molecular genetics of bacteria

Larry Snyder and Wendy Champness

Contents

Preface xv

Introduction 1

The Biological Universe 3

The Eubacteria 3
The Archaea 3
The Eukaryotes 4
The Prokaryotes and the Eukaryotes 5

What Is Genetics? 5

Bacterial Genetics 6

Bacteria Are Haploid 6
Short Generation Times 6
Asexual Reproduction 6
Colony Growth on Agar Plates 7
Colony Purification 7
Serial Dilutions 7
Selections 7
Storing Stocks of Bacterial Strains 7
Genetic Exchange 7

Phage Genetics 8

Phages Are Haploid 8
Selections with Phages 8
Crosses with Phages 8

A Brief History of Bacterial Molecular Genetics 8

Inheritance in Bacteria 8
Transformation 9
Conjugation 9
Transduction 9

Recombination within Genes 9
Semiconservative DNA Replication 10
mRNA 10
The Genetic Code 10
The Operon Model 10
Enzymes for Molecular Biology 10

What's Ahead 10

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

Mechanism of DNA Replication 17

Deoxyribonucleotide Precursor Synthesis 17 Deoxyribonucleotide Polymerization 18 Semiconservative Replication 21 Replication of Double-Stranded DNA 21

Replication Errors 25

Editing 25
Methyl-Directed Mismatch Repair 27
Role of Editing and Mismatch Repair in Maintaining
Replication Fidelity 27

Replication of the Bacterial Chromosome and Cell Division 29

Structure of the Bacterial Chromosome 29
Replication of the Bacterial Chromosome 30
Initiation of Chromosome Replication 30
Termination of Chromosome Replication 34
Chromosome Segregation 35
Where Are the Replication Forks? 42
Cell Division 43
Coordination of Cell Division with Replication of the Chromosome 44
Timing of Initiation of Replication 45

The Bacterial Nucleoid 48

Supercoiling in the Nucleoid 48 Topoisomerases 50

The Bacterial Genome 51

Antibiotics That Affect Replication and DNA Structure 53

Antibiotics That Block Precursor Synthesis 53
Antibiotics That Block Polymerization of
Deoxynucleotides 54
Antibiotics That Affect DNA Structure 54
Antibiotics That Affect Gyrase 55

Molecular Biology Manipulations with DNA 55

Restriction Endonucleases 55
Hybridizations 58
Applications of the Enzymes Used in DNA Replication 61
Random Shotgun Sequencing of Bacterial Genomes 61
Site-Specific Mutagenesis 62
Polymerase Chain Reaction 63

BOX 1.1 Linear Chromosomes in Bacteria 32

BOX 1.2 Restarting Replication Forks 37

BOX 1.3 The Bacterial Cytoskeleton and Bacterial Cell Biology 40

BOX 1.4 Features of Bacterial Genomes 52

BOX 1.5 Bacterial Genome Sequencing 62

SUMMARY 67
QUESTIONS FOR THOUGHT 68
PROBLEMS 68
SUGGESTED READING 69

CHAPTER 2

Bacterial Gene Expression: Transcription, Translation, and Protein Folding 71

Overview 71

The Structure and Function of RNA 72

Types of RNA 72
RNA Precursors 72
RNA Structure 72
RNA Processing and Modification 74

Transcription 74

Structure of Bacterial RNA Polymerase 74 Overview of Transcription 75 Details of Transcription 76 rRNAs and tRNAs 85

Proteins 8	36
------------	----

Protein Structure 87
Translation 89
Details of Protein Synthesis 90
The Genetic Code 93
Translation Initiation 97
Translation Termination 102
Polycistronic mRNA 104
RNases and mRNA Processing and Decay 105

Protein Folding 106

Protein Chaperones 106

Membrane Proteins and Protein Export 109

The Translocase System 110
The Signal Sequence 112
The Targeting Factors 112
Protein Secretion 114
Disulfide Bonds 114

Regulation of Gene Expression 115

Transcriptional Regulation 116
Posttranscriptional Regulation 117

Introns and Inteins 117

Useful Concepts 117

Open Reading Frames 121
Transcriptional and Translational Fusions 122
Bacterial Genome Annotation 125

Antibiotics That Block Transcription and Translation 125

Antibiotic Inhibitors of Transcription 125 Antibiotic Inhibitors of Translation 131

BOX 2.1 Molecular Phylogeny 86

BOX 2.2 Mimicry in Translation 93

BOX 2.3 Exceptions to the Code 95

BOX 2.4 Traffic Jams on mRNA: Removing Stalled Ribosomes with tmRNA 103

BOX 2.5 Stability and Degradation of mRNA 107

BOX 2.6 Selfish DNAs: RNA Introns and Protein Inteins 118

BOX 2.7 Annotation and Comparative Genomics 125

SUMMARY 134

QUESTIONS FOR THOUGHT 136

PROBLEMS 136

SUGGESTED READING 137

CHAPTER 3

Bacterial Genetic Analysis: Forward and Reverse 139

Definitions 139

Terms Used in Genetics 139 Genetic Names 140

Useful Phenotypes in Bacterial Genetics 141

Auxotrophic Mutants 141 Conditional Lethal Mutants 142 Resistant Mutants 143

Inheritance in Bacteria 144

The Luria and Delbrück Experiment 144
The Newcombe Experiment 145
The Lederbergs' Experiment 149

Mutation Rates 150

Calculating Mutation Rates 150 Summary 153

Types of Mutations 153

Base Pair Changes 154
Frameshift Mutations 158
Deletion Mutations 159
Inversion Mutations 160
Tandem Duplication Mutations 161
Insertion Mutations 161

Reversion versus Suppression 163

Intragenic Suppressors 163 Intergenic Suppressors 163 Nonsense Suppressors 164

Genetic Analysis in Bacteria 167

Isolating Mutants 167
Isolating Independent Mutations 168
Selecting Mutants 168
Genetic Mapping by Recombination in
Bacteria 170
Complementation Tests 171
Genetic Crosses in Bacteria 174
Genetic Mapping by Hfr Crosses 176
Mapping of Bacterial Markers by Transduction and
Transformation 181
Other Uses of Transformation and Transduction:

Gene Replacements and Reverse Genetics 186

Strain Construction 184

Isolation of Tandem Duplications of the his Operon in Salmonella enterica Serovar Typhimurium 190

Length of Tandem Duplications 192
Frequency of Spontaneous Duplications 192

BOX 3.1 Statistical Analysis of the Number of Mutants per Culture 148

BOX 3.2 Inversions and the Genetic Map 162

SUMMARY 193

QUESTIONS FOR THOUGHT 194

PROBLEMS 194

SUGGESTED READING 196

CHAPTER 4

Plasmids 197

What Is a Plasmid? 197

Naming Plasmids 198
Functions Encoded by Plasmids 198
Plasmid Structure 199

Properties of Plasmids 201

Replication 201
Functions of the *ori* Region 206
Plasmid Replication Control Mechanisms 209
Mechanisms To Prevent Curing of
Plasmids 222
The Par Systems of Plasmids 227

Constructing a Plasmid Cloning Vector 229

Finding the Plasmid *ori* Region 229

Examples of Plasmid Cloning Vectors 231

Broad-Host-Range Cloning Vectors 234

Using Plasmid Vectors for Gene Replacement and Functional Genomics 235

BOX 4.1 Plasmids and Bacterial Pathogenesis 199

BOX 4.2 Linear Plasmids 205

BOX 4.3 An Incompatibility Group of One's Own 214

BOX 4.4 Plasmid Addiction 223

SUMMARY 240

QUESTIONS FOR THOUGHT 241

PROBLEMS 241

SUGGESTED READING 241

CHAPTER 5

Conjugation 243

Overview 243

Classification of Self-Transmissible Plasmids 244

Mechanism of DNA Transfer during Conjugation in Gram-Negative Bacteria 244

Transfer (*tra*) Genes 244
The *oriT* Sequence 250
Male-Specific Phages 250
Efficiency of Transfer 250

Interspecies Transfer of Plasmids 252

Mobilizable Plasmids 252

Genetic Analysis of Tra Systems in Gram-Negative Bacteria 259

Isolation of tra Mutant Plasmids 260

Complementation Tests To Determine the Number of *tra* Genes 260

Chromosome Transfer by Plasmids 261

Formation of Hfr Strains 263
Transfer of Chromosomal DNA by Integrated Plasmids 263
Chromosome Mobilization 264

Prime Factors 264

Transfer Systems of Gram-Positive Bacteria 266

Plasmid-Attracting Pheromones 267

Other Types of Transmissible Elements 269 Conjugative Transposons 271

BOX 5.1 Gene Exchange between Kingdoms 253
BOX 5.2 Conjugation and Type IV Protein Secretion
Systems 256

BOX 5.3 Conjugation in Streptomycetes 267

BOX 5.4 Conjugative Transposons (Integrating Conjugative Elements) 272

SUMMARY 274

QUESTIONS FOR THOUGHT 275

PROBLEMS 275

SUGGESTED READING 275

CHAPTER 6

Transformation 277

Natural Transformation 278

Discovery of Transformation 278

Competence 278

Regulation of Competence in *B. subtilis* 282
Experimental Evidence for Models of Natural
Transformation 283
Plasmid Transformation and Phage Transfection of
Naturally Competent Bacteria 286
Role of Natural Transformation 286

Importance of Natural Transformation for Forward and Reverse Genetics 288

Artificially Induced Competence 289

Calcium Ion Induction 289 Electroporation 290

BOX 6.1 Antigenic Variation in Neisseria gonorrhoeae 289

SUMMARY 291
QUESTIONS FOR THOUGHT 291
PROBLEMS 291
SUGGESTED READING 292

CHAPTER 7

Lytic Bacteriophages: Development, Genetics, and Generalized Transduction 293

The Bacteriophage Lytic Development Cycle 295

Phage T7: a Phage-Encoded RNA Polymerase 296
Phage T4: Transcriptional Activators, Antitermination,
a New Sigma Factor, and Replication-Coupled
Transcription 301

Phage DNA Replication 306

Phages with Single-Stranded Circular DNA 306
Phage T7: Linear DNA That Forms
Concatemers 314
Phage T4: Another Linear DNA That Forms
Concatemers 315

Phage Lysis 318

Genetic Analysis of Phages 320

Infection of Cells 320
Phage Crosses 321
Recombination and Complementation Tests with Phages 321
Genetic Experiments with the rll Genes of Phage T4 323
Constructing the Genetic Linkage Map of a Phage 332

Generalized Transduction 336

What Makes a Transducing Phage? 336
Shuttle Phasmids 337
Role of Transduction in Bacterial
Evolution 339

BOX 7.1 RNA Phages 297
BOX 7.2 Phage Display 299
BOX 7.3 Protein Priming 308
SUMMARY 339

QUESTIONS FOR THOUGHT 340 PROBLEMS 340 SUGGESTED READING 341

CHAPTER 8

Lysogeny: the λ Paradigm and the Role of Lysogenic Conversion in Bacterial Pathogenesis 343

Phage λ 344

Lytic Development 344 Replication of λ DNA 350

Lysogeny 352

The dI Gene Product 352
Phage λ Integration 353
Maintenance of Lysogeny 354
Immunity to Superinfection 356
Induction of λ 356
Competition between the Lytic and Lysogenic Cycles 359

Specialized Transduction 359

Other Lysogen-Forming Phages 362

Phage P2 362
Phage P4 362
Phages P1 and N15: Plasmid Prophages 364
Phage Mu 365
Use of Lysogen-Forming Phages as Cloning
Vectors 365

Lysogenic Conversion and Bacterial Pathogenesis 366

E. coli and Dysentery: Shiga Toxins 366
Diphtheria 368
Cholera 368
Botulism and Tetanus 369
Synopsis 369

Genetic Experiments with Phage λ 369

Genetics of λ Lysogeny 369 Genetics of the CI Repressor 370 Isolation of λ *nut* Mutations 370 Isolation of Host *nus* Mutations 373

BOX 8.1 Effects of Prophage Integration on the Host 354

BOX 8.2 Retroregulation 358

BOX 8.3 How a Pathogenicity Island Gets Around 365

SUMMARY 373

QUESTIONS FOR THOUGHT 375

PROBLEMS 375

SUGGESTED READING 375

CHAPTER 9

Transposition, Site-Specific Recombination, and Families of Recombinases 377

Transposition 377

Overview of Transposition 378
Structure of Bacterial Transposons 378
Types of Bacterial Transposons 379
Assays of Transposition 384

Mechanisms of Transposition 385

Genetic Requirements for Transposition of Tn3 385 Molecular Models for Transposition of Tn3 and Mu 389 Transposition by Tn10 and Tn5 392

Details of Transposition by the DDE Transposons 395

Details of the Mechanism of Transposition by Tn5 and Tn7 395

Rolling-Circle Transposons 397

Y and S Transposons 397

General Properties of Transposons 397

Target Site Specificity 398
Effects on Genes Adjacent to the Insertion Site 399
Regulation of Transposition 399
Target Immunity 400

Transposon Mutagenesis 400

Transposon Mutagenesis of Plasmids 403
Transposon Mutagenesis of the Bacterial
Chromosome 405

Transposon Mutagenesis of All Bacteria 406
Using Transposon Mutagenesis To Make Random
Gene Fusions 407
In Vivo Cloning 409

Site-Specific Recombination 410

Developmentally Regulated Excision of Intervening DNA 411

Integrases 412 Resolvases 415 DNA Invertases 415

Y and S Recombinases 417

Y Recombinases: Mechanism 417 S Recombinases: Mechanism 421

Importance of Transposition and Site-Specific Recombination in Bacterial Adaptation 423

BOX 9.1 Phage Mu: a Transposon Masquerading as a Phage 390

BOX 9.2 Transposon Mutagenesis In Vitro 402

SUMMARY 424

QUESTIONS FOR THOUGHT 425

PROBLEMS 425

SUGGESTED READING 426

CHAPTER 10

Molecular Mechanisms of Homologous Recombination 429

Overview of Recombination 430

Requirement 1: Pairing between Identical or Very Similar Sequences in the Crossover Region 430

Requirement 2: Complementary Base Pairing between Double-Stranded DNA Molecules 430

Requirement 3: Cutting and Rejoining by Recombination Enzymes 431

Requirement 4: Heteroduplex Formation Involving All Four Strands 431

Molecular Models of Recombination 431

Holliday Double-Strand Invasion Model 431 Single-Strand Invasion Models 433 Double-Strand Break Repair Model 433

The Molecular Basis for Recombination in *E. coli* 435

chi (χ) Sites and the RecBCD Nuclease 436 The RecFOR Pathway 438 Synapse Formation and the RecA Protein 440
The Ruv and RecG Proteins and the Migration and
Cutting of Holliday Junctions 442

Phage Recombination Pathways 446

Rec Proteins of Phages T4 and T7 446
The RecE Pathway of the *rac* Prophage 446
The Phage λ *red* System 446

Genetic Analysis of Recombination in Bacteria 446

Isolating Rec⁻ Mutants of *E. coli* 450
Isolating Mutants with Mutations in Other Recombination Genes 451
Gene Conversion and Other Manifestations of Heteroduplex Formation during Recombination 452

BOX 10.1 Breaking and Entering: Introns and Inteins Move by Double-Strand Break Repair or Retrohoming 434

BOX 10.2 The Three R's: Recombination, Replication, and Repair 439

BOX 10.3 Recombineering: Gene Replacements in *E. coli* with Phage λ Recombination Functions 447

SUMMARY 455
QUESTIONS FOR THOUGHT 456
PROBLEMS 456
SUGGESTED READING 457

CHAPTER 11

DNA Repair and Mutagenesis 459

Evidence for DNA Repair 460

Specific Repair Pathways 461

Deamination of Bases 461
Damage Due to Reactive Oxygen 464
Damage Due to Alkylating Agents 467
Damage Due to UV Irradiation 470

General Repair Mechanisms 471

The Methyl-Directed Mismatch Repair System 471 Nucleotide Excision Repair 477

DNA Damage Tolerance Mechanisms 478

Recombination Repair of Damaged Replication Forks 479 SOS Inducible Repair 484 Mechanism of Induction of SOS Mutagenesis 489 Mechanism of Translesion Synthesis by the UmuD'₂C Complex 490

Summary of Repair Pathways in E. coli 493

Bacteriophage Repair Pathways 494

BOX 11.1 Oxygen: the Enemy Within 465

BOX 11.2 Cancer and Mismatch Repair 475

BOX 11.3 Transcription-Repair Coupling 479
BOX 11.4 Translesion Synthesis and Cancer 486

BOX 11.5 The Ames Test 492

SUMMARY 494
QUESTIONS FOR THOUGHT 496
PROBLEMS 496
SUGGESTED READING 496

CHAPTER 12

Regulation of Gene Expression: Operons 499

Transcriptional Regulation in Bacteria 500

Genetic Evidence for Negative and Positive Regulation 500

Negative Transcriptional Regulation 502 The *E. coli lac* Operon 502

Fine-Structure Analysis of the *lacl* Gene of *E. coli* 506

The *E. coli gal* Operon 512

Negative Regulation of Biosynthetic Operons:
Aporepressors and Corepressors 516

Positive Regulation 518

The *E. coli* L-*ara* Operon 519
The *E. coli* Maltose Operons 525
The *tol* Operons 528

Regulation by Attenuation of Transcription 530

Regulation of the *E. coli trp* Operon by
Attenuation 530
Regulation of the *trp* Operon of *B. subtilis* by
Attenuation 532
Regulation of the *bgl* Operon of *E. coli* 536
Regulation by Secondary-Structure Changes
in the mRNA 536
Riboswitch Regulation 537

Posttranslational Regulation: Feedback Inhibition 539

Feedback Inhibition of the Tryptophan Operon 539 Feedback Inhibition of the Isoleucine-Valine Operon 539

Posttranslation Modifications of Enzymes 540

Operon Analysis for Sequenced Genomes 540

Alleles of Operon Genes 540
Alleles of Regulatory Genes and Elements 543

BOX 12.1 The Helix-Turn-Helix Motif of DNA-Binding Proteins 501

BOX 12.2 Families of Regulators 521

SUMMARY 543
QUESTIONS FOR THOUGHT 544
PROBLEMS 544
SUGGESTED READING 545

CHAPTER 13

Global Regulation: Regulons and Stimulons 547

Catabolite-Sensitive Operons 548

cAMP and the cAMP-Binding Protein 548 Genetic Analysis of Catabolite Regulation in *E. coli* 554

Uses of cAMP in Other Organisms 556 A Bacterial Two-Hybrid System Based on Adenylate Cyclase 557

Regulation of Nitrogen Assimilation 558

Pathways for Nitrogen Assimilation 558
Coordination of Catabolite Repression, the
Ntr System, and the Regulation of Amino
Acid-Degradative Operons 566

Genetic Analysis of Nitrogen Regulation in Enteric Bacteria 566

Stress Responses in Bacteria 568

Heat Shock Regulation 568
General Stress Response in Gram-Negative
Bacteria 571
General Stress Response in Gram-Positive Bacteria 572

Extracytoplasmic (Envelope) Stress Responses 573

Regulation of Porin Synthesis 574
Regulation of the Envelope Stress Response by CpxA-CpxR: a Two-Component Sensor-Kinase Response-Regulator System 582

The Extracytoplasmic Function: *E. coli* Sigma Factor σ^E 583

Iron Regulation in E. coli 584

The Fur Regulon 584
The RyhB RNA 584
The Aconitase Translational Repressor 586

Regulation of Virulence Genes in Pathogenic Bacteria 587

Diphtheria 587 Cholera and Quorum Sensing 587 Whooping Cough 593

Regulation of Ribosome and tRNA Synthesis 595

Ribosomal Proteins 595 Regulation of rRNA and tRNA Synthesis 598

Microarray and Proteomic Analysis of Regulatory Networks 602

Transcriptome Analysis 602
Proteome Analysis 606
From Genes to Regulons to Networks to Genetic Analysis 607

BOX 13.1 cAMP-Independent Catabolite Repression 551

BOX 13.2 Nitrogen Fixation 559 BOX 13.3 Sigma Factors 563

BOX 13.4 Signal Transduction Systems in Bacteria 576

BOX 13.5 Regulatory RNAs 580

SUMMARY 607

QUESTIONS FOR THOUGHT 609

PROBLEMS 609

SUGGESTED READING 610

CHAPTER 14

Bacterial Cell Compartmentalization and Sporulation 613

Analysis of Protein Transport in Escherichia coli 614

Use of the *mal* Genes To Study Protein Transport: Signal Sequences, *sec*, and SRP 615

The Tat Secretion Pathway 620

Genetic Analysis of Transmembrane Domains of Inner Membrane Proteins in Gram-Negative Bacteria 621

Identification of Genes for Transported Proteins by Random *phoA* Fusions 622

Protein Secretion 622

Protein Secretion Systems in Gram-Negative Bacteria 623

Protein Secretion in Gram-Positive Bacteria 630

Sortases 630

Example of a Sortase-Dependent Pathway: Sporulation in Streptomyces coelicolor 632

Genetic Analysis of Sporulation in Bacillus subtilis 635

Identification of Genes That Regulate Sporulation 636

Regulation of Initiation of Sporulation 636

Compartmentalized Regulation of Sporulation Genes 641

Analysis of the Role of Sigma Factors in Sporulation Regulation 642

Intercompartmental Regulation during Development 643

Finding Sporulation Genes: Mutant Hunts, Suppressor Analysis, and Functional Genomics 650

BOX 14.1 Secretion Systems and Motility 626 BOX 14.2 Phosphorelay Activation of the Transcription Factor Spo0A 640

SUMMARY 652

QUESTIONS FOR THOUGHT 652

PROBLEMS 653

SUGGESTED READING 653

Answers to Questions for Thought and Problems 655

Glossary 669

Figure and Table Credits 705

Index 711