Contents

Preface xv Acknowledgments xix About the Authors 1

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

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 *ori* Region 187
Plasmid Replication Control Mechanisms 193
Mechanisms To Prevent Curing of Plasmids 200
The Par Systems of Plasmids 203
Plasmid Cloning Vectors 206

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 (tra) Genes 218

The oriT 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 E. coli 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 337

Transposon Mutagenesis In Vivo 339 Transposon 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 Ruy and RecG Proteins and the Migration and Cutting of Holliday Junctions 371

Recombination between Different DNAs in Bacteria 373

How 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 375 The 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 474

Carbon 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 484 Regulation 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

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 *E. coli* 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