

Detailed Contents

CHEMISTRY AND GENETICS PART

CHAPTER 1	
The Mendelian View of the World 5	
MENDEL'S DISCOVERIES 6 The Principle of Independent Segregation 6 Box 1-1 Mendelian Laws 6 Some Alleles Are Neither Dominant Nor Recessive 8 Principle of Independent Assortment 8 CHROMOSOMAL THEORY OF HEREDITY 8 GENE LINKAGE AND CROSSING OVER 9 Box 1-2 Genes Are Linked to Chromosomes 10 CHROMOSOME MAPPING 12	THE ORIGIN OF GENETIC VARIABILITY THROUGH MUTATIONS 15 EARLY SPECULATIONS ABOUT WHAT GENES ARE AND HOW THEY ACT 16 PRELIMINARY ATTEMPTS TO FIND A GENE-PROTEIN RELATIONSHIP 16 Summary 17 Bibliography 18

CHAPTER 2

Nucleic Acids Convey Genetic Information 19

AVERY'S BOMBSHELL: DNA CAN CARRY GENETIC SPECIFICITY 20

Viral Genes Are Also Nucleic Acids 21

THE DOUBLE HELIX 21

Box 2-1 Chargaff's Rules 23

Finding the Polymerases that Make DNA 24

Experimental Evidence Favors Strand Separation during DNA Replication 26

THE GENETIC INFORMATION WITHIN DNA IS CONVEYED BY THE SEQUENCE OF ITS FOUR NUCLEOTIDE **BUILDING BLOCKS** 28

DNA Cannot Be the Template that Directly Orders Amino Acids during Protein Synthesis 28 Box 2-2 Evidence that Genes Control Amino Acid Sequence in Proteins 29

RNA Is Chemically Very Similar to DNA 30

THE CENTRAL DOGMA 31

The Adaptor Hypothesis of Crick 31

The Test-Tube Synthesis of Proteins 32

The Paradox of the

Nonspecific-Appearing Ribosomes 32

Discovery of Messenger RNA (mRNA) 33

Enzymatic Synthesis of RNA upon DNA Templates 33

Establishing the Genetic Code 35

ESTABLISHING THE DIRECTION OF PROTEIN SYNTHESIS 37

Start and Stop Signals Are Also Encoded within DNA 38

THE ERA OF GENOMICS 38

Summary 39

CHAPTER 3

The Importance of Weak Chemical Interactions 41

CHARACTERISTICS OF CHEMICAL BONDS 41

Chemical Bonds Are Explainable in Quantum-Mechanical Terms

Chemical-Bond Formation Involves a Change in the Form of Energy 43

Equilibrium between Bond Making and Breaking 43

THE CONCEPT OF FREE ENERGY 44

 $K_{\rm eq}$ Is Exponentially Related to ΔG 44

Covalent Bonds Are Very Strong 44

WEAK BONDS IN BIOLOGICAL SYSTEMS

Weak Bonds Have Energies between 1 and 7 kcal/mol 45

Weak Bonds Are Constantly Made and Broken at Physiological Temperatures 45

The Distinction between Polar and Nonpolar Molecules 45

Van der Waals Forces 46

Hydrogen Bonds 47

Some Ionic Bonds Are Hydrogen Bonds 47 Weak Interactions Demand Complementary Molecular Surfaces 48

Water Molecules Form Hydrogen Bonds 49

Weak Bonds between Molecules in Aqueous Solutions 49

Box 3-1 The Uniqueness of Molecular Shapes and the Concept of Selective Stickiness 50

Organic Molecules that Tend to Form Hydrogen Bonds Are Water Soluble

Hydrophobic "Bonds" Stabilize Macromolecules The Advantages of ΔG between 2 and 5 kcal/mol 52 Weak Bonds Attach Enzymes to Substrates 53 Weak Bonds Mediate Most Protein:DNA

and Protein:Protein Interactions 53 Summary 53

Bibliography 54

CHAPTER 4

The Importance of High-Energy Bonds

MOLECULES THAT DONATE ENERGY ARE THERMODYNAMICALLY UNSTABLE 55

ENZYMES LOWER ACTIVATION ENERGIES IN BIOCHEMICAL REACTIONS 57

FREE ENERGY IN BIOMOLECULES 58

High-Energy Bonds Hydrolyze with Large Negative ΔG 58

HIGH-ENERGY BONDS

IN BIOSYNTHETIC REACTIONS

Peptide Bonds Hydrolyze Spontaneously Coupling of Negative with Positive ΔG 61

ACTIVATION OF PRECURSORS

IN GROUP TRANSFER REACTIONS

ATP Versatility in Group Transfer 62

Activation of Amino Acids by Attachment of AMP 63

Nucleic Acid Precursors Are Activated

by the Presence of $\mathbf{Q} \sim \mathbf{Q}$

The Value of **P** ~ **P** Release in Nucleic Acid Synthesis 64

P ~ **P** Splits Characterize Most

Biosynthetic Reactions 65 Summary 67

Weak and Strong Bonds Determine Macromolecular Structure 69

HIGHER-ORDER STRUCTURES ARE DETERMINED BY INTRA- AND INTERMOLECULAR INTERACTIONS 69

DNA Can Form a Regular Helix 69
RNA Forms a Wide Variety of Structures 71
Chemical Features of Protein Building Blocks 71
The Peptide Bond 72

There Are Four Levels of Protein Structure 72 α Helices and β Sheets Are the Common Forms of Secondary Structure 74

Box 5-1 Determination of Protein Structure 75

THE SPECIFIC CONFORMATION
OF A PROTEIN RESULTS FROM ITS
PATTERN OF HYDROGEN BONDS 78

α Helices Come Together to Form Coiled-Coils 80

MOST PROTEINS ARE MODULAR, CONTAINING TWO OR THREE DOMAINS 81

Proteins Are Composed of a Surprisingly Small Number of Structural Motifs 81 Box 5-2 Large Proteins Are Often Constructed of Several Smaller Polypeptide Chains 82 Different Protein Functions Arise from Various Domain Combinations 82

WEAK BONDS CORRECTLY POSITION PROTEINS ALONG DNA AND RNA MOLECULES 84

Proteins Scan along DNA to Locate
a Specific DNA-Binding Site 85
Diverse Strategies for Protein Recognition of RNA

ALLOSTERY: REGULATION OF A PROTEIN'S FUNCTION BY CHANGING ITS SHAPE 87

The Structural Basis of Allosteric Regulation Is Known for Examples Involving Small Ligands, Protein-Protein Interactions, and Protein Modification 88

Not All Regulation of Proteins Is Mediated by Allosteric Events 91

Summary 91 Bibliography 92

86

PART 2 MAINTENANCE OF THE GENOME 93

CHAPTER 6

The Structures of DNA and RNA 97 DNA STRUCTURE 98

DNA Is Composed of Polynucleotide Chains 98
Each Base Has Its Preferred Tautomeric Form 100

The Two Strands of the Double Helix
Are Held Together by Base Pairing in
an Antiparallel Orientation 100
The Two Chains of the Double Helix Ha

The Two Chains of the Double Helix Have

Complementary Sequences 101 Hydrogen Bonding Is Important

for the Specificity of Base Pairing 102

Bases Can Flip Out from the Double Helix 102

DNA Is Usually a Right-Handed Double Helix 103

The Double Helix Has Minor and Major Grooves 103

The Major Groove Is Rich

in Chemical Information 103

Box 6-1 DNA Has 10.5 Base Pairs per Turn of the Helix in Solution: The Mica Experiment 104

The Double Helix Exists in Multiple Conformations 106

DNA Can Sometimes Form a Left-Handed Helix 107

DNA Strands Can Separate (Denature)

and Reassociate 108

Some DNA Molecules Are Circles 111

DNA TOPOLOGY 111

Linking Number Is an Invariant Topological Property of Covalently Closed, Circular DNA 112

DNA SYNTHESIS AT THE **REPLICATION FORK 205**

Box 8-2 ATP Control of Protein Function: Loading a Sliding Clamp 206

Interactions between Replication Fork Proteins Form the E. coli Replisome 210

INITIATION OF DNA REPLICATION 212

Specific Genomic DNA Sequences Direct the Initiation of DNA Replication 212

The Replicon Model of Replication Initiation 212

Replicator Sequences Include Initiator Binding Sites and Easily Unwound DNA 213

BINDING AND UNWINDING: ORIGIN SELECTION AND ACTIVATION BY THE INITIATOR PROTEIN 214

Box 8-3 The Identification of Origins of Replication and Replicators 214

Protein-Protein and Protein-DNA Interactions Direct the Initiation Process 217

Box 8-4 E. coli DNA Replication Is Regulated by DNA·ATP Levels and SeqA 217

Box 8-5 The Replication Factory Hypothesis 221

Eukaryotic Chromosomes Are Replicated Exactly Once per Cell Cycle 223

Pre-Replicative Complex Formation Directs the Initiation of Replication in Eukaryotes 223

Pre-RC Formation and Activation Is Regulated to Allow only a Single Round of Replication during Each Cell Cycle 225

Similarities between Eukaryotic and Prokaryotic DNA Replication Initiation 228

FINISHING REPLICATION 228

Type II Topoisomerases Are Required to Separate Daughter DNA Molecules 228

Lagging Strand Synthesis Is Unable to Copy the Extreme Ends of Linear Chromosomes 229

Telomerase Is a Novel DNA Polymerase that Does Not Require an Exogenous Template 230

Telomerase Solves the End Replication Problem by Extending the 3' End of the Chromosome 232

Summary 232

Bibliography 233

CHAPTER 9

The Mutability and Repair of DNA 235

REPLICATION ERRORS AND THEIR REPAIR 236

The Nature of Mutations 236

Some Replication Errors Escape Proofreading 237

Box 9-1 Expansion of Triple Repeats

Causes Disease 237

Mismatch Repair Removes Errors that Escape Proofreading 238

DNA DAMAGE 242

DNA Undergoes Damage Spontaneously from Hydrolysis and Deamination 242

Box 9-2 The Ames Test 243

DNA Is Damaged by Alkylation, Oxidation, and Radiation 244

Mutations Are also Caused by Base Analogs and Intercalating Agents 245

REPAIR OF DNA DAMAGE 246

Direct Reversal of DNA Damage 247

Base Excision Repair Enzymes Remove Damaged Bases by a Base-Flipping Mechanism 248

Nucleotide Excision Repair Enzymes Cleave Damaged DNA on Either Side of the Lesion 250

Recombination Repairs DNA Breaks by Retrieving Sequence Information from Undamaged DNA 253

Translesion DNA Synthesis Enables Replication to Proceed across DNA Damage 254

Box 9-3 The Y-Family of DNA Polymerases 256

Summary 257

Homologous Recombination at the Molecular Level 259

MODELS FOR HOMOLOGOUS RECOMBINATION 259

The Holliday Model Illustrates Key Steps in Homologous Recombination 260

The Double-Strand Break Repair Model More Accurately Describes Many

Recombination Events 264

Box 10-1 How to Resolve a Recombination Intermediate with Two Holliday Junctions 266

Double-Stranded DNA Breaks Arise by Numerous Means and Initiate Homologous Recombination 267

HOMOLOGOUS RECOMBINATION PROTEIN MACHINES 268

The RecBCD Helicase/Nuclease Processes Broken DNA Molecules for Recombination 269

RecA Protein Assembles on Single-Stranded DNA and Promotes Strand Invasion 272

Newly Base-Paired Partners Are Established within the RecA Filament 274

RecA Homologs Are Present in All Organisms 275

RuvAB Complex Specifically Recognizes Holliday Junctions and Promotes Branch Migration 276

RuvC Cleaves Specific DNA Strands at the Holliday Junction to Finish Recombination 276

HOMOLOGOUS RECOMBINATION IN EUKARYOTES 278

Homologous Recombination Has Additional Functions in Eukaryotes 278

Homologous Recombination Is Required for Chromosome Segregation during Meiosis 279

Programmed Generation of Double-Stranded DNA

Breaks Occurs during Meiosis 279

MRX Protein Processes the Cleaved DNA Endsfor Assembly of the RecA-like Strand-Exchange Proteins 282

Dmc1 Is a RecA-like Protein that Specifically Functions in Meiotic Recombination 282 Many Proteins Function Together to Promote

Meiotic Recombination 284

MATING-TYPE SWITCHING 285

Mating-Type Switching Is Initiated by a Site-Specific Double-Strand Break 286

Mating-Type Switching Is a Gene Conversion Event, Not Associated with Crossing Over 286

GENETIC CONSEQUENCES
OF THE MECHANISM
OF HOMOLOGOUS RECOMBINATION 288

Gene Conversion Occurs because DNA Is Repaired during Recombination 289

Summary 290 Bibliography 291

CHAPTER 11

Site-Specific Recombination and Transposition of DNA 293

CONSERVATIVE SITE-SPECIFIC RECOMBINATION 294

Site-Specific Recombination Occurs at Specific DNA Sequences in the Target DNA 294

Site-Specific Recombinases Cleave and Rejoin DNA Using a Covalent Protein-DNA Intermediate 296

Serine Recombinases Introduce Double-Stranded Breaks in DNA and then Swap Strands to Promote Recombination 298

Tyrosine Recombinases Break and Rejoin One Pair of DNA Strands at a Time 299

Structures of Tyrosine Recombinases Bound to DNA Reveal the Mechanism of DNA Exchange 300

Box 11-1 Application of Site-Specific Recombination to Genetic Engineering 302

BIOLOGICAL ROLES OF SITE-SPECIFIC RECOMBINATION 302

λ Integrase Promotes the Integration and Excision of aViral Genome into the Host Cell Chromosome 303

Phage λ Excision Requires
a New DNA-Bending Protein 304

The Hin Recombinase Inverts a Segment of DNA Al lowing Expression of Alternative Genes 305

Hin Recombination Requires a DNA Enhancer 306

Recombinases Convert Multimeric Circular DNA Molecules into Monomers 307

There Are Other Mechanisms to Direct Recombination to Specific Segments of DNA 310

TRANSPOSITION 310

Some Genetic Elements Move to New Chromosomal Locations by Transposition 310

There Are Three Principal Classes of Transposable Elements 311

DNA Transposons Carry a Transposase Gene, Flanked by Recombination Sites 312

Transposons Exist as Both Autonomous and Nonautonomous Elements 313

Viral-like Retrotransposons and Retroviruses Carry Terminal Repeat Sequences and Two Genes Important for Recombination 313

Poly-A Retrotransposons Look Like Genes 314

DNA Transposition by a Cutand-Paste Mechanism 314

The Intermediate in Cut-and-Paste Transposition Is Finished by Gap Repair 316

There Are Multiple Mechanisms for Cleaving the Nontransferred Strand during DNA Transposition 316

DNA Transposition by a Replicative Mechanism 318

Viral-like Retrotransposons and Retroviruses Move Using an RNA Intermediate 320 DNA Transposases and Retroviral Integrases Are Members of a Protein Superfamily 321

Box 11-2 The Pathway of Retroviral cDNA Formation 322

Poly-A Retrotransposons Move by a "Reverse Splicing" Mechanism 324

EXAMPLES OF TRANSPOSABLE ELEMENTS AND THEIR REGULATION 327

IS4-Family Transposons Are Compact Elements with Multiple Mechanisms for Copy
Number Control 327

Box 11-3 Maize Elements and the Discovery of Transposons 328

Tn10 Transposition Is Coupled to Cellular DNA Replication 329

Phage Mu Is an Extremely Robust Transposon 331

Mu Uses Target Immunity to Avoid Transposing into Its Own DNA 331

Tc1/Mariner Elements Are Extremely Successful DNA Elements in Eukaryotes 334

Yeast Ty Elements Transpose into Safe Havens in the Genome 335

LINEs Promote Their Own Transposition and Even Transpose Cellular RNAs 336

V(D)J RECOMBINATION 337

The Early Events in V(D)J Recombination Occur by a Mechanism Similar to Transposon Excision 339

Summary 342 Bibliography 342

PART 3 EXPRESSION OF THE GENOME 343

CHAPTER 12

Mechanisms of Transcription 347

RNA POLYMERASES AND THE TRANSCRIPTION CYCLE 348

RNA Polymerases Come in Different Forms, but Share Many Features 348 Transcription by RNA Polymerase Proceeds in a Series of Steps 350 Transcription Initiation Involves
Three Defined Steps 352

THE TRANSCRIPTION CYCLE IN BACTERIA 353

Bacterial Promoters Vary in Strength and Sequence, but Have Certain Defining Features 353

The σ Factor Mediates Binding of Polymerase to the Promoter 354 Box 12-1 Consensus Sequences 355 Transition to the Open Complex Involves Structural Changes in RNA Polymerase and in the Promoter DNA 356 Transcription Is Initiated by RNA Polymerase without the Need for a Primer 358 RNA Polymerase Synthesizes Several Short RNAs before Entering the Elongation Phase 358 The Elongating Polymerase Is a Processive Machine that Synthesizes and Proofreads RNA 359 Box 12-2 The Single-Subunit RNA Polymerases 360 Transcription Is Terminated by Signals within the RNA Sequence 361 TRANSCRIPTION IN EUKARYOTES RNA Polymerase II Core Promoters Are Made up of Combinations of Four Different Sequence Elements 363 RNA Polymerase II Forms a Pre-Initiation Complex with General Transcription Factors at the Promoter 364

TBP Binds to and Distorts DNA Using a B Sheet Inserted into the Minor Groove 366 The Other General Transcription Factors also Have Specific Roles in Initiation 367 In Vivo, Transcription Initiation Requires Additional Proteins, Including the Mediator Complex Mediator Consists of Many Subunits. Some Conserved from Yeast to Human 369 A New Set of Factors Stimulate Pol II Elongation and RNA Proofreading 370 Elongating Polymerase Is Associated with a New Set of Protein Factors Required for Various Types of RNA Processing 371 RNA Polymerases I and III Recognize Distinct Promoters, Using Distinct Sets of Transcription Factors, but still Require TBP 374 Summary 376 Bibliography 377

CHAPTER 13

RNA Splicing 379

THE CHEMISTRY OF RNA SPLICING 380

Sequences within the RNA Determine Where Splicing Occurs 380

The Intron Is Removed in a Form Called a Lariat as the Flanking Exons Are Joined 381

Exons from Different RNA Molecules Can Be Fused by Trans-Splicing 383

THE SPLICEOSOME MACHINERY 383

RNA Splicing Is Carried Out by a Large Complex Called the Spliceosome 383

SPLICING PATHWAYS 385

Assembly, Rearrangements, and Catalysis Within the Spliceosome: the Splicing Pathway 385
Self-Splicing Introns Reveal that RNA
Can Catalyze RNA Splicing 387

Group I Introns Release a Linear Intron Rather than a Lariat 388

Box 13-1 Converting Group I Introns into Ribozymes 389

How Does the Spliceosome Find the Splice Sites Reliably? 391

ALTERNATIVE SPLICING 394

Single Genes Can Produce Multiple Products by Alternative Splicing 394

Alternative Splicing Is Regulated by Activators and Repressors 396

Box 13-2 Adenovirus and the Discovery of Splicing 398

A Small Group of Introns Are Spliced by an Alternative Spliceosome Composed of a Different Set of snRNPs 400

EXON SHUFFLING 401

Exons Are Shuffled by Recombination to Produce Genes Encoding New Proteins 400

RNA EDITING 404

RNA Editing Is Another Way of Altering the Sequence of an mRNA 404

CHAPTER 14

Translation 411

MESSENGER RNA 412

Polypeptide Chains Are Specified by Open-Reading Frames 412

Prokaryotic mRNAs Have a Ribosome Binding Site that Recruits the Translational Machinery 413

Eukaryotic mRNAs Are Modified at Their 5' and 3' Ends to Facilitate Translation 414

TRANSFER RNA 415

tRNAs Are Adaptors between Codons and Amino Acids 415

tRNAs Share a Common Secondary Structure that Resembles a Cloverleaf 416

tRNAs Have an L-Shaped

Three-Dimensional Structure 417

ATTACHMENT OF AMINO ACIDS TO tRNA 417

tRNAs Are Charged by the Attachment of an Amino Acid to the 3' Terminal Adenosine Nucleotide via a High-Energy Acyl Linkage 417

Aminoacyl tRNA Synthetases Charge tRNAs in Two Steps 418

Each Aminoacyl tRNA Synthetase Attaches a Single Amino Acid to One or More tRNAs 419

tRNA Synthetases Recognize Unique Structural Features of Cognate tRNAs 420

Aminoacyl-tRNA Formation Is Very Accurate 421 Some Aminoacyl tRNA Synthetases Use an Editing Pocket to Charge tRNAs with High Accuracy 422

The Ribosome Is Unable to Discriminate between Correctly and Incorrectly Charged tRNAs 422

Box 14-1 Selenocysteine 423

THE RIBOSOME 423

The Ribosome Is Composed of a Large and a Small Subunit 425

The Large and Small Subunits Undergo Association and Dissociation during each Cycle of Translation 425

mRNA TRANSPORT 406

Once Processed, mRNA Is Packaged and Exported from the Nucleus into the Cytoplasm for Translation 406

Summary 408 Bibliography 409

New Amino Acids Are Attached to the C-Terminus of the Growing Polypeptide Chain 427

Peptide Bonds Are Formed by Transfer of the Growing Polypeptide Chain from One tRNA to Another 428

Ribosomal RNAs Are Both Structural and Catalytic Determinants of the Ribosome 428

The Ribosome Has Three Binding Sites for tRNA 429

Channels through the Ribosome Allow the mRNA and Growing Polypeptide to Enter and/or Exit the Ribosome 430

INITIATION OF TRANSLATION 432

Prokaryotic mRNAs Are Initially Recruited to the Small Subunit by Base-Pairing to rRNA 433

A Specialized tRNA Charged with a Modified Methionine Binds Directly to the Prokaryotic Small Subunit 433

Three Initiation Factors Direct the Assembly of an Initiation Complex that Contains mRNA and the Initiator tRNA 433

Eukaryotic Ribosomes Are Recruited to the mRNA by the 5' Cap 435

The Start Codon Is Found by Scanning Downstream from the 5' End of the mRNA 437

Translation Initiation Factors Hold Eukaryotic mR-NAs in Circles 438

Box 14-2 uORFs and IRESs: Exceptions that Prove the Rule 439

TRANSLATION ELONGATION 440

Aminoacyl-tRNAs Are Delivered to the A Site by Elongation Factor EF-Tu 441

The Ribosome Uses Multiple Mechanisms to Select Against Incorrect Aminoacyl-tRNAs 441

The Ribosome Is a Ribozyme 442

Peptide Bond Formation and the Elongation Factor EF-G Drive Translocation of the tRNAs and the mRNA 444 EF-G Drives Translocation by Displacing the tRNA Bound to the A Site 445
EF-Tu-GDP and EF-G-GDP Must Exchange GDP for GTP Prior to Participating in a New Round of Elongation 446
A Cycle of Peptide Bond Formation Consumes Two Molecules of GTP and One Molecule of ATP 446

Box 14-3 GTP-Binding Proteins, Conformational Switching, and the Fidelity and Ordering of the Events of Translation 447

TERMINATION OF TRANSLATION 448

Release Factors Terminate Translation in Response to Stop Codons 448

Short Regions of Class I Release Factors Recognize Stop Codons and Trigger Release of the Peptidyl Chain 449

CHAPTER 15

The Genetic Code 461

THE CODE IS DEGENERATE 461

Perceiving Order in the Makeup of the Code 462

Wobble in the Anticodon 463

Three Codons Direct Chain Termination 463

How the Code Was Cracked 464

Stimulation of Amino Acid Incorporation by Synthetic mRNAs 465

Poly-U Codes for Polyphenylalanine 466

Mixed Copolymers Allowed Additional Codon Assignments 467

Transfer RNA Binding to Defined
Trinucleotide Codons 468

Codon Assignments from Repeating Copolymers 468

GDP/GTP Exchange and GTP Hydrolysis Control the Function of the Class II Release Factor 450 The Ribosome Recycling Factor Mimics a tRNA 450 TRANSLATION-DEPENDENT REGULATION OF mRNA AND PROTEIN STABILITY 452

The SsrA RNA Rescues Ribosomes that Translate Broken mRNAs 452

Box 14-4 Antibiotics Arrest Cell Division by Blocking Specific Steps in Translation 453 Eukaryotic Cells Degrade mRNAs that Are Incomplete or that Have Premature Stop Codons 456

Summary 458 Bibliography 459

THREE RULES GOVERN THE GENETIC CODE 469

Three Kinds of Point Mutations Alter the Genetic Code 470 Genetic Proof that the Code Is Read in Units of Three 471

SUPPRESSOR MUTATIONS CAN RESIDE IN THE SAME OR A DIFFERENT GENE 471

Intergenic Suppression Involves Mutant tRNAs 472
Nonsense Suppressors also Read Normal
Termination Signals 474
Proving the Validity of the Genetic Code 474

THE CODE IS NEARLY UNIVERSAL 475

Summary 477 Bibliography 477

PART 4 REGULATION 479

CHAPTER 16

Gene Regulation in Prokaryotes 483

PRINCIPLES OF TRANSCRIPTIONAL REGULATION 483

Gene Expression Is Controlled by Regulatory Proteins 483 Many Promoters Are Regulated by Activators that Help RNA Polymerase Bind DNA and by Repressors that Block that Binding 484 Some Activators Work by Allostery and Regulate Steps after RNA Polymerase Binding 485 Action at a Distance and DNA Looping 486 Cooperative Binding and Allostery Have Many Roles in Gene Regulation 487

Antitermination and Beyond: Not All of Gene Regulation Targets Transcription Initiation 487

REGULATION OF TRANSCRIPTION INITIATION: EXAMPLES FROM BACTERIA 488

An Activator and a Repressor Together Control the *lac* Genes 488

CAP and Lac Repressor Have Opposing Effects on RNA Polymerase Binding to the lac Promoter 489

Box 16-1 Detecting DNA-Binding Sites 490

CAP Has Separate Activating and DNA-Binding Surfaces 492

CAP and Lac Repressor Bind DNA Using a Common Structural Motif 493

Box 16-2 Activator Bypass Experiments 493

The Activities of Lac Repressor and CAP
Are Controlled Allosterically by Their Signals 496

Box 16-3 Jacob, Monod, and the Ideas Behind Gene Regulation 497

Combinatorial Control: CAP Controls
Other Genes As Well 499

Alternative σ Factors Direct RNA Polymerase to Alternative Sets of Promoters 499

NtrC and MerR: Transcriptional Activators that Work by Allostery Rather than by Recruitment 500

NtrC Has ATPase Activity and Works from DNA Sites Far from the Gene 500

MerR Activates Transcription by Twisting Promoter DNA 501

Some Repressors Hold RNA Polymerase at the Promoter Rather than Excluding It 502

AraC and Control of the araBAD Operon by Antiactivation 503

EXAMPLES OF GENE REGULATION AT STEPS AFTER TRANSCRIPTION INITIATION 504

Amino Acid Biosynthetic Operons Are Controlled by Premature Transcription Termination 504

CHAPTER 17

Gene Regulation in Eukaryotes 529

CONSERVED MECHANISMS
OF TRANSCRIPTIONAL REGULATION
FROM YEAST TO MAMMALS 531

Activators Have Separate DNA Binding and Activating Functions 531
Box 17-1 The Two Hybrid Assay 533

Ribosomal Proteins Are Translational Repressors of Their Own Synthesis 506

Box 16-4 Riboswitches 509

THE CASE OF PHAGE λ:
LAYERS OF REGULATION 512

Alternative Patterns of Gene Expression Control Lytic and Lysogenic Growth 513

Regulatory Proteins and Their Binding Sites 514

λ Repressor Binds to Operator Sites Cooperatively 515

Box 16-5 Concentration, Affinity, and Cooperative Binding 516

Repressor and Cro Bind in Different Patterns to Control Lytic and Lysogenic Growth 517

Lysogenic Induction Requires Proteolytic Cleavage of λ Repressor 518

Negative Autoregulation of Repressor Requires Long-Distance Interactions and a Large DNA Loop 519

Another Activator, λcII, Controls the Decision between Lytic and Lysogenic Growth upon Infection of a New Host 520

Box 16-6 Genetic Approaches that Identified Genes Involved in the Lytic/Lysogenic Choice 521

Growth Conditions of *E. coli* Control the Stability of CII Protein and thus the Lytic/Lysogenic Choice 522

Transcriptional Antitermination in λ Development 523

Retroregulation: An Interplay of Controls on RNA Synthesis and Stability Determines in Gene Expression 524

Summary 525 Bibliography 526

536

Eukaryotic Regulators Use a Range of DNA-Binding Domains, but DNA Recognition Involves the Same Principles as Found in Bacteria 534 Activating Regions Are Not Well-Defined Structures

Activators and Repressors Sometimes Come
in Pieces 555
GENE "SILENCING" BY MODIFICATION
OF HISTONES AND DNA 556
Silencing in Yeast Is Mediated by Deacetylation and Methylation of Histones 556
Histone Modifications and
the Histone Code Hypothesis 558
DNA Methylation Is Associated with Silenced Genes
in Mammalian Cells 558
Some States of Gene Expression Are Inherited
through Cell Division even when the Initiating
Signal Is No Longer Present 560
Box 17-3 λ Lysogens and the Epigenetic Switch 562
EUKARYOTIC GENE REGULATION AT STEPS
AFTER TRANSCRIPTION INITIATION 562
Some Activators Control Transcriptional Elongation
rather than Initiation 562
The Regulation of Alternative mRNA Splicing
Can Produce Different Protein Products
in Different Cell Types 563
Expression of the Yeast Transcriptional Activator
Gcn4 Is Controlled at the Level of Translation 565
RNAS IN GENE REGULATION 567
Double-Stranded RNA Inhibits Expression
of Genes Homologous to that RNA 568
Short Interfering RNAs (siRNAs) Are Produced
from dsRNA and Direct Machinery that Switches Off Genes in Various Ways 568
•
MicroRNAs Control the Expression of some Genes
during Development 570
Summary 571 Bibliography 572
Diolography 312
Box 18-1 Microarray Assays: Theory and Practice 577
Gradients of Secreted Signaling Molecules Can
Instruct Cells to Follow Different Pathways of
Development based on Their Location 578
EXAMPLES OF THE THREE STRATEGIES

FOR ESTABLISHING DIFFERENTIAL

in Yeast by Silencing the HO Gene 580

The Localized Ash1 Repressor Controls Mating Type

GENE EXPRESSION 580

Cytoskeleton 576

in Neighboring Cells 576

Cell-to-Cell Contact and Secreted Cell Signaling

Molecules both Elicit Changes in Gene Expression

Box 18-2 Review of Cytoskeleton: Asymmetry and Growth 582

A Localized mRNA Initiates Muscle Differentiation in the Sea Squirt Embryo 584

Cell-to-Cell Contact Elicits Differential
Gene Expression in the Sporulating Bacterium,
B. subtilis 584

Box 18-3 Overview of Ciona Development 585

A Skin-Nerve Regulatory Switch Is Controlled by Notch Signaling in the Insect CNS 587

A Gradient of the Sonic Hedgehog Morphogen Controls the Formation of Different Neurons in the Vertebrate Neural Tube 588

THE MOLECULAR BIOLOGY OF DROSOPHILA EMBRYOGENESIS 590

An Overview of *Drosophila* Embryogenesis 590 A Morphogen Gradient Controls Dorsal-Ventral Patterning of the *Drosophila* Embryo 590 Box 18-4 Overview of

Drosophila Development 592

Box 18-5 The Role of Activator Synergy in Development 597

Segmentation Is Initiated by Localized RNAs at the Anterior and Posterior Poles of the Unfertilized Egg 599

The Bicoid Gradient Regulates the Expression of Segmentation Genes in a

Concentration-Dependent Fashion 601

Hunchback Expression Is also Regulated at the Level of Translation 602

The Gradient of Hunchback Repressor Establishes
Different Limits of Gap Gene Expression 603

Hunchback and Gap Proteins Produce Segmentation Stripes of Gene Expression 604

Box 18-6 Bioinformatics Methods for Identification of Complex Enhancers 605

Gap Repressor Gradients Produce many Stripes of Gene Expression 607

Short-Range Transcriptional Repressors Permit Different Enhancers to Work Independently of one Another within the Complex *eve* Regulatory Region 608

Summary 609 Bibliography 610

CHAPTER 19

Comparative Genomics and the Evolution of Animal Diversity 613

MOST ANIMALS HAVE ESSENTIALLY THE SAME GENES 614

How Does Gene Duplication Give Rise to Biological Diversity? 616

Box 19-1 Gene Duplication and the Importance of Regulatory Evolution 616

Box 19-2 Duplication of Globin Genes Produces New Expression Patterns and Diverse Protein Functions 618

Box 19-3 Creation of New Genes Drives Bacterial Evolution 618

THREE WAYS GENE EXPRESSION IS CHANGED DURING EVOLUTION 619

EXPERIMENTAL MANIPULATIONS THAT ALTER ANIMAL MORPHOLOGY 620

Changes in Pax6 Expression Create Ectopic Eyes 621 Changes in Antp Expression Transform Antennae into Legs 622

Importance of Protein Function: Interconversion of ftz and Antp 622

Subtle Changes in an Enhancer Sequence Can Produce New Patterns of Gene Expression 623

The Misexpression of *Ubx* Changes the Morphology of the Fruit Fly 624

Changes in Ubx Function Modify the Morphology of Fruit Fly Embryos 626

Changes in Ubx Target Enchancers Can Alter Patterns of Gene Expression 627

Box 19-4 The Homeotic Genes of Drosophila Are Organized in Special Chromosome Clusters 627

MORPHOLOGICAL CHANGES IN CRUSTACEANS AND INSECTS 630

Arthropods Are Remarkably Diverse 630

Changes in *Ubx* Expression Explain Modifications in Limbs among the Crustaceans 630

Why Insects Lack Abdominal Limbs 631

Modification of Flight Limbs Might Arise from the Evolution of Regulatory DNA Sequences 632

Box 19-5 Co-option of Gene Networks for Evolutionary Innovation 633

GENOME EVOLUTION AND HUMAN ORIGINS 635

Humans Contain Surprisingly Few Genes 635

The Human Genome Is very Similar to that of the Mouse and Virtually Identical to the Chimp 636

The Evolutionary Origins of Human Speech 637 How FOXP2 Fosters Speech in Humans 637 The Future of Comparative Genome Analysis 638 Summary 639 Bibliography 640

PART 5 METHODS 643

CHAPTER 20

Techniques of Molecular Biology 647

INTRODUCTION 647

NUCLEIC ACIDS 648

Electrophoresis through a Gel Separates DNA and RNA Molecules According to Size 648

Restriction Endopucleases Cleave DNA Molecules

at Particular Sites 649

DNA Hybridization Can Be Used to Identify Specific DNA Molecules 651

Hybridization Probes Can Identify

Electrophoretically-Separated DNAs and RNAs 652

Isolation of Specific Segments of DNA 653

DNA Cloning 654

Cloning DNA in Plasmid Vectors 654

Vector DNA Can Be Introduced into Host Organisms

by Transformation 655

Libraries of DNA Molecules Can Be Created

by Cloning 656

Hybridization Can Be Used to Identify a Specific

Clone in a DNA Library 657

Chemically Synthesized Oligonucleotides 657

The Polymerase Chain Reaction (PCR)

Amplifies DNAs by Repeated Rounds of

DNA Replication in vitro 658

Nested Sets of DNA Fragments

Reveal Nucleotide Sequences 660
Box 20-1 Forensics and the Polymerase

Chain Reaction 661

Shotgun Sequencing a Bacterial Genome 663

The Shotgun Strategy Permits a Partial Assembly of Large Genome Sequences 664

Box 20-2 Sequenators Are Used for High

Throughput Sequencing 665

The Paired-End Strategy Permits the Assembly

of Large Genome Scaffolds 666

Genome-Wide Analyses 667

Comparative Genome Analysis 669

PROTEINS 672

Specific Proteins Can Be Purified

from Cell Extracts 672

Purification of a Protein Requires

a Specific Assay 673

Preparation of Cell Extracts

Containing Active Proteins 673

Proteins Can Be Separated from One Another Using

Column Chromatography 673

Affinity Chromatography Can Facilitate More Rapid

Protein Purification 674

Separation of Proteins on Polyacrylamide Gels 675

Antibodies Visualize Electrophoretically-Separated

Proteins 676

Protein Molecules Can Be Directly Sequenced 676

Proteomics 677

CHAPTER 21

Model	Organisms	681
-------	-----------	-----

BACTERIOPHAGE 682

Assays of Phage Growth 684

The Single-Step Growth Curve 685

Phage Crossses and Complementation Tests 685

Transduction and Recombinant DNA 686

BACTERIA 687

Assays of Bacterial Growth 687

Bacteria Exchange DNA by Sexual Conjugation,

Phage-Mediated Transduction,

and DNA-Mediated Transformation 688

Bacterial Plasmids Can Be Used

as Cloning Vectors 689

Transposons Can Be Used to Generate Insertional

Mutations and Gene and Operon Fusions 689

Studies on the Molecular Biology of Bacteria Have

Been Enhanced by Recombinant DNA

Technology, Whole-Genome Sequencing, and Transcriptional Profiling 690

Biochemical Analysis Is Especially Powerful in Simple

Cells with Well-Developed Tools of Traditional and Molecular Genetics 691

Bacteria Are Accessible to Cytological Analysis 69

Phage and Bacteria Told Us Most of the Fundamental

Things about the Gene 692

BAKER'S YEAST, Saccharomyces cerevisiae 693

The Existence of Haploid and Diploid Cells Facilitate

Genetic Analysis of S. cerevisiae 693

Generating Precise Mutations in Yeast Is Easy 694

S. cerevisiae Has a Small,

Well-Characterized Genome 694

S. cerevisiae Cells Change Shape as They Grow 695

THE NEMATODE WORM, Caenorhabditis elegans 696

C. elegans Has a Very Rapid Life Cycle 696

C. elegans Is Composed of Relatively Few,

Well Studied Cell Lineages 697

The Cell Death Pathway Was Discovered

in C. elegans 698

RNAi Was Discovered in C. elegans 698

THE FRUIT FLY, Drosophila melanogaster 699

Drosophila Has a Rapid Life Cycle 699

The First Genome Maps Were Produced

in Drosophila 700

Genetic Mosaics Permit the Analysis

of Lethal Genes in Adult Flies 702

The Yeast FLP Recombinase Permits the Efficient

Production of Genetic Mosaics 703

It Is Easy to Create Transgenic Fruit Flies

that Carry Foreign DNA 703

THE HOUSE MOUSE, Mus musculus 705

Mouse Embryonic Development Depends on Stem Cells 706

It Is Easy to Introduce Foreign DNA

into the Mouse Embryo 707

Homologous Recombination Permits

the Selective Ablation of Individual Genes 707

Mice Exhibit Epigenetic Inheritance 709