

Contents

UNIT 1 BASIC CHEMICAL AND BIOLOGICAL PRINCIPLES 1

CHAPTER 1 *Cells and Organisms* 2

1. What Is Life?	3
2. Living Creatures Are Made of Cells	4
3. Eubacteria and Archaea Are Genetically Distinct	8
4. Eukaryotic Cells Are Subdivided into Compartments	9
5. The Diversity of Eukaryotes	13
6. Haploidy, Diploidy, and the Eukaryote Cell Cycle	14
7. Organisms Are Classified	15
8. Some Widely-Studied Organisms Serve as Models	16
9. Basic Characteristics of a Model Organism	27
10. Purifying DNA from Model Organisms	27
11. Viruses Are Not Living Cells	29
12. Bacterial Viruses Infect Bacteria	30
13. Human Viral Diseases Are Common	32
14. A Variety of Subcellular Genetic Entities Exist	32
Key Concepts	34
Review Questions	35
Conceptual Questions	36

CHAPTER 2 *Basic Genetics* 37

1. Gregor Mendel, The Father of Classical Genetics	37
2. Genes Determine Each Step in Biochemical Pathways	39
3. Mutants Result from Alterations in Genes	40
4. Phenotypes and Genotypes	41
5. Chromosomes Are Long, Thin Molecules That Carry Genes	42
6. Dominant and Recessive Alleles	45
7. Genes from Both Parents Are Mixed by Sexual Reproduction	48
8. Neighboring Genes Are Linked During Inheritance Unless the DNA Recombines	52
9. Identifying Genes that Cause Human Diseases	57
Key Concepts	58
Review Questions	59
Conceptual Questions	60

CHAPTER 3 *DNA, RNA, and Protein* 62

1. History of DNA as the Genetic Material	62
2. Nucleic Acid Molecules Carry Genetic Information	63
3. Chemical Structure of Nucleic Acids	63
4. Double-Stranded DNA Forms a Double Helix	67

5. Constituents of Chromosomes	75
6. The Central Dogma Outlines the Flow of Genetic Information	78
7. Ribosomes Read the Genetic Code	81
8. Various Classes of RNA Have Different Functions	82
9. Proteins Carry Out Many Cell Functions	84
Key Concepts	91
Review Questions	92
Conceptual Questions	93

CHAPTER 4 *Genomes and DNA* 94

1. Genome Organization	94
2. Repeated Sequences Are a Feature of Eukaryotic DNA	100
3. Palindromes, Inverted Repeats, and Stem and Loop Structures	105
4. Multiple A-Tracts Cause DNA to Bend	106
5. Supercoiling Is Necessary for Packaging of Bacterial DNA	106
6. Separation of DNA Fragments by Electrophoresis	111
7. Alternative Helical Structures of DNA Occur	113
8. Packaging DNA in Eukaryotic Nuclei	116
Key Concepts	121
Review Questions	122
Conceptual Questions	123

CHAPTER 5 *Manipulation of Nucleic Acids* 125

1. Manipulating DNA	126
2. Chemical Synthesis of DNA	135
3. Measuring the Concentration of DNA and RNA with Ultraviolet Light	143
4. Radioactive Labeling of Nucleic Acids	144
5. Fluorescence in the Detection of DNA and RNA	146
6. The Electron Microscope	149
7. Hybridization of DNA and RNA	151
Key Concepts	158
Review Questions	158
Conceptual Questions	159

UNIT 2 THE GENOME 162

CHAPTER 6 *Polymerase Chain Reaction* 163

1. Fundamentals of the Polymerase Chain Reaction	164
2. Inverse PCR	171

3. Randomly Amplified Polymorphic DNA (RAPD)	172
4. Reverse Transcriptase PCR	174
5. Differential Display PCR	175
6. Rapid Amplification of cDNA Ends (RACE)	176
7. PCR in Genetic Engineering	179
8. Directed Mutagenesis	179
9. Engineering Deletions and Insertions by PCR	181
10. Real-Time Fluorescent PCR	182
11. Molecular Beacons and Scorpion Primers	184
12. Use of PCR in Medical Diagnosis	188
13. Environmental Analysis by PCR	189
14. Rescuing DNA from Extinct Life Forms by PCR	190
Key Concepts	191
Review Questions	192
Conceptual Questions	193
CHAPTER 7 Cloning Genes for Analysis	194
1. Properties of Cloning Vectors	195
2. Detecting Insertions in Vectors	199
3. Moving Genes Between Organisms: Shuttle Vectors	201
4. Bacteriophage Lambda Vectors	205
5. Cosmid Vectors	206
6. Yeast Artificial Chromosomes	208
7. Bacterial and P1 Artificial Chromosomes	208
8. Recombineering Increases the Speed of Gene Cloning	209
9. A DNA Library is a Collection of Genes from One Source	212
10. Cloning Complementary DNA Avoids Introns	215
11. Chromosome Walking	217
12. Cloning by Subtractive Hybridization	219
13. Expression Vectors	220
Key Concepts	223
Review Questions	224
Conceptual Questions	225
CHAPTER 8 DNA Sequencing	227
1. DNA Sequencing—General Principles for Chain Termination Sequencing	228
2. Primer Walking Along a Strand of DNA	234
3. Automated Sequencing	235
4. Cycle Sequencing	236
5. The Emergence of DNA Chip Technology	237
6. Pyrosequencing	239
7. Second-Generation Sequencing	239

8. Third-Generation Sequencing	242
9. Nanopore Detectors for DNA	243
Key Concepts	244
Review Questions	246
Conceptual Questions	246
CHAPTER 9 Genomics & Systems Biology	248
1. Large-Scale Mapping with Sequence Tags	249
2. Assembling Small Genomes by Shotgun Sequencing	250
3. Race for the Human Genome	253
4. Survey of the Human Genome	255
5. Pharmacogenomics—Genetically-Individualized Drug Treatment	263
6. Personal Genomics and Comparative Genomics	264
7. Bioinformatics and Computer Analysis	265
8. Systems Biology	266
9. Metagenomics and Community Sampling	268
10. Epigenetics and Epigenomics	268
Key Concepts	269
Review Questions	271
Conceptual Questions	271
UNIT 3 THE CENTRAL DOGMA OF MOLECULAR BIOLOGY	273
CHAPTER 10 Cell Division and DNA Replication	274
1. Cell Division and Reproduction Are Not Always Identical	275
2. DNA Replication Occurs at the Replication Fork	275
3. Properties of DNA Polymerase	279
4. Nucleotides Are the Precursors for DNA Synthesis	280
5. DNA Polymerase Elongates DNA Strands	282
6. The Complete Replication Fork Is Complex	285
7. Discontinuous Synthesis of the Lagging Strand	286
8. Chromosome Replication Initiates at <i>oriC</i>	289
9. Chromosome Replication Terminates at <i>terC</i>	292
10. Cell Division in Bacteria Occurs after Replication of Chromosomes	293
11. The Concept of the Replicon	297
12. Replicating Linear DNA in Eukaryotes	298
13. Cell Division in Higher Organisms	304
Key Concepts	305
Review Questions	307
Conceptual Questions	308

CHAPTER 11 Transcription of Genes	309
1. Genes Are Expressed by Making RNA	310
2. How Is the Beginning of a Gene Recognized?	312
3. Manufacturing the Message	314
4. RNA Polymerase Knows Where to Stop	316
5. How Does the Cell Know Which Genes to Turn On?	318
6. Transcription in Eukaryotes Is More Complex	324
Key Concepts	333
Review Questions	334
Conceptual Questions	335
CHAPTER 12 Processing of RNA	336
1. RNA Is Processed in Several Ways	336
2. Coding and Non-Coding RNA	338
3. Processing of Ribosomal and Transfer RNA	338
4. Eukaryotic Messenger RNA Contains a Cap and a Tail	340
5. Introns Are Removed from RNA by Splicing	344
6. Alternative Splicing Produces Multiple Forms of RNA	349
7. Inteins and Protein Splicing	352
8. Base Modification of rRNA Requires Guide RNA	355
9. RNA Editing Alters the Base Sequence	358
10. Transport of RNA out of the Nucleus	360
11. Degradation of mRNA	361
Key Concepts	366
Review Questions	367
Conceptual Questions	367
CHAPTER 13 Protein Synthesis	369
1. Overview of Protein Synthesis	370
2. Proteins Are Chains of Amino Acids	371
3. Decoding the Genetic Information	376
4. The Ribosome: The Cell's Decoding Machine	381
5. Three Possible Reading Frames Exist	386
6. The tRNA Occupies Three Sites During Elongation of the Polypeptide	389
7. Bacterial mRNA Can Code for Several Proteins	394
8. Some Ribosomes Become Stalled and Are Rescued	395
9. Differences between Eukaryotic and Prokaryotic Protein Synthesis	397
10. Protein Synthesis Is Halted When Resources Are Scarce	401
11. A Signal Sequence Marks a Protein for Export from the Cell	403

12. Protein Synthesis Occurs in Mitochondria and Chloroplasts	405
13. Mistranslation Usually Results in Mistakes in Protein Synthesis	407
14. Many Antibiotics Work by Inhibiting Protein Synthesis	408
15. Post-Translational Modifications of Proteins	408
16. Selenocysteine and Pyrrolysine: Rare Amino Acids	410
17. Degradation of Proteins	412
Key Concepts	415
Review Questions	415
Conceptual Questions	416
CHAPTER 14 Protein Structure and Function	417
1. The Structure of Proteins Reflects Four Levels of Organization	417
2. Determining Protein Structures	428
3. Nucleoproteins, Lipoproteins, and Glycoproteins Are Conjugated Proteins	430
4. Proteins Serve Numerous Cellular Functions	433
5. Protein (Nano)-Machines	436
6. Enzymes Catalyze Metabolic Reactions	437
7. Binding of Proteins to DNA Occurs in Several Different Ways	450
8. Denaturation of Proteins	454
Key Concepts	455
Review Questions	456
Conceptual Questions	457
CHAPTER 15 Proteomics: The Global Analysis of Proteins	459
1. The Proteome	460
2. Antibodies Are Essential Proteomics Tools	464
3. Western Blotting of Proteins	465
4. Isolating Proteins with Chromatography	466
5. Mass Spectrometry for Protein Identification	468
6. Protein-Tagging Systems	470
7. Selection by Phage Display	474
8. Protein Interactions: The Yeast Two-Hybrid System	478
9. Protein Interaction by Co-Immunoprecipitation	483
10. Protein Arrays	484
11. Metabolomics	487
Key Concepts	489
Review Questions	490
Conceptual Questions	491

UNIT 4 REGULATING GENE EXPRESSION 492**CHAPTER 16 Regulation of Transcription in Prokaryotes 493**

1. Gene Regulation Ensures a Physiological Response 493
2. Regulation at the Level of Transcription Involves Several Steps 496
3. Alternative Sigma Factors in Prokaryotes Recognize Different Sets of Genes 497
4. Activators and Repressors Participate in Positive and Negative Regulation 502
5. Two-Component Regulatory Systems 511
6. Specific versus Global Control 515
7. Accessory Factors and Nucleoid-Binding Proteins 517
8. Anti-Termination as a Control Mechanism 520
- Key Concepts 523
- Review Questions 524
- Conceptual Questions 524

CHAPTER 17 Regulation of Transcription in Eukaryotes 526

1. Transcriptional Regulation in Eukaryotes Is More Complex Than in Prokaryotes 526
2. Specific Transcription Factors Regulate Protein-Encoding Genes 527
3. Negative Regulation of Transcription Occurs in Eukaryotes 533
4. Heterochromatin Blocks Access to DNA in Eukaryotes 537
5. Methylation of Eukaryotic DNA Controls Gene Expression 543
6. X-Chromosome Inactivation Occurs in Female XX Animals 547
- Key Concepts 550
- Review Questions 550
- Conceptual Questions 551

CHAPTER 18 Regulation at the RNA Level 553

1. Regulation at the Level of mRNA 553
2. Basic Principles of RNA Interference (RNAi) 564
3. Long Non-coding Regulatory RNA 572
4. CRISPR: Anti-Viral Defense in Bacteria 573

5. Premature Termination Causes Attenuation of RNA Transcription 574
6. Riboswitches—RNA Acting Directly as a Control Mechanism 576
- Key Concepts 579
- Review Questions 579
- Conceptual Questions 580

CHAPTER 19 Analysis of Gene Expression 581

1. Monitoring Gene Expression 581
2. Reporter Genes for Monitoring Gene Expression 582
3. Deletion Analysis of the Upstream Region 590
4. DNA-Protein Complexes Can Be Isolated by Chromatin Immunoprecipitation 594
5. Location of the Start of Transcription by Primer Extension 596
6. Transcriptome Analysis 600
7. DNA Microarrays for Gene Expression 601
8. TaqMan Quantitative PCR to Assay Gene Expression 608
9. Serial Analysis of Gene Expression (SAGE) 610
- Key Concepts 613
- Review Questions 613
- Conceptual Questions 614

UNIT 5 SUBCELLULAR LIFE FORMS 615**CHAPTER 20 Plasmids 616**

1. Plasmids as Replicons 616
2. General Properties of Plasmids 619
3. Plasmid DNA Replicates by Two Alternative Methods 622
4. Many Plasmids Help their Host Cells 628
5. Plasmids may Provide Aggressive Characters 635
6. Ti Plasmids are Transferred from Bacteria to Plants 640
7. The 2 μ Plasmid of Yeast 644
8. Certain DNA Molecules May Behave as Viruses or Plasmids 645
- Key Concepts 646
- Review Questions 647
- Conceptual Questions 648

CHAPTER 21 Viruses 649

1. Viruses Are Infectious Packages of Genetic Information 650
2. The Great Diversity of Viruses 658

3. Viruses with RNA Genomes Have Very Few Genes 666
4. Retroviruses Use both RNA and DNA 672
5. Subviral Infectious Agents 678
- Key Concepts 684
- Review Questions 684
- Conceptual Questions 685

CHAPTER 22 Mobile DNA 686

1. Subcellular Genetic Elements as Gene Creatures 686
2. Most Mobile DNA Consists of Transposable Elements 687
3. Retroelements Make an RNA Copy 703
4. The Multitude of Transposable Elements 708
5. Junk DNA and Selfish DNA 716
- Key Concepts 717
- Review Questions 718
- Conceptual Questions 719

UNIT 6 CHANGING THE DNA BLUEPRINT 720**CHAPTER 23 Mutations and Repair 721**

1. Mutations Alter the DNA Sequence 721
2. The Major Types of Mutation 722
3. Chemical Mutagens Damage DNA 733
4. Overview of DNA Repair 742
5. Mutations Occur More Frequently at Hotspots 758
6. Reversions Are Genetic Alterations That Change the Phenotype Back to Wild-Type 759
7. Site-Directed Mutagenesis 763
- Key Concepts 764
- Review Questions 765
- Conceptual Questions 766

CHAPTER 24 Recombination 767

1. Overview of Recombination 767
2. Molecular Basis of Homologous Recombination 769

3. Site-Specific Recombination 773
4. Recombination in Higher Organisms 776
5. Gene Conversion 778
- Key Concepts 781
- Review Questions 781
- Conceptual Questions 782

CHAPTER 25 Bacterial Genetics 783

1. Reproduction versus Gene Transfer 783
2. Fate of the Incoming DNA after Uptake 784
3. Transformation Is Gene Transfer by Naked DNA 785
4. Gene Transfer by Virus—Transduction 792
5. Transfer of Plasmids between Bacteria 795
6. Gene Transfer among Gram-Positive Bacteria 801
7. Archaeal Genetics 804
8. Whole-Genome Sequencing 805
- Key Concepts 808
- Review Questions 809
- Conceptual Questions 810

CHAPTER 26 Molecular Evolution 812

1. Getting Started—Formation of the Earth 812
2. Oparin's Theory of the Origin of Life 814
3. Origin of Informational Macromolecules 818
4. The Autotrophic Theory of the Origin of Metabolism 823
5. Evolution of DNA, RNA, and Protein Sequences 824
6. Different Proteins Evolve at Very Different Rates 830
7. Symbiotic Origin of Eukaryotic Cells 835
8. DNA Sequencing and Biological Classification 841
9. Evolving Sideways: Horizontal Gene Transfer 847
- Key Concepts 850
- Review Questions 851
- Conceptual Questions 852

Glossary 855**Index 883**