Contents

Preface xvii

Acknowledgments xix

About the Authors xxi

Chapter 1: To the Cell and Beyond: The Realm of Molecular Biology 1

1.1 INTRODUCTION 2

1.2 THE VITAL ROLE OF MICROSCOPY IN BIOLOGY 2

The light microscope led to the first revolution in biology 2

Biochemistry led to the discovery of the importance of macromolecules in life's structure and processes 6

The electron microscope provided another order of resolution 6

1.3 FINE STRUCTURE OF CELLS AND VIRUSES AS REVEALED BY MICROSCOPY 8

1.4 ULTRAHIGH RESOLUTION: BIOLOGY AT THE MOLECULAR LEVEL 10

Fluorescence techniques allow for one approach to ultra-resolution 10

Confocal fluorescence microscopy allows observation of the fluorescence emitted by a particular substance in a cell 10

FIONA provides ultimate optical resolution by use of fluorescence 11

FRET allows distance measurements at the molecular level 12

Single-molecule cryo-electron microscopy is a powerful new technique 12

The atomic force microscope feels molecular structure 13

X-ray diffraction and nuclear magnetic resonance provide resolution to the atomic level 14

Chemical imaging, the new powerful combination of imaging techniques 15

1.5 MOLECULAR GENETICS: ANOTHER FACE OF MOLECULAR BIOLOGY 15

Key concepts 15

Further reading 16

Videos on the Internet 17

Chapter 2: From Classical Genetics to Molecular Genetics 19

2.1 INTRODUCTION 19

2.2 CLASSICAL GENETICS AND THE RULES OF TRAIT INHERITANCE 20

Gregor Mendel developed the formal rules of genetics 20

Mendel's laws have extensions and exceptions 25

Genes are arranged linearly on chromosomes and can be mapped 26

The nature of genes and how they determine phenotypes was long a mystery 27

2.3 THE GREAT BREAKTHROUGH TO MOLECULAR GENETICS 27

Bacteria and bacteriophage exhibit genetic behavior and serve as model systems 27

Transformation and transduction allow transfer of genetic information 29

The Watson–Crick model of DNA structure provided the final key to molecular genetics 30

2.4 MODEL ORGANISMS 31

2.5 WHOLE GENOMES AND EVOLUTION 33

Evolutionary theory: From Darwin to the present day 33

Human-driven evolution: The story of Vavilov 36

The tree of life based on sequencing of thousands of species: Back to the two-domain tree of life 36

Key concepts 36

Further reading 37

Videos on the Internet 37

Chapter 3: Proteins 39

3.1 INTRODUCTION 39

Proteins are macromolecules with enormous variety in size, structure, and function 39

Proteins are essential for the structure and functioning of all organisms 41

3.2 PROTEIN STRUCTURE 41

Proteins are homogeneous polypeptides and amino acids are their building blocks 42

Fred Sanger and the sequence of insulin 42

In proteins, amino acids are covalently connected to form polypeptides 44

3.3 LEVELS OF STRUCTURE IN THE POLYPEPTIDE CHAIN 46

The primary structure of a protein is a unique sequence of amino acids 46

A protein's secondary structure involves regions of regular folding stabilized by hydrogen bonds 50

Each protein has a unique three-dimensional tertiary structure 53
The tertiary structure of most proteins is divided into distinguishable folded domains 55
Algorithms are now used to identify and classify domains in proteins of known sequence 58
Some domains or proteins are intrinsically disordered 62
Quaternary structure involves associations between protein molecules to form aggregated structures 64

3.4 HOW DO PROTEINS FOLD? 65
Folding can be a problem 65
Chaperones help or allow proteins to fold 66

3.5 HOW ARE PROTEINS DESTROYED? 70
The proteasome is the general protein destruction system 71

3.6 THE PROTEOME AND PROTEIN INTERACTION NETWORKS 73
New technologies allow a census of an organism's proteins and their interactions 73
Key concepts 76
Further reading 76
Videos on the Internet 77

Chapter 4: Nucleic Acids 79

4.1 INTRODUCTION 80
Protein sequences are dictated by nucleic acids 80

4.2 CHEMICAL STRUCTURE OF NUCLEIC ACIDS 80
DNA and RNA have similar but different chemical structures 80
Nucleic acids (polynucleotides) are polymers of nucleotides 82

4.3 PHYSICAL STRUCTURES OF DNA 83
Discovery of the B-DNA structure was a breakthrough in molecular biology 83
A number of alternative DNA structures exist 89
Although the double helix is quite rigid, it can be bent by bound proteins 91
DNA can also form folded tertiary structures 91
Closed DNA circles can be twisted into supercoils 92

4.4 PHYSICAL STRUCTURES OF RNA 95
RNA can adopt a variety of complex structures but not the B-form helix 95

4.5 ONE-WAY FLOW OF GENETIC INFORMATION 99

4.6 METHODS USED TO STUDY NUCLEIC ACIDS 100
Key concepts 108
Further reading 108
Videos on the Internet 109

Chapter 5: Recombinant DNA: Principles and Applications 111

5.1 INTRODUCTION 112
Cloning of DNA involves several fundamental steps 112

5.2 CONSTRUCTION OF RECOMBINANT DNA MOLECULES 113
Restriction endonucleases and ligases are essential tools in cloning 113

5.3 VECTORS FOR CLONING 118
Genes coding for selectable markers are inserted into vectors during their construction 118
Bacterial plasmids were the first cloning vectors 121
Recombinant bacteriophages can serve as bacterial vectors 122
Cosmids and phagemids expand the repertoire of cloning vectors 125

5.4 ARTIFICIAL CHROMOSOMES AS VECTORS 125
Bacterial artificial chromosomes meet the need for cloning very large DNA fragments in bacteria 125
Eukaryotic artificial chromosomes provide proper maintenance and expression of very large DNA fragments in eukaryotic cells 125

5.5 EXPRESSION OF RECOMBINANT GENES 126
Expression vectors allow regulated and efficient expression of cloned genes 126
Shuttle vectors can replicate in more than one organism 128

5.6 INTRODUCING RECOMBINANT DNA INTO HOST CELLS 128
Numerous host-specific techniques are used to introduce recombinant DNA molecules into living cells 128

5.7 POLYMERASE CHAIN REACTION AND SITE-DIRECTED MUTAGENESIS 129

5.8 SEQUENCING OF ENTIRE GENOMES 131
Genomic libraries contain the entire genome of an organism as a collection of recombinant DNA molecules 131
There are two approaches for sequencing large genomes 132

5.9 MANIPULATING THE GENETIC CONTENT OF EUKARYOTIC ORGANISMS 133
Making a transgenic mouse involves numerous steps 133
To inactivate, replace, or otherwise modify a particular gene, the vector must be targeted for homologous recombination at that particular site 134

5.10 PRACTICAL APPLICATIONS OF RECOMBINANT DNA TECHNOLOGIES 135
Hundreds of pharmaceutical compounds are produced in recombinant bacteria 135
Plant genetic engineering is a huge but controversial industry 137
Gene therapy is a complex multistep process aiming to correct defective genes or gene functions that are responsible for disease 141
Delivering a gene into sufficient cells within a specific tissue and ensuring its subsequent long-term expression is a challenge 142
CRISPR, the new technology to change genomic DNA sequence at a predefined position 143
Jurassic Park or de-extinction 145
Cloning of whole animals by nuclear transfer 146
Key concepts 147
Further reading 148
Videos on the Internet 149

Chapter 6: Protein–Nucleic Acid Interactions 151

6.1 INTRODUCTION 151

6.2 DNA–PROTEIN INTERACTIONS 152
DNA–protein binding occurs by many modes and mechanisms 152

Site-specific binding is the most widely used mode 154
Most recognition sites fall into a limited number of classes 155
Most specific binding requires the insertion of protein into a DNA groove 156
Some proteins cause DNA looping 157
There are a few major protein motifs of DNA-binding domains 158
Helix–turn–helix motif interacts with the major groove 158
Zinc fingers also probe the major groove 158
Leucine zippers are especially suited for dimeric sites 159

6.3 RNA–PROTEIN INTERACTIONS 159

6.4 STUDYING PROTEIN–NUCLEIC ACID INTERACTIONS 162

Key concepts 168
Further reading 168
Videos on the Internet 169

Chapter 7: The Genetic Code, Genes, and Genomes 171

7.1 GENES AS NUCLEIC ACID REPOSITORIES OF GENETIC INFORMATION 171

Our understanding of the nature of genes is constantly evolving 171
The central dogma states that information flows from DNA to protein 172
It was necessary to separate cellular RNAs to seek the adaptors 174
Messenger RNA, tRNA, and ribosomes constitute the protein factories of the cell 174

7.2 RELATING PROTEIN SEQUENCE TO DNA SEQUENCE IN THE GENETIC CODE 175

The first task was to define the nature of the code 175

7.3 SURPRISES FROM THE EUKARYOTIC CELL: INTRONS AND SPLICING 179

Eukaryotic genes usually contain interspersed noncoding sequences 179

7.4 GENES FROM A NEW AND BROADER PERSPECTIVE 180

Protein-coding genes are complex 180
Genome sequencing has revolutionized the gene concept 180
Mutations, pseudogenes, and alternative splicing all contribute to gene diversity 181

7.5 COMPARING WHOLE GENOMES AND NEW PERSPECTIVES ON EVOLUTION 182

Genome sequencing reveals puzzling features of genomes 182
How are DNA sequence types and functions distributed in eukaryotes? 184
Key concepts 188
Further reading 189
Videos on the Internet 189

Chapter 8: Physical Structure of the Genomic Material 191

8.1 INTRODUCTION 191

8.2 CHROMOSOMES OF VIRUSES AND BACTERIA 192

Generally, viruses are packages for minimal genomes 192
Bacterial chromosomes are organized structures in the cytoplasm 200
DNA-bending proteins and DNA-bridging proteins help to pack bacterial DNA 201

8.3 EUKARYOTIC CHROMATIN 201

Eukaryotic chromosomes are highly condensed DNA–protein complexes segregated into a nucleus 201
The nucleosome is the basic repeating unit of eukaryotic chromatin 203
Histone nonallelic variants and postsynthetic modifications create a heterogeneous population of nucleosomes 206
The nucleosome family is dynamic 211
Nucleosome assembly in vivo uses histone chaperones 212

8.4 HIGHER-ORDER CHROMATIN STRUCTURE 213

Nucleosomes along the DNA form a chromatin fiber 213
The chromatin fiber is folded, but its structure remains controversial 214
The organization of chromosomes in the interphase nucleus is still obscure 216

8.5 MITOTIC CHROMOSOMES 217

Chromosomes condense and separate in mitosis 217
A number of proteins are needed to form and maintain mitotic chromosomes 218
Centromeres and telomeres are chromosome regions with special functions 219
There are a number of models of mitotic chromosome structure 221
Key concepts 225
Further reading 225
Videos on the Internet 226

Chapter 9: Transcription in Bacteria 227

9.1 INTRODUCTION 228

9.2 OVERVIEW OF TRANSCRIPTION 228

There are aspects of transcription common to all organisms 228
Transcription requires the participation of many proteins 229
Transcription is rapid but is often interrupted by pauses 232
Transcription can be visualized by electron microscopy 233

9.3 RNA POLYMERASES AND TRANSCRIPTION CATALYSIS 235

RNA polymerases are a large family of enzymes that produce RNA transcripts of polynucleotide templates 235

9.4 MECHANICS OF TRANSCRIPTION IN BACTERIA 237

Initiation requires a multisubunit polymerase complex, termed the holoenzyme 237
The initiation phase of bacterial transcription is frequently aborted 241
Elongation in bacteria must overcome topological problems 242
There are several mechanisms for transcription termination in bacteria 244
Antisense transcription in bacteria is widespread and might have numerous functions 246
Understanding transcription in bacteria is useful in clinical practice 247
Key concepts 249
Further reading 250
Videos on the Internet 250

Chapter 10: Transcription in Eukaryotes 251

10.1 INTRODUCTION 252

Transcription in eukaryotes is a complex, highly regulated process 252

Eukaryotic cells contain multiple RNA polymerases, each specific for distinct functional subsets of genes 252

10.2 TRANSCRIPTION BY RNA POLYMERASE II 253

The yeast Pol II structure provides insights into transcriptional mechanisms 253

The structure of Pol II is more evolutionarily conserved than its sequence 255

Nucleotide addition during transcription elongation is cyclic 257

Transcription initiation depends on multisubunit protein complexes that assemble at core promoters 257

An additional protein complex is needed to connect Pol II to regulatory proteins 262

Termination of eukaryotic transcription is coupled to polyadenylation of the RNA transcript 262

10.3 TRANSCRIPTION BY RNA POLYMERASE I 263

10.4 TRANSCRIPTION BY RNA POLYMERASE III 264

RNA polymerase III specializes in transcription of small genes 264

10.5 TRANSCRIPTION IN EUKARYOTES: PERVASIVE AND SPATIALLY ORGANIZED 265

Most of the eukaryotic genome is transcribed 265

Transcription in eukaryotes is not uniform within the nucleus 269

Active and inactive genes are spatially separated in the nucleus 270

10.6 METHODS FOR STUDYING EUKARYOTIC TRANSCRIPTION 271

A battery of methods is available for the study of transcription 271

Key concepts 277

Further reading 277

Videos on the Internet 278

Chapter 11: Regulation of Transcription in Bacteria 279

11.1 INTRODUCTION 280

11.2 GENERAL MODELS FOR REGULATION OF TRANSCRIPTION 280

Regulation can occur via differences in promoter strength or use of alternative σ factors 280

Regulation through ligand binding to RNA polymerase is called stringent control 281

11.3 SPECIFIC REGULATION OF TRANSCRIPTION 282

Regulation of specific genes occurs through *cis–trans* interactions with transcription factors 282

Transcription factors are activators and repressors whose own activity is regulated in a number of ways 284

Several transcription factors can act synergistically or in opposition to activate or repress transcription 285

11.4 TRANSCRIPTIONAL REGULATION OF OPERONS IS IMPORTANT TO BACTERIAL PHYSIOLOGY 285

The lac operon is controlled by a dissociable repressor and an activator 285

Control of the *trp* operon involves both repression and attenuation 291

The same protein can serve as an activator or a repressor: the *ara* operon 294

11.5 OTHER MODES OF GENE REGULATION IN BACTERIA 295

DNA supercoiling is involved in both global and local regulation of transcription 295

DNA methylation can provide specific regulation 296

11.6 COORDINATION OF GENE EXPRESSION IN BACTERIA 297

Networks of transcription factors form the basis of coordinated gene expression 298

Key concepts 299

Further reading 299

Videos on the Internet 300

Chapter 12: Regulation of Transcription in Eukaryotes 301

12.1 INTRODUCTION 302

12.2 REGULATION OF TRANSCRIPTION INITIATION: REGULATORY REGIONS AND TRANSCRIPTION FACTORS 302

Core and proximal promoters are needed for basal and regulated transcription 302

Enhancers, silencers, insulators, and locus control regions are all distal regulatory elements 303

Some eukaryotic transcription factors are activators, others are repressors, and still others can be either, depending on context 306

Regulation can use alternative components of the basal transcriptional machinery 307

Mutations in gene regulatory regions and in transcriptional machinery components lead to human diseases 308

12.3 REGULATION OF TRANSCRIPTIONAL ELONGATION 308

The polymerase may stall close to the promoter 308

Transcription elongation rate can be regulated by elongation factors 309

12.4 TRANSCRIPTION REGULATION AND CHROMATIN STRUCTURE 309

What happens to nucleosomes during transcription? 309

12.5 REGULATION OF TRANSCRIPTION BY HISTONE MODIFICATIONS AND VARIANTS 311

Modification of histones provides epigenetic control of transcription 311

Gene expression is often regulated by histone post-translational modifications 312

Readout of histone post-translational modification marks involves specialized protein molecules 313

Post-translational histone marks distinguish transcriptionally active and inactive chromatin regions 314

Some genes are specifically silenced by post-translational modification in some cell lines 315

Polycomb protein complexes silence genes through H3K27 trimethylation and H2AK119 ubiquitylation 316

Heterochromatin formation at telomeres in yeast silences genes through H4K16 deacetylation 318
HP1-mediated gene repression in the majority of eukaryotic organisms involves H3K9 methylation 318
Poly(ADP)ribosylation of proteins is involved in transcriptional regulation 319
Histone variants H2A.Z, H3.3, and H2A.Bbd are present in active chromatin 319
MacroH2A is a histone variant prevalent in inactive chromatin 321
Problems caused by chromatin structure can be fixed by remodeling 321
Endogenous metabolites can exert rheostat control of transcription 323

12.6 DNA METHYLATION 324

DNA methylation patterns in genomic DNA may participate in regulation of transcription 325
Carcinogenesis alters the pattern of CpG methylation 327
DNA methylation changes during embryonic development 327
DNA methylation is governed by complex enzymatic machinery 328
There are proteins that read the DNA methylation mark 328

12.7 LONG NONCODING RNAS IN TRANSCRIPTIONAL REGULATION 329

Noncoding RNAs play surprising roles in regulating transcription 329
The sizes and genomic locations of noncoding transcripts are remarkably diverse 330

12.8 METHODS FOR MEASURING THE ACTIVITY OF TRANSCRIPTIONAL REGULATORY ELEMENTS 333

Key concepts 334
Further reading 335
Vidoes on the Internet 336

Chapter 13: Transcription Regulation in the Human Genome 339

13.1 INTRODUCTION 340

Rapid full-genome sequencing allows deep analysis 340

13.2 BASIC CONCEPTS OF ENCODE 340

ENCODE depends on high-throughput, massively processive sequencing and sophisticated computer algorithms for analysis 340
The ENCODE project integrates diverse data relevant to transcription in the human genome 342

13.3 REGULATORY DNA SEQUENCE ELEMENTS 342

Seven classes of regulatory DNA sequence elements make up the transcriptional landscape 342

13.4 SPECIFIC FINDINGS CONCERNING CHROMATIN STRUCTURE FROM ENCODE 343

Millions of DNase I-hypersensitive sites mark regions of accessible chromatin 343
DNase I signatures at promoters are asymmetric and stereotypic 344
Nucleosome positioning at promoters and around TF-binding sites is highly heterogeneous 345
The chromatin environment at regulatory elements and in gene bodies is also heterogeneous and asymmetric 346

13.5 ENCODE INSIGHTS INTO GENE REGULATION 346

Distal control elements are connected to promoters in a complex network 346
Transcription factor binding defines the structure and function of regulatory regions 348
Transcription factors interact in a huge network 349
TF-binding sites and TF structure co-evolve 351
DNA methylation patterns show a complex relationship with transcription 352

13.6 ENCODE OVERVIEW 353

What have we learned from ENCODE, and where is it leading? 353
Certain methods are essential to ENCODE project studies 354

13.7 BEYOND THE ENCODE PROJECT 356

Key concepts 357
Further reading 357
Videos on the Internet 358

Chapter 14: RNA Processing 359

14.1 INTRODUCTION 360

Most RNA molecules undergo post-transcriptional processing 360
There are four general categories of processing 360
Eukaryotic RNAs exhibit much more processing than bacterial RNAs 360

14.2 PROCESSING OF TRNAS AND RRNAS 361

tRNA processing is similar in all organisms 361
All three mature ribosomal RNA molecules are cleaved from a single long precursor RNA 361

14.3 PROCESSING OF EUKARYOTIC MRNA: END MODIFICATIONS 364

Eukaryotic mRNA capping is co-transcriptional 364
Polyadenylation at the 3′-end serves a number of functions 364
Chemical modifications of eukaryotic RNAs and their roles 366

14.4 PROCESSING OF EUKARYOTIC MRNA: SPLICING 368

The splicing process is complex and requires great precision 368
Splicing is carried out by spliceosomes 368
Splicing can produce alternative mRNAs 369
Tandem chimerism links exons from separate genes 371
Trans-splicing combines exons residing in the two complementary DNA strands 376

14.5 REGULATION OF SPLICING AND ALTERNATIVE SPLICING 376

Splice sites differ in strength 376
Exon–intron architecture affects splice-site usage 376
Cis–trans interactions may stimulate or inhibit splicing 377
RNA secondary structure can regulate alternative splicing 379
Sometimes alternative splicing regulation needs no auxiliary regulators 379
The rate of transcription and chromatin structure may help regulate splicing 379

14.6 SELF-SPLICING: INTRONS AND RIBOZYMES 381

A fraction of introns is excised by self-splicing RNA 381
There are two classes of self-splicing introns 381

14.7 OVERVIEW: THE HISTORY OF AN MRNA MOLECULE 382
Proceeding from the primary transcript to a functioning mRNA requires a number of steps 382
mRNA is exported from the nucleus to the cytoplasm through nuclear pore complexes 383
RNA sequence can be edited by enzymatic modification even after transcription 383
14.8 RNA QUALITY CONTROL AND DEGRADATION 385
Bacteria, archaea, and eukaryotes all have mechanisms for RNA quality control 385
Archaea and eukaryotes utilize specific pathways to deal with different RNA defects 386
14.9 BIOGENESIS AND FUNCTIONS OF SMALL SILENCING RNAS 386
All ssRNAs are produced by processing from larger precursors 386
Key concepts 390
Further reading 393
Videos on the Internet 394

Chapter 15: Translation: The Players 395
15.1 INTRODUCTION 396
15.2 A BRIEF OVERVIEW OF TRANSLATION 396
Three participants are needed for translation to occur 396
15.3 TRANSFER RNA 398
tRNA molecules fold into four-arm cloverleaf structures 398
tRNAs are aminoacylated by a set of specific enzymes, aminoacyl-tRNA synthetases 400
Aminoacylation of tRNA is a two-step process 400
Quality control or proofreading occurs during the amino-acylation reaction 401
Insertion of noncanonical amino acids into polypeptide chains is guided by stop codons 402
15.4 MESSENGER RNA 407
The Shine–Dalgarno sequence in bacterial mRNAs aligns the message on the ribosome 407
Eukaryotic mRNAs do not have Shine–Dalgarno sequences but more complex 5′- and 3′-untranslated regions 408
Overall translation efficiency depends on a number of factors 410
15.5 RIBOSOMES 410
The ribosome is a two-subunit structure comprising rRNAs and numerous ribosomal proteins 411
Functional ribosomes require both subunits, with specific complements of RNA and protein molecules 411
The small subunit can accept mRNA but must join with the large subunit for peptide synthesis to occur 413
Ribosome assembly has been studied both *in vivo* and *in vitro* 414
The expanding "riboverse" 417
Key concepts 419
Further reading 420
Videos on the Internet 420

Chapter 16: Translation: The Process 421
16.1 INTRODUCTION 422
16.2 AN OVERVIEW OF TRANSLATION: HOW FAST AND HOW ACCURATE? 422
16.3 ADVANCED METHODOLOGY FOR THE ANALYSIS OF TRANSLATION 424
Cryo-EM allows visualization of discrete kinetic states of ribosomes 424
X-ray crystallography provides the highest resolution 425
Single-pair fluorescence resonance energy transfer allows dynamic studies at the single-particle level 427
16.4 INITIATION OF TRANSLATION 427
Initiation of translation begins on a free small ribosomal subunit 427
Cryo-EM provides details of initiation complexes 428
Start site selection in eukaryotes is complex 429
16.5 TRANSLATIONAL ELONGATION 430
Decoding means matching the codon to the anticodon-carrying aminoacyl-tRNA 430
Accommodation denotes a relaxation of distorted tRNA to allow peptide bond formation 432
Peptide bond formation is accelerated by the ribosome 432
The formation of hybrid states is an essential part of translocation 434
Structural information on bacterial elongation factors provides insights into mechanisms 436
There is an exit tunnel for the peptide chain in the ribosome 438
Translation elongation in eukaryotes involves even more factors 439
Ribosome stalling during translation elongation 439
16.6 TERMINATION OF TRANSLATION 440
RF3 aids in removing RF1 andRF2 441
Ribosomes are recycled after termination 442
Our views of translation continue to evolve 443
Key concepts 443
Further reading 444
Videos on the Internet 445

Chapter 17: Regulation of Translation 447
17.1 INTRODUCTION 448
17.2 REGULATION OF TRANSLATION BY CONTROLLING RIBOSOME NUMBER 448
Ribosome numbers in bacteria are responsive to the environment 448
Ribosome numbers in eukaryotes: Control and consequences of dysregulation 449
Synthesis of ribosomal components in bacteria is coordinated 450
Regulation of the synthesis of ribosomal components in eukaryotes involves chromatin structure 451
17.3 REGULATION OF TRANSLATION INITIATION 454
Regulation of translation initiation is ubiquitous and remarkably varied 454
Regulation may depend on protein factors binding to the 5′- or 3′-ends of mRNA 454
Cap-dependent regulation is the major pathway for controlling initiation 455
Initiation may utilize internal ribosome entry sites 455
5′–3′-UTR interactions provide a novel mechanism that regulates initiation in eukaryotes 457

Riboswitches are RNA sequence elements that regulate initiation in response to stimuli 457
Repeat-associated non-AUG translation 459
MicroRNAs can bind to mRNA, thereby regulating translation 460
17.4 REGULATION OF THE ELONGATION PROCESS 461
17.5 mRNA STABILITY AND DECAY IN EUKARYOTES 463
The two major pathways of decay for nonfaulty mRNA molecules start with mRNA deadenylation 464
The 5′ → 3′ pathway is initiated by the activities of the decapping enzyme Dcp2 465
The 3′ → 5′ pathway uses the exosome, followed by a different decapping enzyme, DcpS 466
There are additional pathways for mRNA degradation 468
Unused mRNA is sequestered in P bodies and stress granules 468
Cells have several mechanisms that destroy faulty mRNA molecules 471
mRNA molecules that contain premature stop codons are degraded through nonsense-mediated decay or NMD 472
No-go decay (NGD) functions when the ribosome stalls during elongation 473
Non-stop decay or NSD functions when mRNA does not contain a stop codon 473
17.6 SUMMARY OF TRANSLATION REGULATION 474
Key concepts 474
Further reading 474
Videos on the Internet 476
Chapter 18: Protein Processing and Modification 477
18.1 INTRODUCTION 478
18.2 STRUCTURE OF BIOLOGICAL MEMBRANES 478
Biological membranes are protein-rich lipid bilayers 478
Numerous proteins are associated with biomembranes 479
18.3 PROTEIN TRANSLOCATION THROUGH BIOLOGICAL MEMBRANES 479
Protein translocation can occur during or after translation 480
Membrane translocation in bacteria and archaea primarily functions for secretion 480
Membrane translocation in eukaryotes serves a multitude of functions 481
Integral membrane proteins have special mechanisms for membrane insertion 482
Vesicles transport proteins between compartments in eukaryotic cells 484
18.4 PROTEOLYTIC PROTEIN PROCESSING: CUTTING, SPLICING, AND DEGRADATION 485
Proteolytic cleavage is sometimes used to produce mature proteins from precursors 485
Some proteases can catalyze protein splicing 486
Controlled proteolysis is also used to destroy proteins no longer needed 488
18.5 POST-TRANSLATIONAL CHEMICAL MODIFICATIONS OF SIDE CHAINS 489
Modification of side chains can affect protein structure and function 489
Phosphorylation plays a major role in signaling 491
Acetylation mainly modifies interactions 493
Several classes of glycosylated proteins contain added sugar moieties 494
Mechanisms of glycosylation depend on the type of modification 500
Ubiquitylation adds single or multiple ubiquitin molecules to proteins through an enzymatic cascade 502
Specificity of ubiquitin targeting is determined by a special class of enzymes 504
The structure of protein-ubiquitin conjugates determines the biological role of the modification 509
Polyubiquitin marks proteins for degradation by the proteasome 509
Sumoylation adds single or multiple SUMO molecules to proteins 510
18.6 PROTEIN CO-TRANSLATIONAL FOLDING 512
18.7 THE GENOMIC ORIGIN OF PROTEINS 513
Key concepts 513
Further reading 514
Videos on the Internet 515
Chapter 19: DNA Replication in Bacteria 517
19.1 INTRODUCTION 518
19.2 FEATURES OF DNA REPLICATION SHARED BY ALL ORGANISMS 518
Replication on both strands creates a replication fork 518
Mechanistically, synthesis of new DNA chains requires a template, a polymerase, and a primer 520
DNA replication requires the simultaneous action of two DNA polymerases 520
Other protein factors are obligatory at the replication fork 521
19.3 DNA REPLICATION IN BACTERIA 523
Bacterial chromosome replication is bidirectional, from a single origin of replication 523
DNA polymerase III catalyzes replication in bacteria 523
Sliding clamp β, or processivity factor, is essential for processivity 523
The clamp loader organizes the replisome 523
The full complement of proteins in the replisome is organized in a complex and dynamic way 524
DNA polymerase I is necessary for maturation of Okazaki fragments 528
19.4 THE PROCESS OF BACTERIAL REPLICATION 530
The replisome is a dynamic structure during elongation 530
19.5 INITIATION AND TERMINATION OF BACTERIAL REPLICATION 532
Initiation involves both specific DNA sequence elements and numerous proteins 532
Termination of replication also employs specific DNA sequences and protein factors that bind to them 534
19.6 DNA REPLICATION AND BACTERIAL CELL CYCLE 536
19.7 BACTERIOPHAGE AND PLASMID REPLICATION 540
Rolling-circle replication is an alternative mechanism 542
Phage replication can involve both bidirectional and rolling-circle mechanisms 543

Key concepts 543
Further reading 546
Videos on the Internet 547

Chapter 20: DNA Replication in Eukaryotes 549

20.1 INTRODUCTION 550

20.2 REPLICATION INITIATION IN EUKARYOTES 550

Replication initiation in eukaryotes proceeds from multiple origins 550
Eukaryotic origins of replication have diverse DNA and chromatin structure depending on the biological species 553
There is a defined scenario for formation of initiation complexes 559
Re-replication must be prevented 561
Histone methylation regulates onset of replication licensing 561

20.3 REPLICATION ELONGATION IN EUKARYOTES 561

Eukaryotic replisomes both resemble and significantly differ from those of bacteria 561
Other components of the bacterial replisome have functional counterparts in eukaryotes 564
Eukaryotic elongation has some special dynamic features 565

20.4 REPLICATION OF CHROMATIN 565

Chromatin structure is dynamic during replication 565
Histone chaperones may play multiple roles in replication 566
Both old and newly synthesized histones are required in replication 567
Epigenetic information in chromatin must also be replicated 568

20.5 THE DNA END-REPLICATION PROBLEM AND ITS RESOLUTION 570

Telomerase solves the end-replication problem 570
Alternative lengthening of telomeres pathway is active in telomerase-deficient cells 572

20.6 MITOCHONDRIAL DNA REPLICATION 573

Are circular mitochondrial genomes myth or reality? 574
Models of mitochondrial genome replication are contentious 574

20.7 REPLICATION IN VIRUSES THAT INFECT EUKARYOTES 575

Retroviruses use reverse transcriptase to copy RNA into DNA 575
Key concepts 578
Further reading 579
Videos on the Internet 580

Chapter 21: DNA Recombination 581

21.1 INTRODUCTION 582

21.2 HOMOLOGOUS RECOMBINATION 582

Homologous recombination plays a number of roles in bacteria 583
Homologous recombination has multiple roles in mitotic cells 584
Meiotic exchange is essential to eukaryotic evolution 584

21.3 HOMOLOGOUS RECOMBINATION IN BACTERIA 584

End resection requires the RecBCD complex 585
Strand invasion and strand exchange both depend on RecA 586
Much concerning homologous recombination is still not understood 587
Holliday junctions are the essential intermediary structures in HR 589

21.4 HOMOLOGOUS RECOMBINATION IN EUKARYOTES 590

Proteins involved in eukaryotic recombination resemble their bacterial counterparts 590
HR malfunction is connected with many human diseases 591
Meiotic recombination allows exchange of genetic information between homologous chromosomes in meiosis 593

21.5 NONHOMOLOGOUS RECOMBINATION 596

Transposable elements or transposons are mobile DNA sequences that change positions in the genome 596
Many transposons are transcribed but only a few have known functions 596
There are several types of transposons 598
DNA class II transposons can use either of two mechanisms to transpose themselves 601
Retrotransposons, or class I transposons, require an RNA intermediate 602

21.6 SITE-SPECIFIC RECOMBINATION 602

Bacteriophage λ integrates into the bacterial genome by site-specific recombination 603
Immunoglobulin gene rearrangements also occur through site-specific recombination 603
Key concepts 616
Further reading 616
Videos on the Internet 617

Chapter 22: DNA Repair 619

22.1 INTRODUCTION 620

22.2 TYPES OF LESIONS IN DNA 622

Natural agents, from both within and outside a cell, can change the information content of DNA 622

22.3 PATHWAYS AND MECHANISMS OF DNA REPAIR 624

DNA lesions are countered by a number of mechanisms of repair 624
Thymine dimers are directly repaired by DNA photolyase 626
The enzyme O^6-alkylguanine alkyltransferase is involved in the repair of alkylated bases 628
Nucleotide excision repair is active on helix-distorting lesions 628
The role of TFIIH in NER 629
Base excision repair corrects damaged bases 630
Mismatch repair corrects errors in base pairing 630
Methyl-directed mismatch repair in bacteria uses methylation on adenines as a guide 631
Mismatch repair pathways in eukaryotes may be directed by strand breaks during DNA replication 632
Repair of double-strand breaks can be error-free or error-prone 633
Homologous recombination repairs double-strand breaks faithfully 633
Nonhomologous end-joining restores the continuity of the DNA double helix in an error-prone process 634

22.4 TRANSLESION SYNTHESIS 635

Many repair pathways utilize RecQ helicases 637

22.5 CHROMATIN AS AN ACTIVE PLAYER IN DNA REPAIR **637**

Histone variants and their post-translational modifications are specifically involved in DNA repair 638

22.6 SUMMARY OF DNA REPAIR **644**

22.7 OVERVIEW: THE ROLE OF DNA REPAIR IN LIFE **645**

Key concepts 652

Further reading 653

Videos on the Internet 654

Glossary **655**

Index **695**