

Contents

<i>Preface</i>	<i>xv</i>
<i>Acknowledgments</i>	<i>xix</i>
<i>About the Authors</i>	<i>1</i>

Introduction 3

The Biological Universe 5

The Bacteria	5
The Archaea	7
The Eukaryotes	7

What Is Genetics? 8

Bacterial Genetics 8

Bacteria Are Haploid	9
Short Generation Times	9
Asexual Reproduction	9
Colony Growth on Agar Plates	9
Colony Purification	9
Serial Dilutions	9
Selections	10
Storing Stocks of Bacterial Strains	10
Genetic Exchange	10

Phage Genetics 10

Phages Are Haploid	11
Selections with Phages	11
Crosses with Phages	11

A Brief History of Bacterial Molecular Genetics 11

Inheritance in Bacteria	11
Transformation	11
Conjugation	12
Transduction	12
Recombination within Genes	12
Semiconservative DNA Replication	12
mRNA	12
The Genetic Code	12

The Operon Model	12
Enzymes for Molecular Biology	12
Synthetic Genomics	13
What Is Ahead	13



The Bacterial Chromosome: DNA Structure, Replication, and Segregation 17

DNA Structure 17

The Deoxyribonucleotides	17
The DNA Chain	18
The 5' and 3' Ends	18
Base Pairing	20
Antiparallel Construction	20
The Major and Minor Grooves	21

The Mechanism of DNA Replication 21

Deoxyribonucleotide Precursor Synthesis	21
Replication of the Bacterial Chromosome	21
Replication of Double-Stranded DNA	26

Replication Errors 30

Editing	30
RNA Primers and Editing	31

Impediments to DNA Replication 31

Damaged DNA and DNA Polymerase III	31
Mechanisms To Deal with Impediments on Template DNA Strands	32
Physical Blocks to Replication Forks	32

Replication of the Bacterial Chromosome and Cell Division 32

Structure of Bacterial Chromosomes	34
Replication of the Bacterial Chromosome	34
Initiation of Chromosome Replication	34
RNA Priming of Initiation	35
Termination of Chromosome Replication	35
Chromosome Segregation	37
Coordination of Cell Division with Replication of the Chromosome	47
Timing of Initiation of Replication	49

The Bacterial Nucleoid 51

Supercoiling in the Nucleoid	51
Topoisomerases	52

The Bacterial Genome 55

BOX 1.1 Structural Features of Bacterial Genomes	37
BOX 1.2 Antibiotics That Affect Replication and DNA Structure	54



Bacterial Gene Expression: Transcription, Translation, Protein Folding, and Localization 61

Overview 61

The Structure and Function of RNA 62

Types of RNA	62
RNA Precursors	62
RNA Structure	62
RNA Processing and Modification	64
Transcription	64
Structure of Bacterial RNA Polymerase	64
Overview of Transcription	65
Details of Transcription	67
rRNAs and tRNAs	74
RNA Degradation	77
RNases	77
The Structure and Function of Proteins	78
Protein Structure	78
Translation	80
Structure of the Bacterial Ribosome	80
Overview of Translation	83
Details of Protein Synthesis	84
The Genetic Code	92
Polycistronic mRNA	96
Protein Folding and Degradation	98
Protein Chaperones	98
Protein Degradation	101
Protein Localization	101
The Translocase System	101
The Signal Sequence	103
The Targeting Factors	103
The Tat Secretion Pathway	104
Disulfide Bonds	105
Protein Secretion and Export	105
Protein Secretion Systems in Bacteria with an Outer Membrane	106
Protein Secretion in Bacteria That Lack an Outer Membrane	110
Sortases	110
Regulation of Gene Expression	111
Transcriptional Regulation	112
Posttranscriptional Regulation	113
What You Need To Know	114
Open Reading Frames	115
Transcriptional and Translational Fusions	115
BOX 2.1 Antibiotic Inhibitors of Transcription	72
BOX 2.2 Molecular Phylogeny	75
BOX 2.3 Antibiotic Inhibitors of Translation	81
BOX 2.4 Mimicry in Translation	91
BOX 2.5 Exceptions to the Code	94



Bacterial Genetic Analysis: Fundamentals and Current Approaches 123

Definitions 123

Terms Used in Genetics	123
Genetic Names	124
Auxotrophic and Catabolic Mutants	125
Conditional-Lethal Mutants	126
Resistant Mutants	128

Inheritance in Bacteria 128

The Luria and Delbrück Experiment	129
Mutants Are Clonal	130
Esther and Joshua Lederberg's Experiment	130

Mutation Rates 132

Calculating Mutation Rates	133
Calculating the Mutation Rate from the Rate of Increase in the Proportion of Mutants	135

Types of Mutations 136

Properties of Mutations	136
Base Pair Changes	136
Frameshift Mutations	140
Deletion Mutations	141
Tandem-Duplication Mutations	143
Inversion Mutations	144
Insertion Mutations	145

Reversion versus Suppression 147

Intragenic Suppressors	147
Intergenic Suppressors	147

Genetic Analysis in Bacteria 151

Isolating Mutants	151
Genetic Characterization of Mutants	155
Complementation Tests	160
Genetic Crosses in Bacteria	166
Mapping of Bacterial Markers by Transduction and Transformation	168
Other Uses of Transformation and Transduction	171
Genetic Mapping by Hfr Crosses	172

Perspective 176

BOX 3.1 Inversions and the Genetic Map 146



Plasmids 181

What Is a Plasmid? 181

Naming Plasmids	182
Functions Encoded by Plasmids	182
Plasmid Structure	183

Properties of Plasmids 184

Replication	184
Functions of the <i>ori</i> Region	187
Plasmid Replication Control Mechanisms	193
Mechanisms To Prevent Curing of Plasmids	200
The Par Systems of Plasmids	203
Plasmid Cloning Vectors	206

Examples of Plasmid Cloning Vectors	208
Broad-Host-Range Cloning Vectors	210
BOX 4.1 Linear Chromosomes and Plasmids in Bacteria	188
BOX 4.2 Determining the Inc Group	191
BOX 4.3 Toxin-Antitoxin Systems and Plasmid Maintenance	201



Conjugation 215

Overview 215

Classification of Self-Transmissible Plasmids and Integrating Elements	217
The Fertility Plasmid	217

Mechanism of DNA Transfer during Conjugation in *Proteobacteria* 218

Transfer (<i>tra</i>) Genes	218
The <i>oriT</i> Sequence	221
Efficiency of Transfer	222
Interspecies Transfer of Plasmids	225
Conjugation and Type IV Secretion Systems Capable of Translocating Proteins	225
Mobilizable Plasmids	229

Chromosome Transfer by Plasmids 230

Formation of Hfr Strains of <i>E. coli</i>	230
Transfer of Chromosomal DNA by Integrated Plasmids	230
Chromosome Mobilization	231
Prime Factors	231

Diversity in Transfer Systems 233

Integrating Conjugative Elements 234

SXT/R391 ICE	234
ICEBs1	236
Tn916	237
TnGBS1 and TnGBS2	240

BOX 5.1 Pilus-Specific Phages 220

BOX 5.2 Delivery of Conditional Plasmids by Conjugation 223

BOX 5.3 Gene Exchange between Domains 226

BOX 5.4 Conjugation and Synthetic Genomics 232



Transformation 245

Natural Transformation 246

Discovery of Transformation	246
Overview of Natural Transformation	247
DNA Uptake Mechanisms	247
Specificity of DNA Uptake	251
DNA Processing after Uptake	253
Natural Transformation as a Tool	253
Regulation of Natural Competence	254
Identification of Competence in Other Organisms	258
Role of Natural Transformation	258

Artificially Induced Competence 260

Chemical Induction 260
Electroporation 261
Protoplast Transformation 261

BOX 6.1 Experimental Measurements of DNA Uptake 248

BOX 6.2 Genetic Evidence for Single-Stranded DNA Uptake 252

BOX 6.3 Role of Natural Transformation in Pathogens 260



Bacteriophages and Transduction 265

Lytic Development 268

The Lytic Cycle 268
Transcriptional Regulation of Phage Gene Expression 268
Phage Genome Replication and Packaging 279
Host Cell Lysis 289

Lysogenic Development 292

The λ System 292
Other Lysogenic Systems 299

Genetic Analysis of Phages 302

Infection of Cells 302
Phage Crosses 303
Recombination and Complementation Tests with Phages 303
The Genetic-Linkage Map of a Phage 305

Phage-Mediated Genetic Transfer 306

Generalized Transduction 306
Specialized Transduction 308
Lysogenic Conversion and Bacterial Pathogenesis 310

Host Defenses Against Phage Infection 313

Restriction-Modification Systems 313
Abi Systems 313
CRISPR/Cas Systems 314
Small Molecules and Phage Defense 314
Phage versus Phage 314

Phages as Tools 315

Cloning Vectors 315
Phage Display 315
Phage Therapy 317

BOX 7.1 Phage Genomics 266

BOX 7.2 Phage T7-Based Tools 271

BOX 7.3 Protein Priming 285



Transposition, Site-Specific Recombination, and Families of Recombinases 321

Transposition 321

Overview of Transposition 322
Structure of Bacterial DNA Transposons 322
Types of Bacterial DNA Transposons 323
Assays of Transposition 326

Mechanisms of Transposition 328

DDE Transposons 328

HUH Transposons 332

General Properties of Transposons 334

Transposition Regulation 334

Target Site Specificity 335

Effects on Genes Adjacent to the Insertion Site 337

Target Immunity 337

Transposon Mutagenesis 337Transposon Mutagenesis *In Vivo* 339Transposon Mutagenesis *In Vitro* 340

Transposon Mutagenesis of Plasmids 341

Transposon Mutagenesis of the Bacterial Chromosome 341

Transposon Mutagenesis of All Bacteria 342

Using Transposon Mutagenesis To Make Random Gene Fusions 342

Site-Specific Recombination 343

Integrases 343

Resolvases 345

DNA Invertases 345

Y and S Recombinases 347

Y Recombinases: Mechanism 347

S Recombinases: Mechanism 351

Group II Mobile Introns: Elements That Move Using an RNA Intermediate 352

Importance of Transposition and Site-Specific Recombination in Bacterial Adaptation 354

BOX 8.1 Mobile Elements and DNA Replication 333

BOX 8.2 Transposons and Genomics 338

**Molecular Mechanisms of Homologous Recombination 359****Homologous Recombination and DNA Replication in Bacteria 360**

Early Evidence for the Interdependence of Homologous Recombination and DNA Replication 361

The Molecular Basis for Recombination in *E. coli* 361*chi* (χ) Sites and the RecBCD Complex 361

The RecF Pathway 367

Synapse Formation and the RecA Protein 368

The Ruv and RecG Proteins and the Migration and Cutting of Holliday Junctions 371

Recombination between Different DNAs in Bacteria 373How Are Linear DNA Fragments Recombined into the *E. coli* Chromosome? 373

Recombination during Natural Transformation 375

Phage Recombination Pathways 375

Rec Proteins of Phages T4 and T7 375

The RecE Pathway of the *rac* Prophage 375The Phage λ Red System 375

Recombineering: Gene Replacements in *E. coli* with Phage λ Recombination Functions 376

Gene Conversion and Other Manifestations of Heteroduplex Formation during Recombination 379

BOX 9.1 Discovery of χ sites 364

BOX 9.2 Other Types of Double-Strand Break Repair in Bacteria 365



DNA Repair and Mutagenesis 385

Evidence for DNA Repair 386

Specific Repair Pathways 387

Deamination of Bases 387

Damage Due to Reactive Oxygen 389

Damage Due to Alkylating Agents 393

Damage Due to UV Irradiation 395

General Repair Mechanisms 396

Base Analogs 396

Frameshift Mutagens 397

Mismatch Repair 398

Nucleotide Excision Repair 403

DNA Damage Tolerance Mechanisms 405

Homologous Recombination and DNA Replication 405

SOS-Inducible Repair 409

Mechanism of TLS by the Pol V Mutasome 416

Other Specialized Polymerases and Their Regulation 417

Summary of Repair Pathways in *E. coli* 418

Bacteriophage Repair Pathways 418

BOX 10.1 The Role of Reactive Oxygen Species in Cancer and Degenerative Diseases 391

BOX 10.2 DNA Repair and Cancer 401

BOX 10.3 The Ames Test 417



Regulation of Gene Expression: Genes and Operons 425

Transcriptional Regulation in Bacteria 426

Genetic Evidence for Negative and Positive Regulation 427

Negative Regulation of Transcription Initiation 428

Negative Inducible Systems 428

Negative Repressible Systems 437

Molecular Mechanisms of Transcriptional Repression 439

Positive Regulation of Transcription Initiation 439

Positive Inducible Systems 440

Positive Repressible Systems 447

Molecular Mechanisms of Transcriptional Activation 447

Regulation by Transcription Attenuation 449

Modulation of RNA Structure 449

Changes in Processivity of RNA Polymerase 459

Regulation of mRNA Degradation 460

Protein-Dependent Effects on RNA Stability 460

RNA-Dependent Effects on RNA Stability 461

Regulation of Translation 461

Regulation of Translation Initiation 462

Translational Regulation in the Exit Channel of the Ribosome 464

Regulation of Translation Termination 465

Posttranslational Regulation 467

Posttranslational Protein Modification 467

Regulation of Protein Turnover 467

Feedback Inhibition of Enzyme Activity 468

Why Are There So Many Mechanisms of Gene Regulation? 469

BOX 11.1 The Helix-Turn-Helix Motif of DNA-Binding Proteins 427

BOX 11.2 Families of Regulators 442

**Global Regulation: Regulons and Stimulons 473****Carbon Catabolite Regulation 474**Carbon Catabolite Regulation in *E. coli*: Catabolite Activator Protein (CAP) and cAMP 474Carbon Catabolite Regulation in *B. subtilis*: CcpA and Hpr 481**Regulation of Nitrogen Assimilation 482**

Pathways for Nitrogen Assimilation 483

Regulation of Nitrogen Assimilation Pathways in *E. coli* by the Ntr System 484Regulation of Nitrogen Assimilation in *B. subtilis* 491**Regulation of Ribosome Components and tRNA Synthesis 491**

Ribosomal Protein Gene Regulation 492

Regulation of rRNA and tRNA Synthesis 493

Stringent Response 494

Stress Responses in Bacteria 498

Heat Shock Regulation 498

General Stress Response in Enteric Bacteria 501

General Stress Response in *Firmicutes* 505

Extracytoplasmic (Envelope) Stress Responses 506

Iron Regulation in *E. coli* 510

The Fur Regulon 510

The RyhB sRNA 512

The Aconitase Translational Repressor 512

Regulation of Virulence Genes in Pathogenic Bacteria 513

Diphtheria 513

Cholera and Quorum Sensing 514

Whooping Cough 519

Developmental Regulation: Sporulation in *B. subtilis* 520

Identification of Genes That Regulate Sporulation 522

Regulation of Sporulation Initiation 522

Compartmentalized Regulation of Sporulation Genes 524

The Role of Sigma Factors in Sporulation Regulation	524
Intercompartmental Regulation during Development	525
Other Sporulation Systems	529
BOX 12.1 cAMP-Independent Carbon Catabolite Regulation in <i>E. coli</i>	477
BOX 12.2 Nitrogen Fixation	483
BOX 12.3 Signal Transduction Systems in Bacteria	486
BOX 12.4 Sigma Factors	488
BOX 12.5 Regulatory RNAs	503



Genomes and Genomic Analysis 535

The Bacterial Genome 535

DNA Sequencing 537

Advanced Genome-Sequencing Techniques	545
Polymerase Chain Reaction	547

Barriers to Horizontal Transfer: Genome Gatekeepers and Molecular Biologist's Toolkit 549

Restriction Endonucleases	549
Techniques for Nontraditional Cloning and Assembly	553
CRISPR/Cas Systems	559
Final Thoughts	568

Box 13.1 Annotation and Comparative Genomics 538

Box 13.2 Special Problems in Genetic Analysis of Operons 542

Box 13.3 Synthesizing and Cloning Complete Bacterial Genomes 560

Glossary 573

Index 599