Contents

Glossary **xxi**

Chapter 1
Introduction and Overview **1**

1.1 The Biological Importance of DNA 1
1.2 The Origins of Nucleic Acids Research 2
1.3 Early Structural Studies on Nucleic Acids 2
1.4 The Discovery of the Structure of DNA 4
1.5 The Advent of Molecular Biology 7
1.6 The Partnership of Chemistry and Biology 8
1.7 Frontiers in Nucleic Acids Research 10
References 11

Chapter 2
DNA and RNA Structure **13**

2.1 Structures of Components 14
2.1.1 Nucleosides and Nucleotides 14
2.1.2 Physical Properties of Nucleosides and Nucleotides 16
2.1.3 Spectroscopic Properties of Nucleosides and Nucleotides 19
2.1.4 Shapes of Nucleotides 20
2.2 Standard DNA Structures 24
2.2.1 Primary Structure of DNA 24
2.2.2 Secondary Structure of DNA 24
2.2.3 A-DNA 27
2.2.4 The B-DNA Family 30
2.2.5 Z-DNA 31
2.3 Real DNA Structures 33
2.3.1 Sequence-Dependent Modulation of DNA Structure 33
2.3.2 Mismatched Base–Pairs 36
2.3.3 Unusual DNA Structures 38
2.3.4 B–Z Junctions and B–Z Transitions 45
2.3.5 Circular DNA and Supercoiling 46
2.3.6 Triple-Stranded DNA 49
2.3.7 Other Non-Canonical DNA Structures 52

2.4 Structures of RNA Species 55
2.4.1 Primary Structure of RNA 56
2.4.2 Secondary Structure of RNA: A-RNA and A′-RNA 57
2.4.3 RNA·DNA Duplexes 59
2.4.4 RNA Bulges, Hairpins and Loops 61
2.4.5 Triple-Stranded RNAs 64
2.5 Dynamics of Nucleic Acid Structures 64
2.5.1 Helix-Coil Transitions of Duplexes 64
2.5.2 DNA Breathing 66
2.5.3 Energetics of the B–Z Transition 67
2.5.4 Rapid DNA Motions 68
2.6 Higher-Order DNA Structures 68
2.6.1 Nucleosome Structure 68
2.6.2 Chromatin Structure 69
References 72

Chapter 3
Nucleosides and Nucleotides **77**

3.1 Chemical Synthesis of Nucleosides 77
3.1.1 Formation of the Glycosylic Bond 79
3.1.2 Building the Base onto a C-1 Substituent of the Sugar 87
3.1.3 Synthesis of Acyclonucleosides 90
3.1.4 Syntheses of Base and Sugar-Modified Nucleosides 92
3.2 Chemistry of Esters and Anhydrides of Phosphorus Oxyacids 100
3.2.1 Phosphate Esters 100
3.2.2 Hydrolysis of Phosphate Esters 101
3.2.3 Synthesis of Phosphate Diesters and Monoesters 107
3.3 Nucleoside Esters of Polyphosphates 111
3.3.1 Structures of Nucleoside Polyphosphates and Co-Enzymes 111
3.3.2 Synthesis of Nucleoside Polyphosphate Esters 113
3.4 Biosynthesis of Nucleotides 116
3.4.1 Biosynthesis of Purine Nucleotides 116
3.4.2 Biosynthesis of Pyrimidine Nucleotides 119
3.4.3 Nucleoside Di- and Triphosphates 121
3.4.4 Deoxyribonucleotides 121
3.5 Catabolism of Nucleotides 122
3.6 Polymerisation of Nucleotides 124
3.6.1 DNA Polymerases 124
3.6.2 RNA Polymerases 125
3.7 Therapeutic Applications of Nucleoside Analogues 125
3.7.1 Anti-Cancer Chemotherapy 125
3.7.2 Anti-Viral Chemotherapy 129
References 136

Chapter 4
Synthesis of Oligonucleotides **143**

4.1 Synthesis of Oligodeoxyribonucleotides 143
4.1.1 Overall Strategy for Chemical Synthesis 144

4.1.2 Protected 2′-Deoxyribonucleoside Units 144
4.1.3 Ways of Making an Internucleotide Bond 147
4.1.4 Solid-Phase Synthesis 150
4.2 Synthesis of Oligoribonucleotides 153
4.2.1 Protected Ribonucleoside Units 154
4.2.2 Oligoribonucleotide Synthesis 155
4.3 Enzymatic Synthesis of Oligonucleotides 156
4.3.1 Enzymatic Synthesis of Oligodeoxyribonucleotides 156
4.3.2 Enzymatic Synthesis of Oligoribonucleotides 157
4.4 Synthesis of Modified Oligonucleotides 158
4.4.1 Modified Nucleobases 158
4.4.2 Modifications of the 5′- and 3′-Termini 159
4.4.3 Backbone and Sugar Modifications 160
References 165

Chapter 5
Nucleic Acids in Biotechnology **167**

5.1 DNA Sequence Determination 168
5.1.1 Principles of DNA Sequencing 168
5.1.2 Automated Fluorescent DNA Sequencing 169
5.1.3 RNA Sequencing by Reverse Transcription 170
5.2 Gene Cloning 170
5.2.1 Classical Cloning 170
5.2.2 The Polymerase Chain Reaction 173
5.3 Enzymes Useful in Gene Manipulation 174
5.3.1 Restriction Endonucleases 174
5.3.2 Other Nucleases 175
5.3.3 Polynucleotide Kinase 176
5.3.4 Alkaline Phosphatase 176
5.3.5 DNA Ligase 176
5.4 Gene Synthesis 177
5.4.1 Classical Gene Synthesis 177
5.4.2 Gene Synthesis by the Polymerase Chain Reaction 178
5.5 The Detection of Nucleic Acid Sequences by Hybridisation 178
5.5.1 Parameters that Affect Nucleic Acid Hybridisation 179
5.5.2 Southern and Northern Blot Analyses 180
5.5.3 DNA Fingerprinting 181
5.5.4 DNA Microarrays 184
5.5.5 *In Situ* Analysis of RNA in Whole Organisms 188
5.6 Gene Mutagenesis 188
5.6.1 Site-Specific *In Vitro* Mutagenesis 188
5.6.2 Random Mutagenesis 191
5.6.3 Gene Therapy 192
5.7 Oligonucleotides as Reagents and Therapeutics 193
5.7.1 Antisense and Steric Block Oligonucleotides 193
5.7.2 RNA Interference 197
5.7.3 *In Vitro* Selection 198
5.8 DNA Footprinting 203
References 205

Chapter 6
Genes and Genomes **209**

6.1 Gene Structure 210
6.1.1 Conventional Eukaryotic Gene Structure – The β Globin Gene as an Example 211
6.1.2 Complex Gene Structures 211
6.2 Gene Families 213
6.3 Intergenic DNA 215
6.4 Chromosomes 216
6.4.1 Eukaryotic Chromosomes 216
6.4.2 Packaging of DNA in Eukaryotic Chromosomes 216
6.4.3 Prokaryotic Chromosomes 218
6.4.4 Plasmid and Plastid Chromosomes 218
6.4.5 Eukaryotic Chromosome Structural Features 218
6.4.6 Viral Genomes 219
6.5 DNA Sequence and Bioinformatics 220
6.5.1 Finding Genes 220
6.5.2 Genome Maps 222
6.5.3 Molecular Marker Maps 222
6.5.4 Molecular Marker Types 222
6.5.5 Composite Maps for Genomes 223
6.6 Copying DNA 223
6.6.1 A Comparison of Transcription with DNA Replication 223
6.6.2 Transcription in Prokaryotes 224
6.6.3 Transcription in Eukaryotes 226
6.6.4 DNA Replication 231
6.6.5 Telomerases, Transposons and the Maintenance of Chromosome Ends 235
6.7 DNA Mutation and Genome Repair 236
6.7.1 Types of DNA Mutation 236
6.7.2 Mechanisms of DNA Repair 236
6.8 DNA Recombination 238
6.8.1 Homologous DNA Recombination 238
6.8.2 Site-Specific Recombination 242
6.8.3 Transposition and Transposable Elements 242
References 249

Chapter 7
RNA Structure and Function **253**

7.1 RNA Structural Motifs 253
7.1.1 Basic Structural Features of RNA 254
7.1.2 Base Pairings in RNA 255
7.1.3 RNA Multiple Interactions 256
7.1.4 RNA Tertiary Structure 257
7.2 RNA Processing and Modification 263
7.2.1 Protecting and Targeting the Transcript: Capping and Polyadenylation 263
7.2.2 Splicing and Trimming the RNA 264
7.2.3 Editing the Sequence of RNA 269
7.2.4 Modified Nucleotides Increase the Diversity of RNA Functional Groups 271
7.2.5 RNA Removal and Decay 272

7.3 RNAs in the Protein Factory: Translation 273
7.3.1 Messenger RNA and the Genetic Code 273
7.3.2 Transfer RNA and Aminoacylation 275
7.3.3 Ribosomal RNAs and the Ribosome 276
7.4 RNAs Involved in Export and Transport 280
7.4.1 Transport of RNA 280
7.4.2 RNA that Transports Protein: the Signal Recognition Particle 280
7.5 RNAs and Epigenetic Phenomena 281
7.5.1 RNA Mobile Elements 281
7.5.2 SnoRNAs: Guides for Modification of Ribosomal RNA 282
7.5.3 Small RNAs Involved in Gene Silencing and Regulation 283
7.6 RNA Structure and Function in Viral Systems 283
7.6.1 RNA as an Engine Part: The Bacteriophage Packaging Motor 283
7.6.2 RNA as a Catalyst: Self-Cleaving Motifs from Viral RNA 285
7.6.3 RNA Tertiary Structure and Viral Function 287
References 290

Chapter 8
Covalent Interactions of Nucleic Acids with Small Molecules and Their Repair 295

8.1 Hydrolysis of Nucleosides, Nucleotides and Nucleic Acids 296
8.2 Reduction of Nucleosides 296
8.3 Oxidation of Nucleosides, Nucleotides and Nucleic Acids 297
8.4 Reactions with Nucleophiles 298
8.5 Reactions with Electrophiles 298
8.5.1 Halogenation of Nucleic Acid Residues 298
8.5.2 Reactions with Nitrogen Electrophiles 300
8.5.3 Reactions with Carbon Electrophiles 300
8.5.4 Metallation Reactions 302
8.6 Reactions with Metabolically Activated Carcinogens 303
8.6.1 Aromatic Nitrogen Compounds 304
8.6.2 *N*-Nitroso Compounds 306
8.6.3 Polycyclic Aromatic Hydrocarbons 307
8.7 Reactions with Anti-Cancer Drugs 308
8.7.1 Aziridine Antibiotics 310
8.7.2 Pyrrolo[1,4]benzodiazepines, P[1,4]Bs 311
8.7.3 Enediyne Antibiotics 313
8.7.4 Antibiotics Generating Superoxide 316
8.8 Photochemical Modification of Nucleic Acids 316
8.8.1 Pyrimidine Photoproducts 316
8.8.2 Psoralen–DNA Photoproducts 319
8.8.3 Purine Photoproducts 320
8.8.4 DNA and the Ozone Barrier 321
8.9 Effects of Ionizing Radiation on Nucleic Acids 322
8.9.1 Deoxyribose Products in Aerobic Solution 322
8.9.2 Pyrimidine Base Products in Solution 322
8.9.3 Purine Base Products 322
8.10 Biological Consequences of DNA Alkylation 323
8.10.1 *N*-Alkylated Bases 323
8.10.2 *O*-Alkylated Lesions 325

8.11 DNA Repair 325
8.11.1 Direct Reversal of Damage 326
8.11.2 Base Excision Repair of Altered Residues 328
8.11.3 Mechanisms and Inhibitors of DNA Glycohydrolases 329
8.11.4 Nucleotide Excision Repair 329
8.11.5 Crosslink Repair 330
8.11.6 Base Mismatch Repair 330
8.11.7 Preferential Repair of Transcriptionally Active DNA 331
8.11.8 Post-replication Repair 332
8.11.9 Bypass Mutagenesis 332
References 334

Chapter 9
Reversible Small Molecule-Nucleic Acid Interactions 341

9.1 Introduction 342
9.2 Binding Modes and Sites of Interaction 342
9.3 Counter-Ion Condensation and Polyelectrolyte Theory 343
9.3.1 Intercalation and Polyelectrolyte Theory 345
9.4 Non-specific Outside-Edge Interactions 345
9.5 Hydration Effects and Water–DNA Interactions 346
9.5.1 Cation Binding in the Minor Groove 347
9.6 DNA Intercalation 347
9.6.1 The Classical Model 347
9.6.2 The Anthracycline Antibiotic Daunomycin 350
9.6.3 The Neighbour Exclusion Principle 353
9.6.4 Apportioning the Free Energy for DNA Intercalation Reactions 354
9.6.5 Bisintercalation 355
9.6.6 Nonclassical Intercalation: The Threading Mode 358
9.7 Interactions in the Minor Groove 361
9.7.1 General Characteristics of Groove Binding 361
9.7.2 Netropsin and Distamycin 361
9.7.3 Lexitropsins 364
9.7.4 Hairpin Polyamides 365
9.7.5 Hoechst 33258 366
9.8 Intercalation *Versus* Minor Groove Binding 370
9.9 Co-operativity in Ligand–DNA Interactions 372
9.10 Small Molecule Interactions with Higher-Order DNA 372
9.10.1 Triplex DNA and its Interactions with Small Molecules 372
9.10.2 Quadruplex DNA and its Interactions with Small Molecules 375
References 379

Chapter 10
Protein-Nucleic Acid Interactions 383

10.1 Features of DNA Recognized by Proteins 384
10.2 The Physical Chemistry of Protein–Nucleic Acid Interactions 387
10.2.1 Hydrogen-Bonding Interactions 387
10.2.2 Salt Bridges 389

10.2.3 The Hydrophobic Effect 389
10.2.4 How Dispersions Attract: van der Waals Interactions and Base Stacking 390
10.3 Representative DNA Recognition Motifs 391
10.3.1 The Tree of Life and its Fruitful Proteins 391
10.3.2 The Structural Economy of α-Helical Motifs 392
10.3.3 Zinc-Bearing Motifs 393
10.3.4 The Orientations of α-Helices in the DNA Major Groove 394
10.3.5 Minor Groove Recognition *via* α-Helices 394
10.3.6 β-Motifs 394
10.3.7 Loops and Others Elements 395
10.3.8 Single-Stranded DNA Recognition 396
10.4 Kinetic and Thermodynamic Aspects of Protein–Nucleic Acid Interactions 398
10.4.1 The Delicate Balance of Sequence-Specificity 398
10.4.2 The Role of Water 398
10.4.3 Specific versus Non-Specific Complexes 400
10.4.4 Electrostatic Effects 400
10.4.5 DNA Conformability 400
10.4.6 Co-operativity through Protein–Protein and DNA–Protein Interactions 402
10.4.7 Kinetic and Non-Equilibrium Aspects of DNA Recognition 403
10.5 The Specificity of DNA Enzymes 404
10.5.1 Restriction Enzymes: Recognition through the Transition State 404
10.5.2 DNA-Repair Endonucleases 405
10.5.3 DNA Glycosylases 406
10.5.4 Photolyases 407
10.5.5 Structure-Selective Nucleases 407
10.6 DNA Packaging 408
10.6.1 Nucleosomes and Chromatin of the Eukaryotes 408
10.6.2 Packaging and Architectural Proteins in Archaebacteria and Eubacteria 409
10.7 Polymerases 409
10.7.1 DNA-Directed DNA Polymerases 409
10.7.2 DNA-Directed RNA Polymerases 410
10.8 Machines that Manipulate Duplex DNA 413
10.8.1 Helicases 413
10.8.2 DNA Pumps 413
10.8.3 DNA Topoisomerases 416
10.9 RNA–Protein Interactions and RNA-Mediated Assemblies 416
10.9.1 Single-Stranded RNA Recognition 417
10.9.2 Duplex RNA Recognition 417
10.9.3 Transfer RNA Synthetases 417
10.9.4 Small Interfering RNA Recognition 421
Web Resources 421
References 422

Chapter 11
Physical and Structural Techniques Applied to Nucleic Acids **427**

11.1 Spectroscopic Techniques 428
11.1.1 Ultraviolet Absorption 428
11.1.2 Fluorescence 429

11.1.3 Circular and Linear Dichroism 431
11.1.4 Infrared and Raman Spectroscopy 432
11.2 Nuclear Magnetic Resonance 433
11.3 X-ray Crystallography 438
11.4 Hydrodynamic and Separation Methods 439
11.4.1 Centrifugation 439
11.4.2 Light Scattering 441
11.4.3 Gel Electrophoresis 442
11.4.4 Microcalorimetry 443
11.5 Microscopy 446
11.5.1 Electron Microscopy 446
11.5.2 Scanning Probe Microscopy 447
11.6 Mass Spectrometry 449
11.6.1 Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry 450
11.6.2 Electrospray Ionization Mass Spectrometry 450
11.7 Molecular Modelling and Dynamics 451
11.7.1 Molecular Mechanics and Energy Minimisation 453
11.7.2 Molecular Dynamics 453
11.7.3 Mesoscopic Modelling 454
References 455

Subject Index **459**