# **Brief Contents**

### PART 1



- 1 The Mendelian View of the World, 5
- 2 Nucleic Acids Convey Genetic Information, 21

### PART 2



### STRUCTURE AND STUDY OF MACROMOLECULES, 45

- 3 The Importance of Weak and Strong Chemical Bonds, 51
- 4 The Structure of DNA, 77
- 5 The Structure and Versatility of RNA, 107
- 6 The Structure of Proteins, 121
- 7 Techniques of Molecular Biology, 147

### PART 3



### **MAINTENANCE OF THE GENOME, 193**

- 8 Genome Structure, Chromatin, and the Nucleosome, 199
- 9 The Replication of DNA, 257
- 10 The Mutability and Repair of DNA, 313
- 11 Homologous Recombination at the Molecular Level, 341
- 12 Site-Specific Recombination and Transposition of DNA, 377

### PART 4



### **EXPRESSION OF THE GENOME, 423**

- 13 Mechanisms of Transcription, 429
- 14 RNA Splicing, 467
- 15 Translation, 509
- 16 The Genetic Code, 573
- 17 The Origin and Early Evolution of Life, 593

### PART 5



### **REGULATION**, 609

- 18 Transcriptional Regulation in Prokaryotes, 615
- 19 Transcriptional Regulation in Eukaryotes, 657
- 20 Regulatory RNAs, 701
- **21** Gene Regulation in Development and Evolution, 733
- 22 Systems Biology, 775

### PART 6



- 1 Model Organisms, 797
- 2 Answers, 831

Index, 845

# **Detailed Contents**

### PART 1: HISTORY, 1

### $|\mathbf{f}| = 1$ The Mendelian View of the World, 5

### MENDEL'S DISCOVERIES, 6

The Principle of Independent Segregation, 6 ADVANCED CONCEPTS BOX 1-1 Mendelian Laws, 6 Some Alleles Are neither Dominant nor Recessive, 7 Principle of Independent Assortment, 8

CHROMOSOMAL THEORY OF HEREDITY, 8

### GENE LINKAGE AND CROSSING OVER, 9

KEY EXPERIMENTS BOX 1-2 Genes Are Linked to Chromosomes, 10

CHROMOSOME MAPPING, 11

### THE ORIGIN OF GENETIC VARIABILITY THROUGH MUTATIONS, 13

EARLY SPECULATIONS ABOUT WHAT GENES ARE AND HOW THEY ACT, 15

PRELIMINARY ATTEMPTS TO FIND A GENE– PROTEIN RELATIONSHIP, 16

SUMMARY, 17

**BIBLIOGRAPHY**, 17

**QUESTIONS, 18** 

### 2 Nucleic Acids Convey Genetic Information, 21

AVERY'S BOMBSHELL: DNA CAN CARRY GENETIC SPECIFICITY, 22

Viral Genes Are Also Nucleic Acids, 23

#### THE DOUBLE HELIX, 24

KEY EXPERIMENTS BOX 2-1 Chargaff's Rules, 26

Finding the Polymerases That Make DNA, 26

Experimental Evidence Favors Strand Separation during DNA Replication, 27

### THE GENETIC INFORMATION WITHIN DNA IS CONVEYED BY THE SEQUENCE OF ITS FOUR NUCLEOTIDE BUILDING BLOCKS, 30

KEY EXPERIMENTS BOX 2-2 Evidence That Genes Control Amino Acid Sequences in Proteins, 31

DNA Cannot Be the Template That Directly Orders Amino Acids during Protein Synthesis, 32

RNA Is Chemically Very Similar to DNA, 32

THE CENTRAL DOGMA, 33

The Adaptor Hypothesis of Crick, 34 Discovery of Transfer RNA, 34 The Paradox of the Nonspecific-Appearing Ribosomes, 35 Discovery of Messenger RNA (mRNA), 35 Enzymatic Synthesis of RNA upon DNA Templates, 35 Establishing the Genetic Code, 37

## ESTABLISHING THE DIRECTION OF PROTEIN SYNTHESIS, 38

Start and Stop Signals Are Also Encoded within DNA, 40

THE ERA OF GENOMICS, 40

SUMMARY, 41

**BIBLIOGRAPHY**, 42

## PART 2: STRUCTURE AND STUDY OF MACROMOLECULES, 45

## 3 The Importance of Weak and Strong Chemical Bonds, 51

### CHARACTERISTICS OF CHEMICAL BONDS, 51

Chemical Bonds Are Explainable in Quantum-Mechanical Terms, 52

Chemical-Bond Formation Involves a Change in the Form of Energy, 53

Equilibrium between Bond Making and Breaking, 53

### THE CONCEPT OF FREE ENERGY, 54

 $K_{eq}$  Is Exponentially Related to  $\Delta G$ , 54 Covalent Bonds Are Very Strong, 54

### WEAK BONDS IN BIOLOGICAL SYSTEMS, 55

Weak Bonds Have Energies between 1 and 7 kcal/mol, 55

Weak Bonds Are Constantly Made and Broken at Physiological Temperatures, 55

The Distinction between Polar and Nonpolar Molecules, 55

van der Waals Forces, 56

Hydrogen Bonds, 57

Some Ionic Bonds Are Hydrogen Bonds, 58

Weak Interactions Demand Complementary Molecular Surfaces, 58

Water Molecules Form Hydrogen Bonds, 59

Weak Bonds between Molecules in Aqueous Solutions, 59

Organic Molecules That Tend to Form Hydrogen Bonds Are Water Soluble, 60

Hydrophobic "Bonds" Stabilize Macromolecules, 60

ADVANCED CONCEPTS BOX 3-1 The Uniqueness of Molecular Shapes and the Concept of Selective Stickiness, 61

The Advantage of  $\Delta G$  between 2 and 5 kcal/mol, 62



## 4 The Structure of DNA, 77

### DNA STRUCTURE, 78

DNA Is Composed of Polynucleotide Chains, 78

Each Base Has Its Preferred Tautomeric Form, 80

The Two Strands of the Double Helix Are Wound around Each Other in an Antiparallel Orientation, 81

The Two Chains of the Double Helix Have Complementary Sequences, 81 Weak Bonds Attach Enzymes to Substrates, 62 Weak Bonds Mediate Most Protein-DNA and Protein-Protein Interactions, 62

#### HIGH-ENERGY BONDS, 63

MOLECULES THAT DONATE ENERGY ARE THERMODYNAMICALLY UNSTABLE, 63

## ENZYMES LOWER ACTIVATION ENERGIES IN BIOCHEMICAL REACTIONS, 65

FREE ENERGY IN BIOMOLECULES, 66

High-Energy Bonds Hydrolyze with Large Negative  $\Delta G$ , 66

## HIGH-ENERGY BONDS IN BIOSYNTHETIC REACTIONS, 67

Peptide Bonds Hydrolyze Spontaneously, 68 Coupling of Negative with Positive  $\Delta G$ , 69

### ACTIVATION OF PRECURSORS IN GROUP TRANSFER REACTIONS, 69

ATP Versatility in Group Transfer, 70

Activation of Amino Acids by Attachment of AMP, 70

Nucleic Acid Precursors Are Activated by the Presence of  $\mathbf{P} \sim \mathbf{P}$ , 71

The Value of  $\mathbf{P} \sim \mathbf{P}$  Release in Nucleic Acid Synthesis, 72

P ~ P Splits Characterize Most Biosynthetic Reactions, 73

SUMMARY, 74

**BIBLIOGRAPHY**, 75

QUESTIONS, 75

- The Double Helix Is Stabilized by Base Pairing and Base Stacking, 82
- Hydrogen Bonding Is Important for the Specificity of Base Pairing, 83
- Bases Can Flip Out from the Double Helix, 83

DNA Is Usually a Right-Handed Double Helix, 83

KEY EXPERIMENTS BOX 4-1 DNA Has 10.5 bp per Turn of the Helix in Solution: The Mica Experiment, 84 The Double Helix Has Minor and Major Grooves, 84

The Major Groove Is Rich in Chemical Information, 85

The Double Helix Exists in Multiple Conformations, 86

DNA Can Sometimes Form a Left-Handed Helix, 87

KEY EXPERIMENTS BOX 4-2 How Spots on an X-Ray Film Reveal the Structure of DNA, 88

DNA Strands Can Separate (Denature) and Reassociate, 89

Some DNA Molecules Are Circles, 92

#### DNA TOPOLOGY, 93

- Linking Number Is an Invariant Topological Property of Covalently Closed, Circular DNA, 93
- Linking Number Is Composed of Twist and Writhe, 93
- Lk<sup>o</sup> Is the Linking Number of Fully Relaxed cccDNA under Physiological Conditions, 94

DNA in Cells Is Negatively Supercoiled, 95

Nucleosomes Introduce Negative Supercoiling in Eukaryotes, 96 Topoisomerases Can Relax Supercoiled DNA, 97

- Prokaryotes Have a Special Topoisomerase That Introduces Supercoils into DNA, 97
- Topoisomerases Also Unknot and Disentangle DNA Molecules, 98
- Topoisomerases Use a Covalent Protein DNA Linkage to Cleave and Rejoin DNA Strands, 99
- Topoisomerases Form an Enzyme Bridge and Pass DNA Segments through Each Other, 100

DNA Topoisomers Can Be Separated by Electrophoresis, 102

- Ethidium Ions Cause DNA to Unwind, 102
- KEY EXPERIMENTS BOX 4-3 Proving that DNA Has a Helical Periodicity of ~10.5 bp per Turn from the Topological Properties of DNA Rings, 103

SUMMARY, 103

**BIBLIOGRAPHY**, 104

QUESTIONS, 104

### 5 The Structure and Versatility of RNA, 107

RNA CONTAINS RIBOSE AND URACIL AND IS USUALLY SINGLE-STRANDED, 107

RNA CHAINS FOLD BACK ON THEMSELVES TO FORM LOCAL REGIONS OF DOUBLE HELIX SIMILAR TO A-FORM DNA, 108

RNA CAN FOLD UP INTO COMPLEX TERTIARY STRUCTURES, 110

NUCLEOTIDE SUBSTITUTIONS IN COMBINATION WITH CHEMICAL PROBING PREDICT RNA STRUCTURE, 111

MEDICAL CONNECTIONS BOX 5-1 An RNA Switch Controls Protein Synthesis by Murine Leukemia Virus, 112



## 6 The Structure of Proteins, 121

### THE BASICS, 121

Amino Acids, 121

The Peptide Bond, 122

Polypeptide Chains, 123

Three Amino Acids with Special Conformational Properties, 124

ADVANCED CONCEPT BOX 6-1 Ramachandran Plot: Permitted Combinations of Main-Chain Torsion Angles φ and ψ, 124

## DIRECTED EVOLUTION SELECTS RNAs THAT BIND SMALL MOLECULES, 114

### SOME RNAs ARE ENZYMES, 114

TECHNIQUES BOX 5-2 Creating an RNA Mimetic of the Green Fluorescent Protein by Directed Evolution, 115

- The Hammerhead Ribozyme Cleaves RNA by the Formation of a 2', 3' Cyclic Phosphate, 116
- A Ribozyme at the Heart of the Ribosome Acts on a Carbon Center, 118

### SUMMARY, 118

**BIBLIOGRAPHY**, 118

**QUESTIONS**, 118

#### **IMPORTANCE OF WATER, 125**

## PROTEIN STRUCTURE CAN BE DESCRIBED AT FOUR LEVELS, 126

### **PROTEIN DOMAINS, 130**

Polypeptide Chains Typically Fold into One or More Domains, 130

ADVANCED CONCEPTS BOX 6-2 Glossary of Terms, 130 Basic Lessons from the Study of Protein Structures, 131

#### xviii Detailed Contents

Classes of Protein Domains, 132

Linkers and Hinges, 133

Post-Translational Modifications, 133

ADVANCED CONCEPTS BOX 6-3 The Antibody Molecule as an Illustration of Protein Domains, 133

FROM AMINO-ACID SEQUENCE TO THREE-DIMENSIONAL STRUCTURE, 134

Protein Folding, 134

KEY EXPERIMENTS BOX 6-4 Three-Dimensional Structure of a Protein Is Specified by Its Amino Acid Sequence (Anfinsen Experiment), 135

Predicting Protein Structure from Amino Acid Sequence, 135

CONFORMATIONAL CHANGES IN PROTEINS, 136

Mark

## 7 Techniques of Molecular Biology, 147

#### NUCLEIC ACIDS: BASIC METHODS, 148

- Gel Electrophoresis Separates DNA and RNA Molecules according to Size, 148
- Restriction Endonucleases Cleave DNA Molecules at Particular Sites, 149
- DNA Hybridization Can Be Used to Identify Specific DNA Molecules, 151
- Hybridization Probes Can Identify Electrophoretically Separated DNAs and RNAs, 151
- Isolation of Specific Segments of DNA, 153

DNA Cloning, 154

Vector DNA Can Be Introduced into Host Organisms by Transformation, 155

- Libraries of DNA Molecules Can Be Created by Cloning, 156
- Hybridization Can Be Used to Identify a Specific Clone in a DNA Library, 156
- Chemical Synthesis of Defined DNA Sequences, 157
- The Polymerase Chain Reaction Amplifies DNAs by Repeated Rounds of DNA Replication In Vitro, 158
- Nested Sets of DNA Fragments Reveal Nucleotide Sequences, 158
- TECHNIQUES BOX 7-1 Forensics and the Polymerase Chain Reaction, 160
- Shotgun Sequencing a Bacterial Genome, 162
- The Shotgun Strategy Permits a Partial Assembly of Large Genome Sequences, 162
- KEY EXPERIMENTS BOX 7-2 Sequenators Are Used for High-Throughput Sequencing, 163
- The Paired-End Strategy Permits the Assembly of Large-Genome Scaffolds, 165
- The \$1000 Human Genome Is within Reach, 167

PROTEINS AS AGENTS OF SPECIFIC MOLECULAR RECOGNITION, 137

Proteins That Recognize DNA Sequence, 137 Protein – Protein Interfaces, 140 Proteins That Recognize RNA, 141

## ENZYMES: PROTEINS AS CATALYSTS, 141

**REGULATION OF PROTEIN ACTIVITY, 142** 

SUMMARY, 143

**BIBLIOGRAPHY**, 144

### QUESTIONS, 144

### GENOMICS, 168

- Bioinformatics Tools Facilitate the Genome-Wide Identification of Protein-Coding Genes, 169
- Whole-Genome Tiling Arrays Are Used to Visualize the Transcriptome, 169
- Regulatory DNA Sequences Can Be Identified by Using Specialized Alignment Tools, 171
- Genome Editing Is Used to Precisely Alter Complex Genomes, 172

#### PROTEINS, 173

Specific Proteins Can Be Purified from Cell Extracts, 173

- Purification of a Protein Requires a Specific Assay, 173
- Preparation of Cell Extracts Containing Active Proteins, 174
- Proteins Can Be Separated from One Another Using Column Chromatography, 174
- Separation of Proteins on Polyacrylamide Gels, 176
- Antibodies Are Used to Visualize Electrophoretically Separated Proteins, 176
- Protein Molecules Can Be Directly Sequenced, 177

#### PROTEOMICS, 179

Combining Liquid Chromatography with Mass Spectrometry Identifies Individual Proteins within a Complex Extract, 179

- Proteome Comparisons Identify Important Differences between Cells, 181
- Mass Spectrometry Can Also Monitor Protein Modification States, 181
- Protein Protein Interactions Can Yield Information regarding Protein Function, 182

### NUCLEIC ACID-PROTEIN INTERACTIONS, 182

- The Electrophoretic Mobility of DNA Is Altered by Protein Binding, 183
- DNA-Bound Protein Protects the DNA from Nucleases and Chemical Modification, 184
- Chromatin Immunoprecipitation Can Detect Protein Association with DNA in the Cell, 185
- Chromosome Conformation Capture Assays Are Used to Analyze Long-Range Interactions, 187
- In Vitro Selection Can Be Used to Identify a Protein's DNA- or RNA-Binding Site, 189

**BIBLIOGRAPHY**, 190

QUESTIONS, 190

### PART 3: MAINTENANCE OF THE GENOME, 193

### 8 Genome Structure, Chromatin, and the Nucleosome, 199

## GENOME SEQUENCE AND CHROMOSOME DIVERSITY, 200

- Chromosomes Can Be Circular or Linear, 200
- Every Cell Maintains a Characteristic Number of Chromosomes, 201
- Genome Size Is Related to the Complexity of the Organism, 202
- The *E. coli* Genome Is Composed Almost Entirely of Genes, 203
- More Complex Organisms Have Decreased Gene Density, 204
- Genes Make Up Only a Small Proportion of the Eukaryotic Chromosomal DNA, 205
- The Majority of Human Intergenic Sequences Are Composed of Repetitive DNA, 207

## CHROMOSOME DUPLICATION AND SEGREGATION, 208

- Eukaryotic Chromosomes Require Centromeres, Telomeres, and Origins of Replication to Be Maintained during Cell Division, 208
- Eukaryotic Chromosome Duplication and Segregation Occur in Separate Phases of the Cell Cycle, 210
- Chromosome Structure Changes as Eukaryotic Cells Divide, 212
- Sister-Chromatid Cohesion and Chromosome Condensation Are Mediated by SMC Proteins, 214
- Mitosis Maintains the Parental Chromosome Number, 214
- During Gap Phases, Cells Prepare for the Next Cell Cycle Stage and Check That the Previous Stage Is Completed Correctly, 217
- Meiosis Reduces the Parental Chromosome Number, 217
- Different Levels of Chromosome Structure Can Be Observed by Microscopy, 219

THE NUCLEOSOME, 220

- Nucleosomes Are the Building Blocks of Chromosomes, 220
- Histones Are Small, Positively Charged Proteins, 221
- The Atomic Structure of the Nucleosome, 224
- Histones Bind Characteristic Regions of DNA within the Nucleosome, 224
- KEY EXPERIMENTS BOX 8-1 Micrococcal Nuclease and the DNA Associated with the Nucleosome, 226
- Many DNA Sequence–Independent Contacts Mediate the Interaction between the Core Histones and DNA, 227
- The Histone Amino-Terminal Tails Stabilize DNA Wrapping around the Octamer, 227
- Wrapping of the DNA around the Histone Protein Core Stores Negative Superhelicity, 228

### HIGHER-ORDER CHROMATIN STRUCTURE, 229

- Heterochromatin and Euchromatin, 229
- KEY EXPERIMENTS BOX 8-2 Nucleosomes and Superhelical Density, 230
- Histone H1 Binds to the Linker DNA between Nucleosomes, 232
- Nucleosome Arrays Can Form More Complex Structures: The 30-nm Fiber, 232
- The Histone Amino-Terminal Tails Are Required for the Formation of the 30-nm Fiber, 234
- Further Compaction of DNA Involves Large Loops of Nucleosomal DNA, 234
- Histone Variants Alter Nucleosome Function, 234

#### **REGULATION OF CHROMATIN STRUCTURE, 236**

- The Interaction of DNA with the Histone Octamer Is Dynamic, 236
- Nucleosome-Remodeling Complexes Facilitate Nucleosome Movement, 237
- Some Nucleosomes Are Found in Specific Positions: Nucleosome Positioning, 240

- The Amino-Terminal Tails of the Histones Are Frequently Modified, 241
- Protein Domains in Nucleosome-Remodeling and -Modifying Complexes Recognize Modified Histones, 244
- KEY EXPERIMENTS BOX 8-3 Determining Nucleosome Position in the Cell, 245
- Specific Enzymes Are Responsible for Histone Modification, 248
- Nucleosome Modification and Remodeling Work Together to Increase DNA Accessibility, 249

### NUCLEOSOME ASSEMBLY, 249

- Nucleosomes Are Assembled Immediately after DNA Replication, 249
- Assembly of Nucleosomes Requires Histone "Chaperones", 253

SUMMARY, 254

**BIBLIOGRAPHY**, 255

QUESTIONS, 255



### 9 The Replication of DNA, 257

- THE CHEMISTRY OF DNA SYNTHESIS, 258
  - DNA Synthesis Requires Deoxynucleoside Triphosphates and a Primer:Template Junction, 258
  - DNA Is Synthesized by Extending the 3' End of the Primer, 259
  - Hydrolysis of Pyrophosphate Is the Driving Force for DNA Synthesis, 260

### THE MECHANISM OF DNA POLYMERASE, 260

- DNA Polymerases Use a Single Active Site to Catalyze DNA Synthesis, 260
- TECHNIQUES BOX 9-1 Incorporation Assays Can Be Used to Measure Nucleic Acid and Protein Synthesis, 261
- DNA Polymerases Resemble a Hand That Grips the Primer:Template Junction, 263
- DNA Polymerases Are Processive Enzymes, 265
- Exonucleases Proofread Newly Synthesized DNA, 267

MEDICAL CONNECTIONS BOX 9-2 Anticancer and Antiviral Agents Target DNA Replication, 268

### THE REPLICATION FORK, 269

- Both Strands of DNA Are Synthesized Together at the Replication Fork, 269
- The Initiation of a New Strand of DNA Requires an RNA Primer, 270
- RNA Primers Must Be Removed to Complete DNA Replication, 271
- DNA Helicases Unwind the Double Helix in Advance of the Replication Fork, 272
- DNA Helicase Pulls Single-Stranded DNA through a Central Protein Pore, 273
- Single-Stranded DNA-Binding Proteins Stabilize ssDNA before Replication, 273
- Topoisomerases Remove Supercoils Produced by DNA Unwinding at the Replication Fork, 275
- Replication Fork Enzymes Extend the Range of DNA Polymerase Substrates, 275

## THE SPECIALIZATION OF DNA POLYMERASES, 277

- DNA Polymerases Are Specialized for Different Roles in the Cell, 277
- Sliding Clamps Dramatically Increase DNA Polymerase Processivity, 278
- Sliding Clamps Are Opened and Placed on DNA by Clamp Loaders, 281
- ADVANCED CONCEPTS BOX 9-3 ATP Control of Protein Function: Loading a Sliding Clamp, 282

### DNA SYNTHESIS AT THE REPLICATION FORK, 283

Interactions between Replication Fork Proteins Form the *E. coli* Replisome, 286

### INITIATION OF DNA REPLICATION, 288

Specific Genomic DNA Sequences Direct the Initiation of DNA Replication, 288

### The Replicon Model of Replication Initiation, 288

Replicator Sequences Include Initiator-Binding Sites and Easily Unwound DNA, 289

KEY EXPERIMENTS BOX 9-4 The Identification of Origins of Replication and Replicators, 290

### BINDING AND UNWINDING: ORIGIN SELECTION AND ACTIVATION BY THE INITIATOR PROTEIN, 293

- Protein Protein and Protein DNA Interactions Direct the Initiation Process, 293
- ADVANCED CONCEPTS BOX 9-5 E. coli DNA Replication Is Regulated by DnaA·ATP Levels and SeqA, 294
- Eukaryotic Chromosomes Are Replicated Exactly Once per Cell Cycle, 297
- Helicase Loading Is the First Step in the Initiation of Replication in Eukaryotes, 298
- Helicase Loading and Activation Are Regulated to Allow Only a Single Round of Replication during Each Cell Cycle, 300

Similarities between Eukaryotic and Prokaryotic DNA Replication Initiation, 301

#### FINISHING REPLICATION, 302

Type II Topoisomerases Are Required to Separate Daughter DNA Molecules, 303

- Lagging-Strand Synthesis Is Unable to Copy the Extreme Ends of Linear Chromosomes, 303
- Telomerase Is a Novel DNA Polymerase That Does Not Require an Exogenous Template, 305
- Telomerase Solves the End Replication Problem by Extending the 3' End of the Chromosome, 305

- MEDICAL CONNECTIONS BOX 9-6 Aging, Cancer, and the Telomere Hypothesis, 307
- Telomere-Binding Proteins Regulate Telomerase Activity and Telomere Length, 307
- Telomere-Binding Proteins Protect Chromosome Ends, 308

SUMMARY, 310

**BIBLIOGRAPHY**, 311

QUESTIONS, 312



## 10 The Mutability and Repair of DNA, 313

**REPLICATION ERRORS AND THEIR REPAIR, 314** 

The Nature of Mutations, 314

Some Replication Errors Escape Proofreading, 315

- MEDICAL CONNECTIONS BOX 10-1 Expansion of Triple Repeats Causes Disease, 316
- Mismatch Repair Removes Errors That Escape Proofreading, 316

### DNA DAMAGE, 320

- DNA Undergoes Damage Spontaneously from Hydrolysis and Deamination, 320
- MEDICAL CONNECTIONS BOX 10-2 The Ames Test, 321
- DNA Is Damaged by Alkylation, Oxidation, and Radiation, 322
- ADVANCED CONCEPTS BOX 10-3 Quantitation of DNA Damage and Its Effects on Cellular Survival and Mutagenesis, 323
- Mutations Are Also Caused by Base Analogs and Intercalating Agents, 323
- **REPAIR AND TOLERANCE OF DNA DAMAGE, 324** 
  - Direct Reversal of DNA Damage, 325

- Base Excision Repair Enzymes Remove Damaged Bases by a Base-Flipping Mechanism, 326
- Nucleotide Excision Repair Enzymes Cleave Damaged DNA on Either Side of the Lesion, 328

MEDICAL CONNECTIONS BOX 10-4 Linking Nucleotide Excision Repair and Translesion Synthesis to a Genetic Disorder in Humans, 330

- Recombination Repairs DNA Breaks by Retrieving Sequence Information from Undamaged DNA, 330
- DSBs in DNA Are Also Repaired by Direct Joining of Broken Ends, 331
- MEDICAL CONNECTIONS BOX 10-5 Nonhomologous End Joining, 332
- Translesion DNA Synthesis Enables Replication to Proceed across DNA Damage, 333
- ADVANCED CONCEPTS BOX 10-6 The Y Family of DNA Polymerases, 336

SUMMARY, 338

**BIBLIOGRAPHY**, 338

QUESTIONS, 339

### 11 Homologous Recombination at the Molecular Level, 341

## DNA BREAKS ARE COMMON AND INITIATE RECOMBINATION, 342

MODELS FOR HOMOLOGOUS RECOMBINATION, 342

- Strand Invasion Is a Key Early Step in Homologous Recombination, 344
- Resolving Holliday Junctions Is a Key Step to Finishing Genetic Exchange, 346

The Double-Strand Break – Repair Model Describes Many Recombination Events, 346

## HOMOLOGOUS RECOMBINATION PROTEIN MACHINES, 349

- ADVANCED CONCEPTS BOX 11-1 How to Resolve a Recombination Intermediate with Two Holliday Junctions, 350
- The RecBCD Helicase/Nuclease Processes Broken DNA Molecules for Recombination, 351

Chi Sites Control RecBCD, 354

RecA Protein Assembles on Single-Stranded DNA and Promotes Strand Invasion, 355

- Newly Base-Paired Partners Are Established within the RecA Filament, 356
- RecA Homologs Are Present in All Organisms, 359
- The RuvAB Complex Specifically Recognizes Holliday Junctions and Promotes Branch Migration, 359
- RuvC Cleaves Specific DNA Strands at the Holliday Junction to Finish Recombination, 361

HOMOLOGOUS RECOMBINATION IN EUKARYOTES, 362

- Homologous Recombination Has Additional Functions in Eukaryotes, 362
- Homologous Recombination Is Required for Chromosome Segregation during Meiosis, 362
- Programmed Generation of Double-Stranded DNA Breaks Occurs during Meiosis, 363
- MRX Protein Processes the Cleaved DNA Ends for Assembly of the RecA-Like Strand-Exchange Proteins, 364
- Dmc1 Is a RecA-Like Protein That Specifically Functions in Meiotic Recombination, 366
- Many Proteins Function Together to Promote Meiotic Recombination, 366

- MEDICAL CONNECTIONS BOX 11-2 The Product of the Tumor Suppressor Gene BRCA2 Interacts with Rad51 Protein and Controls Genome Stability, 367
- MEDICAL CONNECTIONS BOX 11-3 Proteins Associated with Premature Aging and Cancer Promote an Alternative Pathway for Holliday Junction Processing, 368

#### MATING-TYPE SWITCHING, 369

- Mating-Type Switching Is Initiated by a Site-Specific Double-Strand Break, 370
- Mating-Type Switching Is a Gene Conversion Event and Not Associated with Crossing Over, 370

## GENETIC CONSEQUENCES OF THE MECHANISM OF HOMOLOGOUS RECOMBINATION, 371

One Cause of Gene Conversion Is DNA Repair during Recombination, 373

SUMMARY, 374

**BIBLIOGRAPHY**, 375

QUESTIONS, 376

## $\langle \zeta \rangle$ 12 Site-Specific Recombination and Transposition of DNA, 377

## CONSERVATIVE SITE-SPECIFIC RECOMBINATION, 378

- Site-Specific Recombination Occurs at Specific DNA Sequences in the Target DNA, 378
- Site-Specific Recombinases Cleave and Rejoin DNA Using a Covalent Protein-DNA Intermediate, 380
- Serine Recombinases Introduce Double-Strand Breaks in DNA and Then Swap Strands to Promote Recombination, 382
- Structure of the Serine Recombinase DNA Complex Indicates that Subunits Rotate to Achieve Strand Exchange, 383
- Tyrosine Recombinases Break and Rejoin One Pair of DNA Strands at a Time, 383
- Structures of Tyrosine Recombinases Bound to DNA Reveal the Mechanism of DNA Exchange, 384
- MEDICAL CONNECTIONS BOX 12-1 Application of Site-Specific Recombination to Genetic Engineering, 386

## BIOLOGICAL ROLES OF SITE-SPECIFIC RECOMBINATION, 386

- λ Integrase Promotes the Integration and Excision of a Viral Genome into the Host-Cell Chromosome, 386
- Bacteriophage  $\lambda$  Excision Requires a New DNA-Bending Protein, 389

- The Hin Recombinase Inverts a Segment of DNA Allowing Expression of Alternative Genes, 389
- Hin Recombination Requires a DNA Enhancer, 390
- Recombinases Convert Multimeric Circular DNA Molecules into Monomers, 391
- There Are Other Mechanisms to Direct Recombination to Specific Segments of DNA, 391
- ADVANCED CONCEPTS BOX 12-2 The Xer Recombinase Catalyzes the Monomerization of Bacterial Chromosomes and of Many Bacterial Plasmids, 392

### **TRANSPOSITION**, 393

- Some Genetic Elements Move to New Chromosomal Locations by Transposition, 393
- There Are Three Principal Classes of Transposable Elements, 395
- DNA Transposons Carry a Transposase Gene, Flanked by Recombination Sites, 395
- Transposons Exist as Both Autonomous and Nonautonomous Elements, 396

Virus-Like Retrotransposons and Retroviruses Carry Terminal Repeat Sequences and Two Genes Important for Recombination, 396

- Poly-A Retrotransposons Look Like Genes, 396
- DNA Transposition by a Cut-and-Paste Mechanism, 397

- The Intermediate in Cut-and-Paste Transposition is Finished by Gap Repair, 398
- There Are Multiple Mechanisms for Cleaving the Nontransferred Strand during DNA Transposition, 399
- DNA Transposition by a Replicative Mechanism, 401
- Virus-Like Retrotransposons and Retroviruses Move Using an RNA Intermediate, 403
- DNA Transposases and Retroviral Integrases Are Members of a Protein Superfamily, 403
- Poly-A Retrotransposons Move by a "Reverse Splicing" Mechanism, 405

## EXAMPLES OF TRANSPOSABLE ELEMENTS AND THEIR REGULATION, 406

- KEY EXPERIMENTS BOX 12-3 Maize Elements and Discovery of Transposons, 408
- IS4 Family Transposons Are Compact Elements with Multiple Mechanisms for Copy Number Control, 409

Phage Mu Is an Extremely Robust Transposon, 411

- Mu Uses Target Immunity to Avoid Transposing into Its Own DNA, 411
- Tc1/mariner Elements Are Highly Successful DNA Elements in Eukaryotes, 411
- ADVANCED CONCEPTS BOX 12-4 Mechanism of Transposition Target Immunity, 413
- Yeast Ty Elements Transpose into Safe Havens in the Genome, 414
- LINEs Promote Their Own Transposition and Even Transpose Cellular RNAs, 414

#### V(D)J RECOMBINATION, 416

The Early Events in V(D)J Recombination Occur by a Mechanism Similar to Transposon Excision, 418

SUMMARY, 420

**BIBLIOGRAPHY**, 420

QUESTIONS, 421

### PART 4: EXPRESSION OF THE GENOME, 423

### 13 Mechanisms of Transcription, 429

RNA POLYMERASES AND THE TRANSCRIPTION CYCLE, 430

- RNA Polymerases Come in Different Forms but Share Many Features, 430
- Transcription by RNA Polymerase Proceeds in a Series of Steps, 432
- Transcription Initiation Involves Three Defined Steps, 434

#### THE TRANSCRIPTION CYCLE IN BACTERIA, 434

- Bacterial Promoters Vary in Strength and Sequence but Have Certain Defining Features, 434
- TECHNIQUES BOX 13-1 Consensus Sequences, 436
- The  $\sigma$  Factor Mediates Binding of Polymerase to the Promoter, 437
- Transition to the Open Complex Involves Structural Changes in RNA Polymerase and in the Promoter DNA, 438
- Transcription Is Initiated by RNA Polymerase without the Need for a Primer, 440
- During Initial Transcription, RNA Polymerase Remains Stationary and Pulls Downstream DNA into Itself, 441
- Promoter Escape Involves Breaking Polymerase Promoter Interactions and Polymerase Core– $\sigma$ Interactions, 442

- The Elongating Polymerase Is a Processive Machine That Synthesizes and Proofreads RNA, 442
- ADVANCED CONCEPTS BOX 13-2 The Single-Subunit RNA Polymerases, 443
- RNA Polymerase Can Become Arrested and Need Removing, 445

## Transcription Is Terminated by Signals within the RNA Sequence, 445

#### **TRANSCRIPTION IN EUKARYOTES, 448**

- RNA Polymerase II Core Promoters Are Made Up of Combinations of Different Classes of Sequence Element, 448
- RNA Polymerase II Forms a Preinitiation Complex with General Transcription Factors at the Promoter, 449
- Promoter Escape Requires Phosphorylation of the Polymerase "Tail," 449
- TBP Binds to and Distorts DNA Using a  $\beta$  Sheet Inserted into the Minor Groove, 451
- The Other General Transcription Factors Also Have Specific Roles in Initiation, 452
- In Vivo, Transcription Initiation Requires Additional Proteins, Including the Mediator Complex, 453

#### xxiv Detailed Contents

Mediator Consists of Many Subunits, Some Conserved from Yeast to Human, 454

A New Set of Factors Stimulates Pol II Elongation and RNA Proofreading, 455

Elongating RNA Polymerase Must Deal with Histones in Its Path, 456

Elongating Polymerase Is Associated with a New Set of Protein Factors Required for Various Types of RNA Processing, 457

Transcription Termination Is Linked to RNA Destruction by a Highly Processive RNase, 460

## 7

## 14 RNA Splicing, 467

THE CHEMISTRY OF RNA SPLICING, 469

- Sequences within the RNA Determine Where Splicing Occurs, 469
- The Intron Is Removed in a Form Called a Lariat as the Flanking Exons Are Joined, 470
- KEY EXPERIMENTS BOX 14-1 Adenovirus and the Discovery of Splicing, 471

### THE SPLICEOSOME MACHINERY, 473

RNA Splicing Is Performed by a Large Complex Called the Spliceosome, 473

#### SPLICING PATHWAYS, 474

Assembly, Rearrangements, and Catalysis within the Spliceosome: The Splicing Pathway, 474

Spliceosome Assembly Is Dynamic and Variable and Its Disassembly Ensures That the Splicing Reaction Goes Only Forward in the Cell, 476

Self-Splicing Introns Reveal That RNA Can Catalyze RNA Splicing, 477

Group I Introns Release a Linear Intron Rather Than a Lariat, 478

KEY EXPERIMENTS BOX 14-2 Converting Group I Introns into Ribozymes, 479

How Does the Spliceosome Find the Splice Sites Reliably?, 480

### VARIANTS OF SPLICING, 482

Exons from Different RNA Molecules Can Be Fused by *Trans*-Splicing, 482

A Small Group of Introns Is Spliced by an Alternative Spliceosome Composed of a Different Set of snRNPs, 483

### **ALTERNATIVE SPLICING, 483**

Single Genes Can Produce Multiple Products by Alternative Splicing, 483

## TRANSCRIPTION BY RNA POLYMERASES I AND III, 462

- RNA Pol I and Pol III Recognize Distinct Promoters but Still Require TBP, 462
- Pol I Transcribes Just the rRNA Genes, 462

Pol III Promoters Are Found Downstream from the Transcription Start Site, 463

SUMMARY, 463

**BIBLIOGRAPHY**, 464

**QUESTIONS**, 465

- Several Mechanisms Exist to Ensure Mutually Exclusive Splicing, 486
- The Curious Case of the *Drosophila Dscam* Gene: Mutually Exclusive Splicing on a Grand Scale, 487
- Mutually Exclusive Splicing of *Dscam* Exon 6 Cannot Be Accounted for by Any Standard Mechanism and Instead Uses a Novel Strategy, 488
- KEY EXPERIMENTS BOX 14-3 Identification of Docking Site and Selector Sequences, 490
- Alternative Splicing Is Regulated by Activators and Repressors, 491
- Regulation of Alternative Splicing Determines the Sex of Flies, 493
- An Alternative Splicing Switch Lies at the Heart of Pluripotency, 495

#### **EXON SHUFFLING, 497**

Exons Are Shuffled by Recombination to Produce Genes Encoding New Proteins, 497

MEDICAL CONNECTIONS BOX 14-4 Defects in Pre-mRNA Splicing Cause Human Disease, 497

#### **RNA EDITING, 500**

RNA Editing Is Another Way of Altering the Sequence of an mRNA, 500

- Guide RNAs Direct the Insertion and Deletion of Uridines, 501
- MEDICAL CONNECTIONS BOX 14-5 Deaminases and HIV, 503

mRNA TRANSPORT, 503

Once Processed, mRNA Is Packaged and Exported from the Nucleus into the Cytoplasm for Translation, 503

SUMMARY, 505

**BIBLIOGRAPHY**, 506



### 15 Translation, 509

#### MESSENGER RNA, 510

- Polypeptide Chains Are Specified by Open Reading Frames, 510
- Prokaryotic mRNAs Have a Ribosome-Binding Site That Recruits the Translational Machinery, 512
- Eukaryotic mRNAs Are Modified at their 5' and 3' Ends to Facilitate Translation, 512

#### **TRANSFER RNA, 513**

tRNAs Are Adaptors between Codons and Amino Acids, 513

- ADVANCED CONCEPTS BOX 15-1 CCA-Adding Enzymes: Synthesizing RNA without a Template, 513
- tRNAs Share a Common Secondary Structure That Resembles a Cloverleaf, 514

tRNAs Have an L-Shaped Three-Dimensional Structure, 514

### ATTACHMENT OF AMINO ACIDS TO tRNA, 515

tRNAs Are Charged by the Attachment of an Amino Acid to the 3'-Terminal Adenosine Nucleotide via a High-Energy Acyl Linkage, 515

- Aminoacyl-tRNA Synthetases Charge tRNAs in Two Steps, 515
- Each Aminoacyl-tRNA Synthetase Attaches a Single Amino Acid to One or More tRNAs, 515

tRNA Synthetases Recognize Unique Structural Features of Cognate tRNAs, 517

Aminoacyl-tRNA Formation Is Very Accurate, 518

Some Aminoacyl-tRNA Synthetases Use an Editing Pocket to Charge tRNAs with High Accuracy, 518

The Ribosome Is Unable to Discriminate between Correctly and Incorrectly Charged tRNAs, 519

#### THE RIBOSOME, 519

- ADVANCED CONCEPTS BOX 15-2 Selenocysteine, 520
- The Ribosome Is Composed of a Large and a Small Subunit, 521
- The Large and Small Subunits Undergo Association and Dissociation during Each Cycle of Translation, 522
- New Amino Acids Are Attached to the Carboxyl Terminus of the Growing Polypeptide Chain, 523
- Peptide Bonds Are Formed by Transfer of the Growing Polypeptide Chain from One tRNA to Another, 524
- Ribosomal RNAs Are Both Structural and Catalytic Determinants of the Ribosome, 524
- The Ribosome Has Three Binding Sites for tRNA, 525

Channels through the Ribosome Allow the mRNA and Growing Polypeptide to Enter and/or Exit the Ribosome, 527

### **INITIATION OF TRANSLATION, 528**

- Prokaryotic mRNAs Are Initially Recruited to the Small Subunit by Base Pairing to rRNA, 528
- A Specialized tRNA Charged with a Modified Methionine Binds Directly to the Prokaryotic Small Subunit, 528
- Three Initiation Factors Direct the Assembly of an Initiation Complex That Contains mRNA and the Initiator tRNA, 529
- Eukaryotic Ribosomes Are Recruited to the mRNA by the 5' Cap, 530
- Translation Initiation Factors Hold Eukaryotic mRNAs in Circles, 532

ADVANCED CONCEPTS BOX 15-3 uORFs and IRESs: Exceptions That Prove the Rule, 533

The Start Codon Is Found by Scanning Downstream from the 5' End of the mRNA, 535

#### **TRANSLATION ELONGATION, 535**

Aminoacyl-tRNAs Are Delivered to the A-Site by Elongation Factor EF-Tu, 537

- The Ribosome Uses Multiple Mechanisms to Select against Incorrect Aminoacyl-tRNAs, 537
- The Ribosome Is a Ribozyme, 538
- Peptide-Bond Formation Initiates Translocation in the Large Subunit, 541
- EF-G Drives Translocation by Stabilizing Intermediates in Translocation, 542
- EF-Tu-GDP and EF-G-GDP Must Exchange GDP for GTP before Participating in a New Round of Elongation, 543
- A Cycle of Peptide-Bond Formation Consumes Two Molecules of GTP and One Molecule of ATP, 543

### **TERMINATION OF TRANSLATION, 544**

- Release Factors Terminate Translation in Response to Stop Codons, 544
- Short Regions of Class I Release Factors Recognize Stop Codons and Trigger Release of the Peptidyl Chain, 544

ADVANCED CONCEPTS BOX 15-4 GTP-Binding Proteins, Conformational Switching, and the Fidelity and Ordering of the Events of Translation, 546

GDP/GTP Exchange and GTP Hydrolysis Control the Function of the Class II Release Factor, 547

The Ribosome Recycling Factor Mimics a tRNA, 548

#### **REGULATION OF TRANSLATION, 549**

Protein or RNA Binding near the Ribosome-Binding Site Negatively Regulates Bacterial Translation Initiation, 549

Regulation of Prokaryotic Translation: Ribosomal Proteins Are Translational Repressors of Their Own Synthesis, 551

- MEDICAL CONNECTIONS BOX 15-5 Antibiotics Arrest Cell Division by Blocking Specific Steps in Translation, 552
- Global Regulators of Eukaryotic Translation Target Key Factors Required for mRNA Recognition and Initiator tRNA Ribosome Binding, 556
- Spatial Control of Translation by mRNA-Specific 4E-BPs, 556
- An Iron-Regulated, RNA-Binding Protein Controls Translation of Ferritin, 557
- Translation of the Yeast Transcriptional Activator Gcn4 Is Controlled by Short Upstream ORFs and Ternary Complex Abundance, 558
- TECHNIQUES BOX 15-6 Ribosome and Polysome Profiling, 561

## TRANSLATION-DEPENDENT REGULATION OF mRNA AND PROTEIN STABILITY, 563

The SsrA RNA Rescues Ribosomes That Translate Broken mRNAs, 563

MEDICAL CONNECTIONS BOX 15-7 A Frontline Drug in Tuberculosis Therapy Targets SsrA Tagging, 565

Eukaryotic Cells Degrade mRNAs That Are Incomplete or Have Premature Stop Codons, 565

SUMMARY, 567

**BIBLIOGRAPHY**, 570

QUESTIONS, 570



### 16 The Genetic Code, 573

### THE CODE IS DEGENERATE, 573

Perceiving Order in the Makeup of the Code, 575 Wobble in the Anticodon, 575

Three Codons Direct Chain Termination, 577

How the Code Was Cracked, 577

Stimulation of Amino Acid Incorporation by Synthetic mRNAs, 578

Poly-U Codes for Polyphenylalanine, 579

- Mixed Copolymers Allowed Additional Codon Assignments, 579
- Transfer RNA Binding to Defined Trinucleotide Codons, 579
- Codon Assignments from Repeating Copolymers, 581

THREE RULES GOVERN THE GENETIC CODE, 582

Three Kinds of Point Mutations Alter the Genetic Code, 582

Genetic Proof That the Gode Is Read in Units of Three, 583

SUPPRESSOR MUTATIONS CAN RESIDE IN THE SAME OR A DIFFERENT GENE, 584

Intergenic Suppression Involves Mutant tRNAs, 584

Nonsense Suppressors Also Read Normal Termination Signals, 585

Proving the Validity of the Genetic Code, 586

### THE CODE IS NEARLY UNIVERSAL, 587

ADVANCED CONCEPTS BOX 16-1 Expanding the Genetic Code, 589

SUMMARY, 590

**BIBLIOGRAPHY**, 590

QUESTIONS, 591

### 17 The Origin and Early Evolution of Life, 593

WHEN DID LIFE ARISE ON EARTH?, 594

WHAT WAS THE BASIS FOR PREBIOTIC ORGANIC CHEMISTRY?, 595

DID LIFE EVOLVE FROM AN RNA WORLD?, 599

CAN SELF-REPLICATING RIBOZYMES BE CREATED BY DIRECTED EVOLUTION?, 599

## DOES DARWINIAN EVOLUTION REQUIRE SELF-REPLICATING PROTOCELLS?, 603 DID LIFE ARISE ON EARTH?, 606 SUMMARY, 607 BIBLIOGRAPHY, 607 QUESTIONS, 607

### 18 Transcriptional Regulation in Prokaryotes, 615

#### PRINCIPLES OF TRANSCRIPTIONAL REGULATION, 615

- Gene Expression Is Controlled by Regulatory Proteins, 615
- Most Activators and Repressors Act at the Level of Transcription Initiation, 616
- Many Promoters Are Regulated by Activators That Help RNA Polymerase Bind DNA and by Repressors That Block That Binding, 616
- Some Activators and Repressors Work by Allostery and Regulate Steps in Transcriptional Initiation after RNA Polymerase Binding, 618
- Action at a Distance and DNA Looping, 618
- Cooperative Binding and Allostery Have Many Roles in Gene Regulation, 619
- Antitermination and Beyond: Not All of Gene Regulation Targets Transcription Initiation, 620

## REGULATION OF TRANSCRIPTION INITIATION: EXAMPLES FROM PROKARYOTES, 620

- An Activator and a Repressor Together Control the *lac* Genes, 620
- CAP and Lac Repressor Have Opposing Effects on RNA Polymerase Binding to the *lac* Promoter, 622
- CAP Has Separate Activating and DNA-Binding Surfaces, 622
- CAP and Lac Repressor Bind DNA Using a Common Structural Motif, 623
- KEY EXPERIMENTS BOX 18-1 Activator Bypass Experiments, 624
- The Activities of Lac Repressor and CAP Are Controlled Allosterically by Their Signals, 626
- Combinatorial Control: CAP Controls Other Genes As Well, 627
- KEY EXPERIMENTS BOX 18-2 Jacob, Monod, and the Ideas behind Gene Regulation, 628
- Alternative σ Factors Direct RNA Polymerase to Alternative Sets of Promoters, 630
- NtrC and MerR: Transcriptional Activators That Work by Allostery Rather than by Recruitment, 630
- NtrC Has ATPase Activity and Works from DNA Sites Far from the Gene, 631
- MerR Activates Transcription by Twisting Promoter DNA, 632

- Some Repressors Hold RNA Polymerase at the Promoter Rather than Excluding It, 633
- AraC and Control of the *araBAD* Operon by Antiactivation, 634

MEDICAL CONNECTIONS 18-3 Blocking Virulence by Silencing Pathways of Intercellular Communication, 635

## THE CASE OF BACTERIOPHAGE $\lambda$ : LAYERS OF REGULATION, 636

- Alternative Patterns of Gene Expression Control Lytic and Lysogenic Growth, 636
- Regulatory Proteins and Their Binding Sites, 638
- $\lambda$  Repressor Binds to Operator Sites Cooperatively, 639
- Repressor and Cro Bind in Different Patterns to Control Lytic and Lysogenic Growth, 640
- ADVANCED CONCEPTS BOX 18-4 Concentration, Affinity, and Cooperative Binding, 641
- Lysogenic Induction Requires Proteolytic Cleavage of  $\lambda$  Repressor, 642
- Negative Autoregulation of Repressor Requires Long-Distance Interactions and a Large DNA Loop, 643
- Another Activator,  $\lambda$  CII, Controls the Decision between Lytic and Lysogenic Growth upon Infection of a New Host, 644
- KEY EXPERIMENTS BOX 18-5 Evolution of the  $\lambda$  Switch, 645
- The Number of Phage Particles Infecting a Given Cell Affects Whether the Infection Proceeds Lytically or Lysogenically, 647
- Growth Conditions of *E. coli* Control the Stability of CII Protein and Thus the Lytic/Lysogenic Choice, 648
- Transcriptional Antitermination in  $\lambda$  Development, 648
- KEY EXPERIMENTS BOX 18-6 Genetic Approaches That Identified Genes Involved in the Lytic/Lysogenic Choice, 649
- Retroregulation: An Interplay of Controls on RNA Synthesis and Stability Determines *int* Gene Expression, 651

### SUMMARY, 652

#### **BIBLIOGRAPHY**, 653



### 19 Transcriptional Regulation in Eukaryotes, 657

### CONSERVED MECHANISMS OF

TRANSCRIPTIONAL REGULATION FROM YEAST TO MAMMALS, 659

- Activators Have Separate DNA-Binding and Activating Functions, 660
- Eukaryotic Regulators Use a Range of DNA-Binding Domains, But DNA Recognition Involves the Same Principles as Found in Bacteria, 661
- Activating Regions Are Not Well-Defined Structures, 663

TECHNIQUES BOX 19-1 The Two-Hybrid Assay, 664

## RECRUITMENT OF PROTEIN COMPLEXES TO GENES BY EUKARYOTIC ACTIVATORS, 665

- Activators Recruit the Transcriptional Machinery to the Gene, 665
- TECHNIQUES BOX 19-2 The ChIP-Chip and ChIP-Seq Assays Are the Best Method for Identifying Enhancers, 666
- Activators Also Recruit Nucleosome Modifiers That Help the Transcriptional Machinary Bind at the Promoter or Initiate Transcription, 667
- Activators Recruit Additional Factors Needed for Efficient Initiation or Elongation at Some Promoters, 669
- MEDICAL CONNECTIONS BOX 19-3 Histone Modifications, Transcription Elongation, and Leukemia, 670
- Action at a Distance: Loops and Insulators, 672
- Appropriate Regulation of Some Groups of Genes Requires Locus Control Regions, 673

## SIGNAL INTEGRATION AND COMBINATORIAL CONTROL, 675

Activators Work Synergistically to Integrate Signals, 675

Signal Integration: The *HO* Gene Is Controlled by Two Regulators—One Recruits Nucleosome Modifiers, and the Other Recruits Mediator, 675

Signal Integration: Cooperative Binding of Activators at the Human  $\beta$ -Interferon Gene, 676



## 20 Regulatory RNAs, 701

### **REGULATION BY RNAs IN BACTERIA, 701**

Riboswitches Reside within the Transcripts of Genes Whose Expression They Control through Changes in Secondary Structure, 703

RNAs as Defense Agents in Prokaryotes and Archaea, 705

Combinatorial Control Lies at the Heart of the Complexity and Diversity of Eukaryotes, 678

Combinatorial Control of the Mating-Type Genes from *S. cerevisiae*, 680

### TRANSCRIPTIONAL REPRESSORS, 681

## SIGNAL TRANSDUCTION AND THE CONTROL OF TRANSCRIPTIONAL REGULATORS, 682

Signals Are Often Communicated to Transcriptional Regulators through Signal Transduction Pathways, 682

### **KEY EXPERIMENTS BOX 19-4** Evolution of a Regulatory Circuit, 683

Signals Control the Activities of Eukaryotic Transcriptional Regulators in a Variety of Ways, 686

## GENE "SILENCING" BY MODIFICATION OF HISTONES AND DNA, 687

- Silencing in Yeast Is Mediated by Deacetylation and Methylation of Histones, 688
- In Drosophila, HP1 Recognizes Methylated Histones and Condenses Chromatin, 689
- Repression by Polycomb Also Uses Histone Methylation, 690
- ADVANCED CONCEPTS BOX 19-5 Is There a Histone Code?, 691
- DNA Methylation Is Associated with Silenced Genes in Mammalian Cells, 692

### EPIGENETIC GENE REGULATION, 694

Some States of Gene Expression Are Inherited through Cell Division Even When the Initiating Signal Is No Longer Present, 694

### MEDICAL CONNECTIONS BOX 19-6 Transcriptional Repression and Human Disease, 696

SUMMARY, 697

**BIBLIOGRAPHY**, 698

- CRISPRs Are a Record of Infections Survived and Resistance Gained, 706
- ADVANCED CONCEPTS BOX 20-1 Amino Acid Biosynthetic Operons Are Controlled by Attenuation, 707
- Spacer Sequences Are Acquired from Infecting Viruses, 710

A CRISPR Is Transcribed as a Single Long RNA, Which Is Then Processed into Shorter RNA Species That Target Destruction of Invading DNA or RNA, 710

## REGULATORY RNAs ARE WIDESPREAD IN EUKARYOTES, 711

Short RNAs That Silence Genes Are Produced from a Variety of Sources and Direct the Silencing of Genes in Three Different Ways, 712

## SYNTHESIS AND FUNCTION OF miRNA MOLECULES, 714

- miRNAs Have a Characteristic Structure That Assists in Identifying Them and Their Target Genes, 714
- An Active miRNA Is Generated through a Two-Step Nucleolytic Processing, 716
- Dicer Is the Second RNA-Cleaving Enzyme Involved in miRNA Production and the Only One Needed for siRNA Production, 717

## SILENCING GENE EXPRESSION BY SMALL RNAs, 718

Incorporation of a Guide Strand RNA into RISC Makes the Mature Complex That Is Ready to Silence Gene Expression, 718

- Small RNAs Can Transcriptionally Silence Genes by Directing Chromatin Modification, 719
- RNAi Is a Defense Mechanism That Protects against Viruses and Transposons, 721
- KEY EXPERIMENTS BOX 20-2 Discovery of miRNAs and RNAi, 722
- RNAi Has Become a Powerful Tool for Manipulating Gene Expression, 725
- MEDICAL CONNECTIONS BOX 20-3 microRNAs and Human Disease, 727

## LONG NON-CODING RNAs AND X-INACTIVATION, 728

- Long Non-Coding RNAs Have Many Roles in Gene Regulation, Including *Cis* and *Trans* Effects on Transcription, 728
- X-Inactivation Creates Mosaic Individuals, 728
- Xist Is a Long Non-Coding RNA That Inactivates a Single X Chromosome in Female Mammals, 729

### SUMMARY, 730

**BIBLIOGRAPHY**, 731

QUESTIONS, 732

## 21 Gene Regulation in Development and Evolution, 733

MEDICAL CONNECTIONS BOX 21-1 Formation of iPS Cells, 734

### THREE STRATEGIES BY WHICH CELLS ARE INSTRUCTED TO EXPRESS SPECIFIC SETS OF GENES DURING DEVELOPMENT, 735

- Some mRNAs Become Localized within Eggs and Embryos Because of an Intrinsic Polarity in the Cytoskeleton, 735
- Cell-to-Cell Contact and Secreted Cell-Signaling Molecules Both Elicit Changes in Gene Expression in Neighboring Cells, 736
- Gradients of Secreted Signaling Molecules Can Instruct Cells to Follow Different Pathways of Development Based on Their Location, 737

#### EXAMPLES OF THE THREE STRATEGIES FOR ESTABLISHING DIFFERENTIAL GENE EXPRESSION, 738

- The Localized Ash1 Repressor Controls Mating Type in Yeast by Silencing the *HO* Gene, 738
- A Localized mRNA Initiates Muscle Differentiation in the Sea Squirt Embryo, 740
- ADVANCED CONCEPTS BOX 21-2 Review of Cytoskeleton: Asymmetry and Growth, 741
- Cell-to-Cell Contact Elicits Differential Gene Expression in the Sporulating Bacterium, *Bacillus subtilis*, 743

A Skin-Nerve Regulatory Switch Is Controlled by Notch Signaling in the Insect Central Nervous System, 743

A Gradient of the Sonic Hedgehog Morphogen Controls the Formation of Different Neurons in the Vertebrate Neural Tube, 744

## THE MOLECULAR BIOLOGY OF *DROSOPHILA* EMBRYOGENESIS, 746

An Overview of Drosophila Embryogenesis, 746

- A Regulatory Gradient Controls Dorsoventral Patterning of the Drosophila Embryo, 747
- ADVANCED CONCEPTS BOX 21-3 Overview of Drosophila Development, 748
- Segmentation Is Initiated by Localized RNAs at the Anterior and Posterior Poles of the Unfertilized Egg, 751
- KEY EXPERIMENTS BOX 21-4 Activator Synergy, 752
- Bicoid and Nanos Regulate hunchback, 753
- Multiple Enhancers Ensure Precision of *hunchback* Regulation, 754
- The Gradient of Hunchback Repressor Establishes Different Limits of Gap Gene Expression, 754
- MEDICAL CONNECTIONS BOX 21-5 Stem Cell Niche, 755
- ADVANCED CONCEPTS BOX 21-6 Gradient Thresholds, 757

Hunchback and Gap Proteins Produce Segmentation Stripes of Gene Expression, 758

- KEY EXPERIMENTS BOX 21-7 cis-Regulatory Sequences in Animal Development and Evolution, 759
- Gap Repressor Gradients Produce Many Stripes of Gene Expression, 760
- Short-Range Transcriptional Repressors Permit Different Enhancers to Work Independently of One Another within the Complex *eve* Regulatory Region, 761

## HOMEOTIC GENES: AN IMPORTANT CLASS OF DEVELOPMENTAL REGULATORS, 762

- Changes in Homeotic Gene Expression Are Responsible for Arthropod Diversity, 763
- Changes in *Ubx* Expression Explain Modifications in Limbs among the Crustaceans, 763
- in the

## 22 Systems Biology, 775

### **REGULATORY CIRCUITS, 776**

### AUTOREGULATION, 776

Negative Autoregulation Dampens Noise and Allows a Rapid Response Time, 777

Gene Expression Is Noisy, 777

Positive Autoregulation Delays Gene Expression, 779

### **BISTABILITY**, 780

Some Regulatory Circuits Persist in Alternative Stable States, 780

Bimodal Switches Vary in Their Persistence, 781

KEY EXPERIMENTS BOX 22-1 Bistability and Hysteresis, 782 ADVANCED CONCEPTS BOX 21-8 Homeotic Genes of Drosophila Are Organized in Special Chromosome Clusters, 764

How Insects Lost Their Abdominal Limbs, 766

Modification of Flight Limbs Might Arise from the Evolution of Regulatory DNA Sequences, 767

### **GENOME EVOLUTION AND HUMAN ORIGINS, 769**

Diverse Animals Contain Remarkably Similar Sets of Genes, 769

Many Animals Contain Anomalous Genes, 769

Synteny Is Evolutionarily Ancient, 770

Deep Sequencing Is Being Used to Explore Human Origins, 772

SUMMARY, 772

**BIBLIOGRAPHY**, 773

QUESTIONS, 774

### FEED-FORWARD LOOPS, 784

Feed-Forward Loops Are Three-Node Networks with Beneficial Properties, 784

Feed-Forward Loops Are Used in Development, 786

#### **OSCILLATING CIRCUITS**, 786

Some Circuits Generate Oscillating Patterns of Gene Expression, 786

Synthetic Circuits Mimic Some of the Features of Natural Regulatory Networks, 789

SUMMARY, 790

BIBLIOGRAPHY, 791

QUESTIONS, 791

### PART 6: APPENDICES, 793

## APPENDIX 1: Model Organisms, 797

### BACTERIOPHAGE, 798

Assays of Phage Growth, 800 The Single-Step Growth Curve, 800 Phage Crosses and Complementation Tests, 801 Transduction and Recombinant DNA, 801

### BACTERIA, 802

Assays of Bacterial Growth, 803

Bacteria Exchange DNA by Sexual Conjugation, Phage-Mediated Transduction, and DNA-Mediated Transformation, 803

Bacterial Plasmids Can Be Used as Cloning Vectors, 805

Transposons Can Be Used to Generate Insertional Mutations and Gene and Operon Fusions, 805

Studies on the Molecular Biology of Bacteria Have Been Enhanced by Recombinant DNA Technology, Whole-Genome Sequencing, and Transcriptional Profiling, 806

Biochemical Analysis Is Especially Powerful in Simple Cells with Well-Developed Tools of Traditional and Molecular Genetics, 806

Bacteria Are Accessible to Cytological Analysis, 807

Phage and Bacteria Told Us Most of the Fundamentals Things about the Gene, 807

Synthetic Circuits and Regulatory Noise, 808

### BAKER'S YEAST, SACCHAROMYCES

### CEREVISIAE, 808

The Existence of Haploid and Diploid Cells Facilitates Genetic Analysis of *S. cerevisiae*, 809

Generating Precise Mutations in Yeast Is Easy, 810

- S. cerevisiae Has a Small, Well-Characterized Genome, 810
- S. cerevisiae Cells Change Shape as They Grow, 810

#### ARABIDOPSIS, 811

- Arabidopsis Has a Fast Life Cycle with Haploid and Diploid Phases, 812
- Arabidopsis Is Easily Transformed for Reverse Genetics, 813
- Arabidopsis Has a Small Genome That Is Readily Manipulated, 813

**Epigenetics**, 814

Plants Respond to the Environment, 815

Development and Pattern Formation, 815

## THE NEMATODE WORM, *CAENORHABDITIS ELEGANS*, 816

- C. elegans Has a Very Rapid Life Cycle, 816
- *C. elegans* Is Composed of Relatively Few, Well-Studied Cell Lineages, 817
- The Cell Death Pathway Was Discovered in *C. elegans*, 818
- RNAi Was Discovered in C. elegans, 818

### THE FRUIT FLY, DROSOPHILA MELANOGASTER, 819

- Drosophila Has a Rapid Life Cycle, 819
- The First Genome Maps Were Produced in Drosophila, 820
- Genetic Mosaics Permit the Analysis of Lethal Genes in Adult Flies, 822
- The Yeast FLP Recombinase Permits the Efficient Production of Genetic Mosaics, 823
- It Is Easy to Create Transgenic Fruit Flies that Carry Foreign DNA, 824

### THE HOUSE MOUSE, MUS MUSCULUS, 825

Mouse Embryonic Development Depends on Stem Cells, 826

- It Is Easy to Introduce Foreign DNA into the Mouse Embryo, 827
- Homologous Recombination Permits the Selective Ablation of Individual Genes, 827

Mice Exhibit Epigenetic Inheritance, 829

#### **BIBLIOGRAPHY**, 830



### APPENDIX 2: Answers, 831

Chapter 1, 831	Chapter 12, 837
Chapter 2, 831	Chapter 13, 838
Chapter 3, 832	Chapter 14, 839
Chapter 4, 833	Chapter 15, 839
Chapter 5, 833	Chapter 16, 840
Chapter 6, 834	Chapter 17, 841
Chapter 7, 834	Chapter 18, 841
Chapter 8, 835	Chapter 19, 843
Chapter 9, 835	Chapter 20, 843
Chapter 10, 836	Chapter 21, 843
Chapter 11, 837	Chapter 22, 844