA double-strand breaks are repaired across different types of bacteria, using systems found in all domains of life, is also included. Chapter 11 was updated to discuss many advances in the field of repair, including an expanded understanding of the regulation of multiple DNA polymerases found in bacteria with the SOS response. Chapters 12 and 13 have been reorganized so that chapter 12 is now focused on mechanisms of regulation of individual genes and operons and chapter 13 is mostly concerned with examples of global regulatory systems that utilize these mechanisms. There is also more emphasis on posttranscriptional regulation in both chapters, and global regulatory mechanisms in *Escherichia coli* are contrasted with those in *Bacillus subtilis*. Chapter 14 is probably the most changed chapter. It now contains our entire discussion of protein export, including the Sec and Tat systems as well as the secretion systems of gram-negative (i.e., *Proteobacteria*) and gram-positive (i.e., *Firmicutes*) bacteria. Most notably, it now contains a new section on bacterial cell biology, including cell wall synthesis and cell division and their regulation, as well as a new box on the evolution of cytoskeletal filaments, and it introduces the use of *Caulobacter crescentus* as a model system for these studies. Chapter 14 finishes with sporulation in *B. subtilis*, probably the best understood bacterial developmental system.

As in earlier editions, we do not mention the names of most investigators who have made major contributions to bacterial molecular genetics. We include only those names that have become icons in the field because they are associated with certain seminal experiments (e.g., Meselson and Stahl or Luria and Delbrück), models (e.g., Jacob and Monod), or structures (e.g., Watson and Crick). Many other names are available in the suggested reading lists, where we give some of the original references to the developments under discussion, and in the credit lines for sources of figures and tables, which are given at the end of the book.

Again we are indebted to a number of people who helped us in various ways. Some read sections of the book at our request and made valuable suggestions. Some, who have used the book for teaching, have pointed out ways to make it more useful for them and their students. Others have noticed factual errors or errors of omission and have pointed out references that helped us check our facts. In addition to those who commented on earlier editions, many of whose contributions have carried over, this list includes Dennis Arvidson, Dominique Belin, Melanie Berkmen, Helmut Bertrand, Lindsay Black, Rob Britton, Yves Brun, Rich Calendar, George Chaconas, Dhruva Chattoraj, Carton Chen, Todd Ciche, Laszlo Csonka, Gary Dunny, Marie Elliot, Laura Frost, Barbara Funnell, Peter Geiduschek,

Graham Hatfull, John Helmann, Ann Hochschild, Susan Lovett, Ken Marinas, Norman Pace, Steven Sandler, Joel Schildbach, Linda Sherwood, Chris Waters, Robert Weiss, Joanne Willey, Steve Winans, Ry Young, and Steve Zinder. Special thanks go to Lee Kroos, who agreed to update an entire section. Yet others furnished original figures that we could incorporate into the text; some of them are mentioned in the figure credits. However, in the end, any mistakes and omissions were all ours.

As with the first three editions, it was a great pleasure to work with the professionals at ASM Press. The former director of ASM Press, Jeff Holtmeier, helped us prepare for the fourth edition. We have been fortunate to continue to work with Kenneth April, the production

manager, who coordinated the entire project. We have also had the good fortune to work again with two of the same professionals who did a masterful job with the first three editions: Susan Brown Schmidler, who created the book and cover design; Terese Winslow, who created the cover illustration; and Elizabeth McGillicuddy, who copyedited the manuscript. We also thank Patrick Lane of ScEYence Studios for bringing an attractive aestheticism to the rendering of our hand-drawn illustrations into the final figures.

LARRY SNYDER
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About the Authors

Larry (Loren R.) Snyder, PhD, is a professor emeritus of microbiology and molecular genetics at Michigan State University, where he taught microbial genetics and microbiology to undergraduate and graduate students for about 40 years. He received his BS in mathematics and zoology at the University of Minnesota in Duluth and his PhD in biophysics at the University of Chicago before doing postdoctoral work at the International Laboratory of Genetics and Biophysics in Naples, Italy, and at the Curie Institute and Faculty of Sciences at the University of Paris as a Jane Coffin Childs postdoctoral fellow. He was a visiting professor at Harvard University and the University of Tel Aviv. Most of his research was on the interaction between bacteriophage T4 and its host, *Escherichia coli*, and was supported by the National Science Foundation (NSF) and the National Institutes of Health (NIH). At Michigan State, he served as acting chair of the Department of Microbiology for one year and was a founding co-principal investigator of the NSF Center for Microbial Ecology and director of the Howard Hughes Medical Institute Undergraduate Research Program. He was awarded the College of Natural Science Alumni Association Meritorious Faculty Award in 2002.

Joseph E. Peters, PhD, is an associate professor of microbiology at Cornell University, where he has been teaching bacterial genetics and microbiology since 2002. He received his BS from Stony Brook University and his PhD from the University of Maryland at College Park. He did postdoctoral work at the Johns Hopkins University School of Medicine, in part as an NSF-Alfred P. Sloan Foundation postdoctoral research fellow in molecular evolution. His research has focused on the intersection of DNA replication, recombination, and repair and how it relates to evolution, especially in the area of transposition. Research in his laboratory is funded by the NSF and NIH. He is the chair of the advisory board for the NSF-funded *E. coli* Genetic Stock Center, the chair of the American Society for Microbiology's Division of Genetics and Molecular Biology, and the director of graduate studies for the field of microbiology at Cornell.

Tina M. Henkin, PhD, is a professor of microbiology, chair of the Department of Microbiology, and Robert W. and Estelle S. Bingham Professor of Biological Sciences at Ohio State University, where she has been teaching bacterial genetics and microbiology since 1995. She received her BA in biology at Swarthmore College and her PhD in genetics at the University of Wisconsin—Madison, and she did postdoctoral work at the Tufts University School of Medicine. Her research focuses on gene regulation and regulatory RNAs in bacteria. Research in her laboratory is funded by the NIH. She is a fellow of the American Academy of Microbiology, the American Association for the Advancement of Science, and the American Academy of Arts and Sciences; a member of the National Academy of Sciences; and a cowinner of the National Academy of Sciences Pfizer Prize in Molecular Biology for her work on riboswitch RNAs.

Wendy Champness, PhD, is a professor emerita of microbiology and molecular genetics at Michigan State University, where she taught microbial genetics and microbiology to undergraduate and graduate students for more than 25 years. She received her BS and PhD degrees at Michigan State, where she was an NSF predoctoral fellow. She did postdoctoral work at the Massachusetts Institute of Technology as a Jane Coffin Childs postdoctoral fellow and was a visiting scientist at the John Innes Research Centre in Norwich, United Kingdom, and at the University of Tel Aviv. Most of her research was on the regulation of antibiotic synthesis genes in *Streptomyces*, and research in her laboratory was supported by grants from the NSF and NIH. She was a charter member of the NSF Center for Microbial Ecology at Michigan State and was a member of the editorial board of the *Journal of Bacteriology* for 12 years as well as an associate editor of the journal *Microbiology*.

Contents

Preface xv

About the Authors xix

Introduction 1

The Biological Universe 3

The Bacteria 3

The Archaea 3

The Eukaryotes 5

Speculations on the Origin of the Three Domains of Life 5

What Is Genetics? 6

Bacterial Genetics 6

Bacteria Are Haploid 7

Short Generation Times 7

Asexual Reproduction 7

Colony Growth on Agar Plates 7

Colony Purification 7

Serial Dilutions 7

Selections 8

Storing Stocks of Bacterial Strains 8

Genetic Exchange 8

Phage Genetics 8

Phages Are Haploid 8

Selections with Phages 9

Crosses with Phages 9

A Brief History of Bacterial Molecular Genetics 9

Inheritance in Bacteria 9
 Transformation 9
 Conjugation 10
 Transduction 10
 Recombination within Genes 10
 Semiconservative DNA Replication 10
 mRNA 10
 The Genetic Code 10
 The Operon Model 10
 Enzymes for Molecular Biology 10

What Is Ahead 11

SUGGESTED READING 11

CHAPTER 1**The Bacterial Chromosome: DNA Structure, Replication, and Segregation 13****DNA Structure 13**

The Deoxyribonucleotides 13
 The DNA Chain 14
 The 5' and 3' Ends 14
 Base Pairing 16
 Antiparallel Construction 17
 The Major and Minor Grooves 17

The Mechanism of DNA Replication 17

Deoxyribonucleotide Precursor Synthesis 17
 Replication of the Bacterial Chromosome 19
 Replication of Double-Stranded DNA 23

Replication Errors 26

Editing 26
 RNA Primers and Editing 27

Impediments to DNA Replication 28

Damaged DNA and DNA Polymerase III 28
 Mechanisms To Deal with Impediments on Template DNA Strands 28
 Physical Blocks to Replication Forks 30

Replication of the Bacterial Chromosome and Cell Division 31

Structure of the Bacterial Chromosome 31
 Replication of the Bacterial Chromosome 31
 Initiation of Chromosome Replication 32

RNA Priming of Initiation 33
 Termination of Chromosome Replication 33
 Chromosome Segregation 34
 Coordination of Cell Division with Replication of the Chromosome 43
 Timing of Initiation of Replication 45

The Bacterial Nucleoid 47

Supercoiling in the Nucleoid 47
 Topoisomerases 49

The Bacterial Genome 50**Antibiotics That Affect Replication and DNA Structure 51**

Antibiotics That Block Precursor Synthesis 51
 Antibiotics That Block Polymerization of Deoxynucleotides 52
 Antibiotics That Affect DNA Structure 52
 Antibiotics That Affect Gyrase 52

Molecular Biology Manipulations with DNA 53

Restriction Endonucleases 53
 Hybridizations 56
 Applications of the Enzymes Used in DNA Replication 58
 Polymerase Chain Reaction 58

BOX 1.1 Structural Features of Bacterial Genomes 37**BOX 1.2 Advanced Genome-Sequencing Techniques 59**

SUMMARY 62
 QUESTIONS FOR THOUGHT 64
 PROBLEMS 64
 SUGGESTED READING 65

CHAPTER 2**Bacterial Gene Expression: Transcription, Translation, and Protein Folding 67****Overview 67****The Structure and Function of RNA 68**

Types of RNA 69
 RNA Precursors 69
 RNA Structure 69
 RNA Processing and Modification 70

Transcription 70

Structure of Bacterial RNA Polymerase 70
 Overview of Transcription 71
 Details of Transcription 75
 rRNAs and tRNAs 81

RNA Degradation 82

RNases 83

The Structure and Function of Proteins 84

Protein Structure 85

Translation 86

Structure of the Bacterial Ribosome 86
 Overview of Translation 88
 Details of Protein Synthesis 90
 The Genetic Code 97

Protein Folding and Degradation 105

Protein Chaperones 105
 Protein Degradation 107

Membrane Proteins and Protein Export 108**Regulation of Gene Expression 108**

Transcriptional Regulation 108
 Posttranscriptional Regulation 109

Genomes and Genomics 109

Annotation and Comparative Genomics 110

What You Need To Know 110

Open Reading Frames 116
 Transcriptional and Translational Fusions 116

Antibiotics That Block Transcription and Translation 116

Antibiotic Inhibitors of Transcription 117
 Antibiotic Inhibitors of Translation 118

BOX 2.1 Molecular Phylogeny 82**BOX 2.2 Mimicry in Translation 96****BOX 2.3 Exceptions to the Code 99****BOX 2.4 Selfish DNAs: RNA Introns and Protein Inteins 102****BOX 2.5 Annotation and Comparative Genomics 110**

SUMMARY 120

QUESTIONS FOR THOUGHT 122

PROBLEMS 122

SUGGESTED READING 123

CHAPTER 3**Bacterial Genetic Analysis: Fundamentals and Current Approaches 125****Definitions 125**

Terms Used in Genetics 125
 Genetic Names 126
 Auxotrophic and Catabolic Mutants 127
 Conditional-Lethal Mutants 128
 Resistant Mutants 130

Inheritance in Bacteria 130

The Luria and Delbrück Experiment 131
 Mutants Are Clonal 132
 The Lederbergs' Experiment 133

Mutation Rates 133

Calculating Mutation Rates 135
 Calculating the Mutation Rate from the Rate of Increase in the Proportion of Mutants 136

Types of Mutations 137

Properties of Mutations 138
 Base Pair Changes 138
 Frameshift Mutations 142
 Deletion Mutations 144
 Tandem-Duplication Mutations 145
 Inversion Mutations 147
 Insertion Mutations 148

Reversion versus Suppression 149

Intragenic Suppressors 149
 Intergenic Suppressors 150

Genetic Analysis in Bacteria 153

Isolating Mutants 153
 Genetic Characterization of Mutants 157
 Complementation Tests 161
 Genetic Crosses in Bacteria 167
 Mapping of Bacterial Markers by Transduction and Transformation 168
 Other Uses of Transformation and Transduction 172
 Genetic Mapping by Hfr Crosses 173

Isolation of Tandem Duplications of the *his* Operon in *Salmonella* 176

Lengths of Tandem Duplications 178
 Frequency of Spontaneous Duplications 179

BOX 3.1 Statistical Analysis of the Number of Mutants per Culture 134**BOX 3.2 Inversions and the Genetic Map 148**

SUMMARY 179

QUESTIONS FOR THOUGHT 181

PROBLEMS 181

SUGGESTED READING 182

CHAPTER 4**Plasmids 183****What Is a Plasmid? 183**

Naming Plasmids 184

Functions Encoded by Plasmids 184

Plasmid Structure 185

Properties of Plasmids 186

Replication 186

Functions of the *ori* Region 189

Plasmid Replication Control Mechanisms 194

Mechanisms To Prevent Curing of Plasmids 203

The Par Systems of Plasmids 205

Plasmid Cloning Vectors 209

Examples of Plasmid Cloning Vectors 210

Broad-Host-Range Cloning Vectors 213

BOX 4.1 Linear Chromosomes and Plasmids in Bacteria 190**BOX 4.2 Toxin-Antitoxin Systems and Plasmid Maintenance 204**

SUMMARY 216

QUESTIONS FOR THOUGHT 217

PROBLEMS 217

SUGGESTED READING 217

CHAPTER 5**Conjugation 219****Overview 219**

Classification of Self-Transmissible Plasmids 220

The Fertility Plasmid 220

Mechanism of DNA Transfer during Conjugation in Gram-Negative Bacteria 221Transfer (*tra*) Genes 221The *oriT* Sequence 225

Male-Specific Phages 226

Efficiency of Transfer 227

Interspecies Transfer of Plasmids 227

Conjugation and Type IV Protein Secretion 228

Mobilizable Plasmids 232

Chromosome Transfer by Plasmids 235

Formation of Hfr Strains 235

Transfer of Chromosomal DNA by Integrated Plasmids 236

Chromosome Mobilization 236

Prime Factors 236

Transfer Systems of Gram-Positive Bacteria 237

Plasmid-Attracting Pheromones 237

Integrating Conjugative Elements 240**BOX 5.1 Gene Exchange between Domains 230**

SUMMARY 242

QUESTIONS FOR THOUGHT 243

PROBLEMS 243

SUGGESTED READING 244

CHAPTER 6**Transformation 247****Natural Transformation 248**

Discovery of Transformation 248

Competence 248

DNA Processing after Uptake 252

Experimental Evidence for Models of Natural Transformation 252

Plasmid Transformation and Phage Transfection of Naturally Competent Bacteria 254

Regulation of Natural Competence 255

Role of Natural Transformation 257

Importance of Natural Transformation for Forward and Reverse Genetics 259

Congression 259

Artificially Induced Competence 260

Chemical Induction 260

Electroporation 261

Protoplast Transformation 261

BOX 6.1 Antigenic Variation in *Neisseria gonorrhoeae* 259

SUMMARY 262

QUESTIONS FOR THOUGHT 262

PROBLEMS 262

SUGGESTED READING 263

CHAPTER 7

Bacteriophages: Lytic Development, Genetics, and Generalized Transduction 265

Regulation of Gene Expression during Lytic Development 270

Phages That Encode Their Own RNA Polymerases 272

T7 Phage-Based Expression Vectors 273

Making Riboprobes and RNA-Processing Substrates 273

Phage T4: Transcriptional Activators, a New Sigma Factor, and Replication-Coupled Transcription 275

Phage DNA Genome Replication and Packaging 279

Phages with Single-Stranded Circular DNA 279

Replication and DNA Packaging: Linear Genomes 285

Phage T7: Linear DNA That Forms Concatemers 285

Phage T4: Another Phage That Forms Concatemers 286

Phage Lysis 289

Single-Protein Lysis 289

Timed Lysis 290

Timing of Lysis by Holins 290

Phage Display 292

Genetic Analysis of Phages 298

Infection of Cells 298

Phage Crosses 299

Recombination and Complementation Tests with Phages 299

Genetic Experiments with the *rII* Genes of Phage T4 301

Constructing the Genetic-Linkage Map of a Phage 307

Phage Defense Mechanisms 309

Restriction-Modification Systems 310

Abi Systems 310

CRISPR Loci 311

Generalized Transduction 314

What Makes a Transducing Phage? 315

Shuttle Phasmids 316

Role of Transduction in Bacterial Evolution 317

BOX 7.1 Phage Genomics 268

BOX 7.2 RNA Phages 271

BOX 7.3 Protein Priming 286

BOX 7.4 Phage Display 294

SUMMARY 318

QUESTIONS FOR THOUGHT 319

PROBLEMS 319

SUGGESTED READING 320

CHAPTER 8

Lysogeny: the λ Paradigm and the Role of Lysogenic Conversion in Bacterial Pathogenesis 323

Phage λ 324

λ Lytic Development 324

Replication of λ DNA 331

Lysogeny by Phage λ 333

The Lytic-versus-Lysogen Decision: the Roles of *cl*, *clI*, and *clII* Gene Products 333

Phage λ Integration 334

Maintenance of λ Lysogeny 335

Immunity to Superinfection 337

Induction of λ 338

Summary of the Lytic and Lysogenic Cycles 340

Specialized Transduction 340

Selection of HFT Particles 342

Other Lysogen-Forming Phages 343

Phage P2 343

Phage P4: a Satellite Virus 343

Prophages That Replicate as Plasmids 345

Phage Mu: a Transposon Masquerading as a Phage 345

Lysogenic Conversion and Bacterial Pathogenesis 345

E. coli and Dysentery: Shiga Toxins 346

Diphtheria 347

Cholera 347

S. aureus and Toxic Shock Syndrome 349

Synopsis 350

Uses of Lysogeny in Genetic Analysis and Biotechnology 350

- Complementation and Gene Expression Studies 350
- Use of Phage Display and Frequency of Mixed Dilysoyons To Detect Protein-Protein Interactions 350

Genetic Experiments with Phage λ 351

- Genetic Analysis of λ Lysogen Formation 351
- Genetics of the CI Repressor: Evidence for the Domain Structure of Proteins 353
- Identification of λ *nut* Sites Involved in Progressive Transcription Antitermination 354
- Isolation of Host *nus* Mutations: *E. coli* Functions Involved in Transcription Elongation-Termination 356

BOX 8.1 Effects of Prophage Insertion on the Host 336

- SUMMARY 357
- QUESTIONS FOR THOUGHT 358
- PROBLEMS 358
- SUGGESTED READING 359

CHAPTER 9

Transposition, Site-Specific Recombination, and Families of Recombinases 361

Transposition 361

- Overview of Transposition 362
- Structure of Bacterial Transposons 362
- Types of Bacterial Transposons 364
- Assays of Transposition 366

Mechanisms of Transposition 368

- Genetic Requirements for Transposition of Tn3 368
- A Molecular Model for Transposition of Tn3 372
- Transposition by Tn10 and Tn5 373

Details of Transposition by the DDE Transposons 376

- Details of the Mechanism of Transposition by Tn5 and Tn7 376

Rolling-Circle Transposons 378

Y and S Transposons 378

General Properties of Transposons 379

- Target Site Specificity 379

- Effects on Genes Adjacent to the Insertion Site 380

- Regulation of Transposition 380

- Target Immunity 381

Transposon Mutagenesis 382

- Transposon Mutagenesis In Vivo 382
- Transposon Mutagenesis In Vitro 382
- Transposon Mutagenesis of Plasmids 385
- Transposon Mutagenesis of the Bacterial Chromosome 386
- Transposon Mutagenesis of All Bacteria 386
- Using Transposon Mutagenesis To Make Random Gene Fusions 387

Site-Specific Recombination 387

- Integrases 387
- Resolvases 390
- DNA Invertases 391

Y and S Recombinases 392

- Y Recombinases: Mechanism 392
- S Recombinases: Mechanism 397

Importance of Transposition and Site-Specific Recombination in Bacterial Adaptation 398

BOX 9.1 Transposons and Genomics 383

- SUMMARY 399
- QUESTIONS FOR THOUGHT 400
- PROBLEMS 400
- SUGGESTED READING 401

CHAPTER 10

Molecular Mechanisms of Homologous Recombination 403

Homologous Recombination and DNA Replication in Bacteria 404

- Early Evidence for the Interdependence of Homologous Recombination and DNA Replication 404

The Molecular Basis for Recombination in *E. coli* 405

- Chi (χ) Sites and the RecBCD Complex 405
- The RecF Pathway 409
- Synapse Formation and the RecA Protein 411
- The Ruv and RecG Proteins and the Migration and Cutting of Holliday Junctions 414

Recombination between Different DNAs in Bacteria 416How Are Linear DNA Fragments Recombined into the *E. coli* Chromosome? 417

Phage Recombination Pathways 417

Rec Proteins of Phages T4 and T7 417

The RecE Pathway of the *rac* Prophage 417The Phage λ Red System 419**Recombineering: Gene Replacements in *E. coli* with Phage λ Recombination Functions 419****Genetic Analysis of Recombination in Bacteria 422**Isolating Rec⁻ Mutants of *E. coli* 422

Isolating Mutants with Mutations in Other Recombination Genes 423

Gene Conversion and Other Manifestations of Heteroduplex Formation during Recombination 426

BOX 10.1 Other Types of Double-Strand Break Repair in Bacteria 410**BOX 10.2 Breaking and Entering: Introns and Inteins Move by Double-Strand Break Repair or Retrohoming 425**

SUMMARY 429

QUESTIONS FOR THOUGHT 430

PROBLEMS 430

SUGGESTED READING 431

CHAPTER 11**DNA Repair and Mutagenesis 433****Evidence for DNA Repair 434****Specific Repair Pathways 435**

Deamination of Bases 435

Damage Due to Reactive Oxygen 438

Damage Due to Alkylating Agents 441

Damage Due to UV Irradiation 443

General Repair Mechanisms 445

Base Analogs 445

Frameshift Mutagens 445

Methyl-Directed Mismatch Repair 445

Nucleotide Excision Repair 452

DNA Damage Tolerance Mechanisms 453

Homologous Recombination and DNA Replication 454

SOS-Inducible Repair 458

Mechanism of TLS by the Pol V Mutasome 463

Other Specialized Polymerases and Their Regulation 464

Summary of Repair Pathways in *E. coli* 466**Bacteriophage Repair Pathways 466****BOX 11.1 The Role of Reactive Oxygen Species in Cancer and Degenerative Diseases 439****BOX 11.2 DNA Repair and Cancer 449****BOX 11.3 The Ames Test 465**

SUMMARY 468

QUESTIONS FOR THOUGHT 469

PROBLEMS 469

SUGGESTED READING 469

CHAPTER 12**Regulation of Gene Expression: Genes and Operons 471****Transcriptional Regulation in Bacteria 472**

Genetic Evidence for Negative and Positive Regulation 474

Negative Regulation of Transcription Initiation 474

Negative Inducible Systems 474

Negative Repressible Systems 484

Molecular Mechanisms of Transcriptional Repression 486

Positive Regulation of Transcription Initiation 487

Positive Inducible Systems 487

Positive Repressible Systems 496

Molecular Mechanisms of Transcriptional Activation 496

Regulation by Transcription Attenuation 497

Modulation of RNA Structure 497

Changes in Processivity of RNA Polymerase 506

Regulation of mRNA Degradation 507

Protein-Dependent Effects on RNA Stability 507

RNA-Dependent Effects on RNA Stability 508

Regulation of Translation 508

Regulation of Translation Initiation 509

Translational Regulation in the Exit Channel of the Ribosome 511

Regulation of Translation Termination 512

Posttranslational Regulation 514

Posttranslational Protein Modification 514

Regulation of Protein Turnover 514

Feedback Inhibition of Enzyme Activity 515

Why Are There So Many Mechanisms of Gene Regulation? 520

Operon Analysis for Sequenced Genomes 521

BOX 12.1 The Helix-Turn-Helix Motif of DNA-Binding Proteins 473

BOX 12.2 Families of Regulators 488

BOX 12.3 Special Problems in Genetic Analysis of Operons 516

SUMMARY 521

QUESTIONS FOR THOUGHT 522

PROBLEMS 522

SUGGESTED READING 523

CHAPTER 13

Global Regulation: Regulons and Stimulons 525

Carbon Catabolite Regulation 526

Catabolite Regulation in *E. coli*: Catabolite Activator Protein (CAP) and cAMP 526

Carbon Catabolite Regulation in *B. subtilis*: CcpA and Hpr 535

Regulation of Nitrogen Assimilation 536

Pathways for Nitrogen Assimilation 536

Regulation of Nitrogen Assimilation Pathways in *E. coli* by the Ntr System 537

Regulation of Nitrogen Assimilation in *B. subtilis* 547

Regulation of Ribosome and tRNA Synthesis 547

Ribosomal Protein Gene Regulation 548

Regulation of rRNA and tRNA Synthesis 550

The Stringent Response 551

Stress Responses in Bacteria 554

Heat Shock Regulation 555

General Stress Response in Gram-Negative Bacteria 558

General Stress Response in Gram-Positive Bacteria 559

Extracytoplasmic (Envelope) Stress Responses 563

Iron Regulation in *E. coli* 568

The Fur Regulon 568

The RyhB sRNA 569

The Aconitase Translational Repressor 570

Regulation of Virulence Genes in Pathogenic Bacteria 571

Diphtheria 572

Cholera and Quorum Sensing 572

Whooping Cough 578

From Genes to Regulons to Networks 579

BOX 13.1 cAMP-Independent Carbon Catabolite Regulation in *E. coli* 529

BOX 13.2 A Bacterial Two-Hybrid System Based on Adenylate Cyclase 531

BOX 13.3 Nitrogen Fixation 537

BOX 13.4 Signal Transduction Systems in Bacteria 539

BOX 13.5 Sigma Factors 542

BOX 13.6 Regulatory RNAs 560

BOX 13.7 Tools for Studying Global Regulation 571

SUMMARY 580

QUESTIONS FOR THOUGHT 582

PROBLEMS 582

SUGGESTED READING 582

CHAPTER 14

Bacterial Cell Biology and Development 585

Membrane Proteins and Protein Export 585

The Translocase System 586

The Signal Sequence 586

The Targeting Factors 588

The Tat Secretion Pathway 590

Disulfide Bonds 590

Use of *mal-lac* Fusions To Study Protein Transport in *E. coli* 591

Genetic Analysis of Transmembrane Domains of Inner Membrane Proteins in Gram-Negative Bacteria 594

Identification of Genes for Inner Membrane Proteins by Random *phoA* Fusions 595