

CONTENTS

Preface	xxiii
Chapter 1: The Beginnings of Molecular Biology	1
1.1 Introduction	1
1.2 Insights into the nature of the heredity material	4
Mendel's laws of inheritance	4
The chromosome theory of inheritance	6
The transforming principle is DNA	6
Creativity in approach leads to the one gene–one enzyme hypothesis	8
The importance of technological advances: the Hershey–Chase experiment	10
1.3 A model for the structure of DNA: the DNA double helix	11
1.4 The central dogma of molecular biology	12
1.5 An evolutionary framework for heredity	13
Chapter summary	15
Analytical questions	15
Bibliography	16
Chapter 2: The Structure of DNA	17
2.1 Introduction	17
2.2 Primary structure: the components of nucleic acids	18
Five-carbon sugars	18
Nitrogenous bases	19
The phosphate functional group	19
Nucleosides and nucleotides	20
Nomenclature of nucleotides	21
The length of RNA and DNA chains	21
Significance of 5' and 3'	21
2.3 Secondary structure of DNA	22
Hydrogen bonds form between the bases	22
Base stacking provides chemical stability to the DNA double helix	23
Structure of the Watson–Crick DNA double helix	24

Distinguishing between features of alternative double-helical structures	26
DNA can undergo reversible strand separation	29
2.4 Unusual DNA secondary structures	31
Slipped structures	31
Cruciform structures	32
Triple helix DNA	33
Disease box 2.1 Friedreich's ataxia and triple helix DNA	34
2.5 Tertiary structure of DNA	34
Supercoiling of DNA	34
What is the significance of supercoiling <i>in vivo</i> ?	35
Chapter summary	37
Analytical questions	37
Bibliography	37
Chapter 3: The Versatility of RNA	39
3.1 Introduction	39
3.2 RNA is involved in a wide range of cellular processes	40
3.3 Structural motifs of RNA	42
Secondary structure of RNA	42
tRNA structure: important insights into RNA structural motifs	44
Common tertiary structure motifs in RNA	46
Kinetics of RNA folding	50
3.4 The discovery of RNA catalysis	51
<i>Tetrahymena</i> self-splicing RNA	52
Focus box 3.1: The RNA World	53
RNase P is a ribozyme	55
Ribozymes catalyze a variety of chemical reactions	56
3.5 RNA-based genomes	59
Eukaryotic RNA viruses	59
Retroviruses	59
Disease box 3.1: Avian flu and swine flu	61
Viroids and other subviral pathogens	62
Chapter summary	62
Analytical questions	63
Bibliography	63

Chapter 4: Protein Structure and Folding	65
4.1 Introduction	65
4.2 Primary structure: amino acids and the genetic code	67
The 22 amino acids found in proteins	67
Protein primary structure	67
Translating the genetic code	68
The 21st and 22nd genetically encoded amino acids	70
Modified nucleotides and codon bias	70
D- and L-amino acids in nature	71
4.3 The three-dimensional structure of proteins	72
Secondary structure	72
Tertiary structure	74
Quaternary structure	77
4.4 Protein function and regulation of activity	77
Enzymes are biological catalysts	77
Regulation of protein activity by post-translational modifications	79
Allosteric regulation of protein activity	81
Macromolecular assemblages	83
4.5 Protein folding and misfolding	83
Molecular chaperones	83
Ubiquitin-mediated protein degradation	85
Protein misfolding diseases	86
Disease box 4.1 Prions	87
Chapter summary	88
Analytical questions	89
Bibliography	89
Chapter 5: Genome Organization and Evolution	91
5.1 Introduction	91
5.2 Genome organization varies in different organisms	92
5.3 Packaging of the eukaryotic genome	95
Histones are small, positively charged proteins	95
Nucleosomes are the fundamental packing unit of chromatin	98
Higher order chromatin structure: the 30-nm fiber	99
Further packaging of DNA involves loop domains	99
Fully condensed chromatin: metaphase chromosomes	100
Organization and expression of the genetic material	102

5.4	The majority of the eukaryotic genome is noncoding	104
	Interspersed elements are primarily transposable elements	104
	Tandem repetitive sequences are arranged in arrays with variable numbers of repeats	105
5.5	Lateral gene transfer in the eukaryotic genome	106
	Organelle genomes reflect an endosymbiont origin	107
	Disease box 5.1 Mitochondrial DNA and disease	108
	Intercompartmental DNA transfer	108
5.6	Prokaryotic and viral genome organization	109
	Bacterial genome organization	110
	Plasmid DNA	110
	Archaeal genome organization	111
	Viral genome organization	112
	Chapter Summary	114
	Analytical questions	114
	Bibliography	115
 Chapter 6: DNA Replication and Telomere Maintenance		 117
6.1	Introduction	117
6.2	Early insights into the mode of bacterial DNA replication	118
	The Meselson-Stahl experiment	118
	Visualization of replicating bacterial DNA	120
6.3	DNA polymerases are the enzymes that catalyze DNA synthesis from 5' to 3'	121
	Semidiscontinuous DNA replication	121
6.4	Multi-protein machines mediate bacterial DNA replication	124
	Bacterial DNA polymerases have multiple functions	124
	Initiation of replication	125
	Replication is mediated by the replisome	125
	Topoisomerases relax supercoiled DNA	126
	Is leading strand synthesis really continuous?	129
6.5	Multi-protein machines trade places during eukaryotic DNA replication	129
	Mapping origins of replication	130
	Selective activation of origins of replication	131
	Replication factories	131
	Histone removal at the origins of replication	132

Prereplication complex formation and replication licensing	133
Focus box 6.1 The naming of genes involved in DNA replication	133
Duplex unwinding at replication forks	134
RNA priming of leading strand and lagging strand DNA synthesis	136
Polymerase switching	136
Elongation of leading strands and lagging strands	136
PCNA: a sliding clamp with many protein partners	136
Proofreading	138
Maturation of nascent DNA strands	139
Histone deposition	141
Topoisomerase untangles the newly synthesized DNA	142
Disease box 6.1 Topoisomerase-targeted anticancer drugs	143
6.6 Alternative modes of circular DNA replication	143
Rolling circle replication	143
Models for organelle DNA replication	144
Disease box 6.2 RNase MRP and cartilage-hair hypoplasia	145
6.7 Telomere maintenance: the role of telomerase in DNA replication, aging, and cancer	146
Telomeres	147
Solution to the end replication problem	147
Maintenance of telomeres by telomerase	148
Other modes of telomere maintenance	148
Regulation of telomerase activity	150
Telomerase, aging, and cancer	150
Disease box 6.3 Dyskeratosis congenita: loss of telomerase activity	153
Chapter summary	155
Analytical questions	156
Bibliography	156
Chapter 7: DNA Repair Pathways	159
7.1 Introduction	159
7.2 Mutations and DNA damage	160
Transitions and transversions can lead to silent, missense, or nonsense mutations	160
Expansion of trinucleotide repeats leads to genetic instability	162
General classes of DNA damage	163
7.3 Lesion bypass	166
7.4 Direct reversal of DNA damage	167
Reversal of thymine-thymine dimers by DNA photolyase	167
Damage reversal by DNA methyltransferase	168

7.5 Repair of single base changes and structural distortions by removal of DNA damage	169
Base excision repair	169
Mismatch repair	171
Disease box 7.1 Hereditary nonpolyposis colorectal cancer: a defect in mismatch repair	172
Nucleotide excision repair	174
Disease box 7.2 Xeroderma pigmentosum and related disorders: defects in nucleotide excision repair	175
7.6 Double-strand break repair by removal of DNA damage	177
Homologous recombination	178
Disease box 7.3 Hereditary breast cancer syndromes: mutations in <i>BRCA1</i> and <i>BRCA2</i>	178
Nonhomologous end-joining	180
Chapter summary	182
Analytical questions	183
Bibliography	183
Chapter 8: Recombinant DNA Technology and Molecular Cloning	185
8.1 Introduction	185
8.2 The beginnings of recombinant DNA technology	186
Insights from bacteriophage lambda (λ) cohesive sites	186
Insights from bacterial modification and restriction systems	186
The first cloning experiments	188
Focus box 8.1 Fear of recombinant DNA molecules	189
8.3 Cutting and joining DNA	190
Major classes of restriction endonucleases	190
Recognition sequences for type II restriction endonucleases	190
Focus box 8.2 <i>EcoRI</i> : kinking and cutting DNA	193
DNA ligase joins linear pieces of DNA	194
8.4 Molecular cloning	195
Choice of vector is dependent on insert size and application	195
Plasmid DNA as a vector	196
Tool box 8.1 Liquid chromatography	199
Bacteriophage lambda (λ) as a vector	201
Artificial chromosome vectors	201
Sources of DNA for cloning	203
Tool box 8.2 Complementary DNA (cDNA) synthesis	203

Tool box 8.3 Polymerase chain reaction (PCR)	205
Constructing DNA libraries	206
8.5 Library screening and probes	207
Types of DNA and RNA probes	207
Labeling of probes	209
Tool box 8.4 Radioactive and nonradioactive labeling methods	210
Tool box 8.5 Nucleic acid labeling	211
Library screening	212
Screening of expression libraries	214
8.6 Restriction mapping and RFLP analysis	214
Restriction mapping	214
Tool box 8.6 DNA and RNA Electrophoresis	215
Restriction fragment length polymorphism (RFLP)	216
Tool box 8.7 Southern blot	218
Disease box 8.1 PCR-RFLP assay for maple syrup urine disease	219
8.7 DNA sequencing	220
Manual DNA sequencing by the Sanger “dideoxy” DNA method	221
Automated DNA sequencing	222
Next-generation sequencing	223
Chapter summary	224
Analytical questions	225
Bibliography	226
Chapter 9: Tools for Analyzing Gene Expression	227
9.1 Introduction	227
Focus box 9.1 Model organisms	228
9.2 Transient and stable transfection assays	230
9.3 Reporter genes	230
Commonly used reporter genes	231
Analysis of gene regulation	232
Purification and detection tags: fusion proteins	233
Tool box 9.1 Production of recombinant proteins	238
Tool box 9.2 Fluorescence, confocal, and multiphoton microscopy	239
9.4 <i>In vitro</i> mutagenesis	240
Deletion mutagenesis by PCR	241
Linker-scanning mutagenesis	242
Site-directed mutagenesis	242

9.5 Analysis at the level of gene transcription: RNA expression and localization	242
Northern blot	244
<i>In situ</i> hybridization	244
RNase protection assay (RPA)	244
Reverse transcription-PCR	245
Quantitative real-time PCR (Q-PCR)	245
9.6 Analysis at the level of translation: protein expression and localization	246
Western blot	246
Tool box 9.3 Protein gel electrophoresis	246
Enzyme-linked immunosorbent assay (ELISA)	249
Tool box 9.4 Antibody production	250
9.7 Antisense technology	251
Antisense oligonucleotides	251
RNA interference (RNAi)	252
9.8 Analysis of DNA–protein interactions	253
Electrophoretic mobility shift assay (EMSA)	253
DNase I footprinting	253
Chromatin immunoprecipitation (ChIP) assay	253
9.9 Analysis of protein–protein interactions	255
Pull-down assay	255
Yeast two-hybrid assay	255
Coimmunoprecipitation assay	257
Fluorescence resonance energy transfer (FRET)	257
9.10 Structural analysis of proteins	257
X-ray crystallography	257
Nuclear magnetic resonance (NMR) spectroscopy	258
Cryoelectron microscopy	259
Atomic force microscopy (AFM)	259
Chapter summary	259
Analytical questions	261
Bibliography	262
Chapter 10: Transcription in Bacteria	263
10.1 Introduction	263
10.2 Mechanism of transcription	264
Bacterial promoter structure	265

Structure of bacterial RNA polymerase	265
Initiation of transcription	267
Elongation	268
Focus box 10.1 Which moves—the RNA polymerase or the DNA?	270
Proofreading	271
Termination of transcription	272
10.3 Insights into gene regulation from the lactose (<i>lac</i>) operon	273
The Jacob-Monod operon model of gene regulation	274
Characterization of the Lac repressor	274
Lactose (<i>lac</i>) operon regulation	275
The <i>lac</i> promoter and <i>lacZ</i> structural gene are widely used in molecular biology research	278
10.4 Mode of action of transcriptional regulators	279
Cooperative binding of proteins to DNA	279
Allosteric modifications and DNA binding	279
DNA looping	281
10.5 Control of gene expression by RNA	282
Differential folding of RNA: transcriptional attenuation of the tryptophan operon	283
Riboswitches	284
Riboswitch ribozymes	285
10.6 Gene regulatory networks	286
Alternative sigma factors	286
Quorum sensing	288
Chapter summary	288
Analytical questions	290
Bibliography	290
Chapter 11: Transcription in Eukaryotes	292
11.1 Introduction	292
11.2 Overview of transcriptional regulation	293
Chromosomal territories and transcription factories	293
Eukaryotes have different types of RNA polymerase	293
11.3 Protein-coding gene regulatory elements	295
Structure and function of promoter elements	296
Structure and function of long-range regulatory elements	298
Focus box 11.1 Position effect and long-range regulatory elements	299
Disease box 11.1 Hispanic thalassemia and DNase I hypersensitive sites	302
Focus box 11.2 Is there a nuclear matrix?	304

11.4	The general transcription machinery	306
	Components of the general transcription machinery	306
	Structure of RNA polymerase II	307
	General transcription factors and preinitiation complex formation	309
	Mediator: a molecular bridge	312
	Initiation of transcription	314
11.5	The role of specific transcription factors in gene regulation	314
	Transcription factors mediate gene-specific transcriptional activation or repression	315
	Transcription factors are modular proteins	315
	DNA-binding domain motifs	316
	Focus box 11.3 Homeoboxes and homeodomains	317
	Disease box 11.2 Greig cephalopolysyndactyly syndrome and Sonic hedgehog signaling	320
	Disease box 11.3 Defective histone acetyltransferases in Rubinstein–Taybi syndrome	322
	Transactivation domain	323
	Dimerization domain	324
11.6	Transcriptional coactivators and corepressors	324
	Chromatin modification complexes	324
	Focus box 11.4 Is there a histone code?	326
	Linker histone variants	328
	Chromatin remodeling complexes	328
11.7	Transcription complex assembly: the enhanceosome model versus the “hit-and-run” model	331
	Order of recruitment of various proteins that regulate transcription	331
	Enhanceosome model	332
	Hit-and-run model	333
	Merging of models	333
11.8	Transcription elongation through nucleosomes	334
	Transcription elongation	335
	Proofreading and backtracking	335
	Transcription elongation through the nucleosomal barrier	336
	Disease box 11.4 Defects in Elongator and Familial Dysautonomia	338
11.9	Nuclear import and export of proteins	339
	Karyopherins mediate nuclear import and export	340
	Nuclear import pathway	341
	Focus box 11.5 The nuclear pore complex	342
	Nuclear export pathway	344

11.10 Regulated nuclear import and signal transduction pathways	345
Regulated nuclear import of NF- κ B	345
Regulated nuclear import of the glucocorticoid receptor	347
Chapter summary	348
Analytical questions	350
Bibliography	351
	437
	438
	439
Chapter 12: Epigenetic Mechanisms of Gene Regulation	354
12.1 Introduction	354
12.2 Epigenetic markers	355
Cytosine DNA methylation marks genes for silencing	355
Disease box 12.1 Cancer and epigenetics	357
Stable maintenance of histone modifications	358
Disease box 12.2 Fragile X mental retardation and aberrant DNA methylation	359
12.3 Genomic imprinting	360
Disease box 12.3 Genomic imprinting and neurodevelopmental disorders	362
Establishing and maintaining the imprint	364
Mechanisms of monoallelic expression	365
Genomic imprinting is essential for normal development	367
Origins of genomic imprinting	368
12.4 X chromosome inactivation	368
Random X chromosome inactivation in mammals	369
Molecular mechanisms for stable maintenance of X chromosome inactivation	370
Is there monoallelic expression of all X-linked genes?	370
12.5 Epigenetic control of transposable elements	371
Barbara McClintock's discovery of mobile genetic elements in maize	372
DNA transposons have a wide host range	373
Disease box 12.4 Jumping genes and human disease	375
DNA transposons move by a "cut-and-paste" mechanism	376
Retrotransposons move by a "copy-and-paste" mechanism	377
Some LTR retrotransposons are active in the mammalian genome	378
Non-LTR retrotransposons include LINEs and SINEs	378
Methylation of transposable elements	379
Heterochromatin formation mediated by RNAi and RNA-directed DNA methylation	380

12.6 Epigenetics and nutritional legacy	381
A diet lacking folic acid can activate a retrotransposon in mice	381
Paternal epigenetic effects	383
12.7 Allelic exclusion	383
Yeast mating-type switching and silencing	383
Antigen switching in trypanosomes	387
Disease box 12.5 Trypanosomiasis: human “sleeping sickness”	388
V(D)J recombination and the adaptive immune response	393
Focus box 12.1 Did the V(D)J system evolve from a transposon?	394
Chapter summary	398
Analytical questions	400
Bibliography	401
Chapter 13: RNA Processing and Post-Transcriptional Gene Regulation	403
13.1 Introduction	403
13.2 The discovery of split genes	404
Focus box 13.1 Intron-encoded small nucleolar RNAs and “inside-out” genes	406
13.3 Splicing occurs by a variety of mechanisms	407
Group I introns require an external G cofactor for splicing	407
Group II introns require an internal bulged A for splicing	408
Mobile group I and II introns	409
Archael introns are spliced by an endoribonuclease	410
Some nuclear tRNA genes contain an intron	410
13.4 Cotranscriptional processing of nuclear pre-mRNA	411
Addition of the 5'-7-methylguanosine cap	413
Termination and polyadenylation	413
Splicing	414
Disease box 13.1 Oculopharyngeal muscular dystrophy: trinucleotide repeat expansion in a poly(A)-binding protein gene	415
Disease box 13.2 Spinal muscular atrophy: defects in snRNP biogenesis	417
Disease box 13.3 <i>Prp8</i> gene mutations cause retinitis pigmentosa	422
13.5 Alternative splicing	423
Effects of alternative splicing on gene expression	424
Focus box 13.2 The <i>Dscam</i> gene: extreme alternative splicing	424
Regulation of alternative splicing	425
Trans-splicing	426

13.6 RNA editing	428
RNA editing in trypanosomes	428
RNA editing in mammals	431
Disease box 13.4 Amyotrophic lateral sclerosis: a defect in RNA editing?	432
13.7 Post-transcriptional gene regulation by RNAi	434
The discovery of RNAi	436
RNAi machinery	437
The discovery of miRNA in <i>Caenorhabditis elegans</i>	438
Processing of miRNAs	439
miRNAs target mRNA for degradation and translational inhibition	440
13.8 RNA turnover in the nucleus and cytoplasm	442
Nuclear exosomes and quality control	442
Quality control and the formation of nuclear export-competent RNPs	442
Cytoplasmic RNA turnover	443
Chapter summary	445
Analytical questions	447
Bibliography	448
Chapter 14: The Mechanism of Translation	451
14.1 Introduction	451
14.2 Ribosome structure and assembly	451
Structure of ribosomes	452
The nucleolus	453
Ribosome biogenesis	455
14.3 Aminoacyl-tRNA synthetases	456
Aminoacyl-tRNA charging	456
Proofreading activity of aminoacyl-tRNA synthetases	457
14.4 Initiation of translation	458
Ternary complex formation and loading onto the 40S ribosomal subunit	459
Loading the mRNA on the 40S ribosomal subunit	459
Scanning and AUG recognition	461
Joining of the 40S and 60S ribosomal subunits	461
Tool box 14.1 Translation toeprinting assays	462
Disease box 14.1 Eukaryotic initiation factor 2B and vanishing white matter	463
14.5 Elongation and events in the ribosome tunnel	463
Decoding the message	463
Peptide bond formation and translocation	466

Peptidyl transferase activity	466
Events in the ribosome tunnel	470
14.6 Termination of translation	471
14.7 Translational and post-translational control	472
Phosphorylation of eIF2 α blocks ternary complex formation	473
eIF2 α phosphorylation is mediated by four distinct protein kinases	474
Chapter summary	476
Analytical questions	477
Bibliography	477
Chapter 15: Genetically Modified Organisms: Use in Basic and Applied Research	479
15.1 Introduction	479
15.2 Transgenic mice	480
Focus box 15.1 OncoMouse patent	481
How to make a transgenic mouse	481
Tool box 15.1 Transposon tagging	484
Inducible transgenic mice	485
15.3 Gene-targeted mouse models	486
Focus box 15.2 A mouse for every need	486
Knockout mice	487
Knockin mice	490
Knockdown mice	490
Conditional knockout and knockin mice	490
15.4 Other applications of transgenic animal technology	492
Focus box 15.3 Transgenic artwork: the GFP bunny	493
Transgenic primates	493
Transgenic livestock	494
Gene pharming	494
15.5 Cloning by nuclear transfer	496
Genetic equivalence of somatic cell nuclei: frog cloning experiments	496
Cloning of mammals by nuclear transfer	497
“Breakthrough of the year”: the cloning of Dolly	497
Method for cloning by nuclear transfer	498
Source of mtDNA in clones	500
Why is cloning by nuclear transfer inefficient?	500
Applications of cloning by nuclear transfer	502
Focus box 15.4 Genetically manipulated pets	502

15.6 Transgenic plants	506
Focus box 15.5 Genetically modified crops: are you eating genetically engineered tomatoes?	506
T-DNA-mediated gene delivery	507
Electroporation and microballistics	508
Chapter summary	508
Analytical questions	509
Bibliography	510
Chapter 16: Genome Analysis: DNA Typing, Genomics, and Beyond	511
16.1 Introduction	511
16.2 DNA typing	512
Focus box 16.1 DNA profiles of marijuana	512
Focus box 16.2 Nonhuman DNA typing	513
DNA polymorphisms: the basis of DNA typing	514
Minisatellite analysis	514
Polymerase chain reaction-based analysis	516
Short tandem repeat analysis	517
Mitochondrial DNA analysis	518
Y chromosome analysis	519
Randomly amplified polymorphic DNA (RAPD) analysis	519
16.3 Genomics, proteomics, and beyond	520
What is bioinformatics?	520
Genomics	523
Proteomics	523
The age of “omics” and systems biology	523
16.4 Whole-genome sequencing	523
Clone-by-clone genome assembly approach	524
Whole-genome shotgun approach	525
Rough drafts versus finished sequences	525
Comparative analysis of genomes	525
Focus box 16.3 Comparative analysis of genomes: insights from pufferfish and chickens	526
What is a gene and how many are there in the human genome?	528
16.5 High-throughput analysis of gene function	529
DNA microarrays	529
Protein arrays	531
Mass spectrometry	531
Focus box 16.4 The nucleolar proteome	533

16.6 Genome-wide association studies	534
Single nucleotide polymorphisms	535
Disease box 16.1 Mapping disease-associated SNPs: Alzheimer's disease	535
Copy number variants (CNVs)	537
Gene polymorphisms and human behavior	537
Aggressive, impulsive, and violent behavior	537
Schizophrenia susceptibility loci	539
Chapter summary	540
Analytical questions	542
Bibliography	543
Chapter 17: Medical Molecular Biology	545
17.1 Introduction	545
17.2 Molecular biology of cancer	546
Activation of proto-oncogenes and oncogenes	547
Focus box 17.1 How cancer cells metastasize: the role of Src	550
Inactivation of tumor-suppressor genes	552
Disease box 17.1 Knudson's two-hit hypothesis and retinoblastoma	553
Focus box 17.2 The discovery of p53	556
Inappropriate expression of microRNAs in cancer	557
Chromosomal rearrangements and cancer	558
Viruses and cancer	560
Disease box 17.2 Human papilloma virus (HPV) and cervical cancer	561
Chemical carcinogenesis	564
17.3 Gene therapy	566
Vectors for somatic cell gene therapy	566
Disease box 17.3 Cancer gene therapy: a "magic bullet?"	566
Disease box 17.4 RNAi therapies	567
Focus box 17.3 Retroviral-mediated gene transfer: how to make a "safe vector"	569
Focus box 17.4 The first gene therapy fatality	571
Enhancement genetic engineering	571
Gene therapy for inherited immunodeficiency syndromes	572
Cystic fibrosis gene therapy	574
HIV-1 gene therapy	575
Focus box 17.5 HIV-1 life cycle	575
The future of gene therapy	577
Chapter summary	578
Analytical questions	580
Bibliography	580
Glossary	582
Index	632