

Contents

PART I Fundamentals and Foundations 1

1 Introduction to Cells and Cell Research 3

1.1 The Origin and Evolution of Cells 4

- How did the first cell arise? 4
- The evolution of metabolism 7
- Prokaryotes 8
- Eukaryotic cells 9
- The origin of eukaryotes 11
- The development of multicellular organisms 13

1.2 Experimental Models in Cell Biology 17

- E. coli* 18
- Yeasts 18
- Caenorhabditis elegans* and *Drosophila melanogaster* 19
- Arabidopsis thaliana* 20
- Vertebrates 21
- Animal cell culture 22
- Key Experiment** HeLa Cells: The First Human Cell Line 24

Viruses 25

Molecular Medicine Viruses and Cancer 26

1.3 Tools of Cell Biology: Microscopy and Subcellular Fractionation 27

- Light microscopy 27
- Fluorescence microscopy and GFP 30
- Following protein movements and interactions 31
- Sharpening the focus and seeing cells in three dimensions 32
- Super-resolution microscopy: breaking the diffraction barrier 33
- Electron microscopy 35
- Subcellular fractionation 36
- DATA ANALYSIS PROBLEM** 40

2 Molecules and Membranes 43

2.1 The Molecules of Cells 43

- Chemical bonds 44
- Carbohydrates 47
- Lipids 49
- Nucleic acids 51
- Proteins 53
- Key Experiment** The Folding of Polypeptide Chains 56

2.2 Enzymes as Biological Catalysts 60

- The catalytic activity of enzymes 61
- Mechanisms of enzymatic catalysis 62
- Coenzymes 64
- Regulation of enzyme activity 66

2.3 Cell Membranes 67

Membrane lipids 68

Membrane proteins 69

Key Experiment The Structure of Cell Membranes 71

Transport across cell membranes 72

DATA ANALYSIS PROBLEM 74**3 Bioenergetics and Metabolism 77****3.1 Metabolic Energy and ATP 77**

The laws of thermodynamics 77

The role of ATP 79

3.2 Glycolysis and Oxidative Phosphorylation 81

Glycolysis 81

The citric acid cycle 83

The derivation of energy from lipids 84

Electron transport and oxidative phosphorylation 86

Chemiosmotic coupling 88

Key Experiment The Chemiosmotic Theory 89

ATP synthesis 95

Synthesis of glucose 96

3.4 The Biosynthesis of Cell Constituents 97

Carbohydrates 97

Lipids 98

Proteins 99

Nucleic acids 101

Key Experiment Antimetabolites, Cancer, and
AIDS 102**DATA ANALYSIS PROBLEM 104****3.3 Photosynthesis 92**

Electron transport 92

4 Fundamentals of Molecular Biology 107**4.1 Heredity, Genes, and DNA 107**

Genes and chromosomes 108

Identification of DNA as the genetic material 111

The structure of DNA 111

Replication of DNA 113

4.2 Expression of Genetic Information 115

The role of messenger RNA 116

The genetic code 116

RNA viruses and reverse transcription 119

Key Experiment The DNA Provirus Hypothesis 120**4.3 Recombinant DNA 121**

Restriction endonucleases 122

Generation of recombinant DNA molecules 124

DNA sequencing 127

Expression of cloned genes 127

4.4 Detection of Nucleic Acids and Proteins 129Amplification of DNA by the polymerase
chain reaction 129

Nucleic acid hybridization 131

Antibodies as probes for proteins 132

4.5 Gene Function in Eukaryotes 135

Gene transfer in plants and animals 136

Mutagenesis of cloned DNAs 139

Introducing mutations into cellular genes 139

Genome engineering by the CRISPR/Cas system 141

Targeting mRNA 141

Key Experiment RNA Interference 143**DATA ANALYSIS PROBLEM 145**

5 Genomics, Proteomics, and Systems Biology 149

5.1 Genomes and Transcriptomes 149

- The genomes of bacteria and yeast 150
- The genomes of *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Arabidopsis thaliana* 151
- The human genome 152
- The genomes of other vertebrates 153
- Key Experiment** The Human Genome 155
- Next-generation sequencing and personal genomes 156
- Global analysis of gene expression 158

5.2 Proteomics 160

- Identification of cell proteins 160
- Global analysis of protein localization 161
- Protein interactions 162

5.3 Systems Biology 165

- Systematic screens of gene function 166
- Regulation of gene expression 167
- Networks 168
- Synthetic biology 170
- Molecular Medicine** Malaria and Synthetic Biology 172

DATA ANALYSIS PROBLEM 174

Part II The Flow of Genetic Information 177

6 Genes and Genomes 179

6.1 The Structure of Eukaryotic Genes 179

- Introns and exons 181
- Key Experiment** The Discovery of Introns 183
- Roles of introns 185

6.2 Noncoding Sequences 187

- Noncoding RNAs 187
- Key Experiment** The ENCODE Project 188
- Repetitive sequences 190
- Gene duplication and pseudogenes 193

6.3 Chromosomes and Chromatin 196

- Chromatin 196
- Centromeres 199
- Telomeres 203

DATA ANALYSIS PROBLEM 204

7 Replication, Maintenance, and Rearrangements of Genomic DNA 207

7.1 DNA Replication 207

- DNA polymerases 208
- The replication fork 208
- The fidelity of replication 216

- Origins and the initiation of replication 217
- Telomeres and telomerase: Maintaining the ends of chromosomes 220

- Key Experiment** Telomerase Is a Reverse Transcriptase 221

7.2 DNA Repair 223

Direct reversal of DNA damage 224

Excision repair 225

Molecular Medicine Colon Cancer and DNA Repair 229

Translesion DNA synthesis 230

Repair of double-strand breaks 231

7.3 DNA Rearrangements and Gene Amplification 233

Antibody genes 233

Gene amplification 239

DATA ANALYSIS PROBLEM 240

8 RNA Synthesis and Processing 243

8.1 Transcription in Bacteria 243

RNA polymerase 244

Bacterial promoters 244

Elongation and termination 245

8.2 Eukaryotic RNA Polymerases and General Transcription Factors 248

Eukaryotic RNA polymerases 248

General transcription factors and initiation of transcription by RNA polymerase II 249

Transcription by RNA polymerases I and III 252

8.3 RNA Processing and Turnover 254

Processing of ribosomal and transfer RNAs 255

Processing of mRNA in eukaryotes 258

Splicing mechanisms 260

Key Experiment The Discovery of snRNPs 263

Alternative splicing 265

Molecular Medicine Splicing Therapy for Duchenne Muscular Dystrophy 267

RNA editing 268

RNA degradation 269

DATA ANALYSIS PROBLEM 271

9 Transcriptional Regulation and Epigenetics 273

9.1 Gene Regulation in *E. coli* 273

The *lac* repressor 273

Positive control of transcription 275

9.2 Transcription Factors in Eukaryotes 276

cis-acting regulatory sequences: promoters and enhancers 276

Transcription factor binding sites 280

Transcriptional regulatory proteins 281

Key Experiment Isolation of a Eukaryotic Transcription Factor 284

Regulation of elongation 285

9.3 Chromatin and Epigenetics 288

Histone modifications 288

Key Experiment The Role of Histone Modification 290

Chromatin remodeling factors 293

Histones and epigenetic inheritance 294

DNA methylation 295

Noncoding RNAs 297

DATA ANALYSIS PROBLEM 298

10 Protein Synthesis, Processing, and Regulation 303

10.1 Translation of mRNA 303

- Transfer RNAs 304
- The ribosome 306
- The organization of mRNAs and the initiation of translation 308
- The process of translation 310
- Regulation of translation 314

10.2 Protein Folding and Processing 318

- Chaperones and protein folding 319
- Protein misfolding diseases 321
- Enzymes that catalyze protein folding 323
- Molecular Medicine** Alzheimer's Disease 324

- Protein cleavage 325
- Attachment of carbohydrates and lipids 326

10.3 Regulation of Protein Function and Stability 328

- Regulation by small molecules 328
- Protein phosphorylation and other modifications 329
- Key Experiment** The Discovery of Tyrosine Kinases 332
- Protein-protein interactions 333
- Protein degradation 333
- DATA ANALYSIS PROBLEM** 336

Part III Cell Structure and Function 339

11 The Nucleus 341

11.1 The Nuclear Envelope and Traffic between the Nucleus and the Cytoplasm 341

- Structure of the nuclear envelope 342
- The nuclear pore complex 344
- Molecular Medicine** Nuclear Lamina Diseases 345
- Selective transport of proteins to and from the nucleus 348
- Key Experiment** Identification of Nuclear Localization Signals 349
- Transport of RNAs 352
- Regulation of nuclear protein import 353

11.2 The Organization of Chromatin 354

- Chromosome territories 355
- Chromatin localization and transcriptional activity 356
- Replication and transcription factories 358

11.3 Nuclear Bodies 360

- The nucleolus and rRNA 361
- Polycomb bodies: Centers of transcriptional repression 363
- Cajal bodies and speckles: Processing and storage of snRNPs 363
- DATA ANALYSIS PROBLEM** 364

12 Protein Sorting and Transport *The Endoplasmic Reticulum, Golgi Apparatus, and Lysosomes* **369**

12.1 The Endoplasmic Reticulum 369

The endoplasmic reticulum and protein secretion 370

Targeting proteins to the endoplasmic reticulum 371

Key Experiment The Signal Hypothesis 373

Insertion of proteins into the ER membrane 376

Protein folding and processing in the ER 381

Quality control in the ER 384

The smooth ER and lipid synthesis 386

Export of proteins and lipids from the ER 388

12.2 The Golgi Apparatus 389

Organization of the Golgi 390

Protein glycosylation within the Golgi 390

Lipid and polysaccharide metabolism in the Golgi 392

Protein sorting and export from the Golgi apparatus 394

12.3 The Mechanism of Vesicular Transport 397

Cargo selection, coat proteins, and vesicle budding 397

Vesicle fusion 399

12.4 Lysosomes 401

Lysosomal acid hydrolases 401

Molecular Medicine Gaucher Disease 402

Endocytosis and lysosome formation 403

Autophagy 405

DATA ANALYSIS PROBLEM 406

13 Mitochondria, Chloroplasts, and Peroxisomes 409

13.1 Mitochondria 409

Organization and function of mitochondria 410

The genetic system of mitochondria 412

Protein import and mitochondrial assembly 414

Molecular Medicine Mitochondrial Replacement Therapy 414

Mitochondrial lipids 418

Transport of metabolites across the inner membrane 419

13.2 Chloroplasts and Other Plastids 420

The structure and function of chloroplasts 421

The chloroplast genome 423

Import and sorting of chloroplast proteins 423

Other plastids 426

13.3 Peroxisomes 427

Functions of peroxisomes 428

Peroxisome assembly 429

Molecular Medicine Peroxisome Biogenesis Disorders 430

DATA ANALYSIS PROBLEM 433

14 The Cytoskeleton and Cell Movement 437

14.1 Structure and Organization of Actin Filaments 437

Assembly and organization of actin filaments 438

Association of actin filaments with the plasma membrane 442

Microvilli 445

Cell surface protrusions and cell movement 446

14.2 Myosin Motors 449

Muscle contraction 449

Contractile assemblies of actin and myosin in nonmuscle cells 453

Unconventional myosins 454

14.3 Microtubules 456

Structure and dynamic organization of microtubules 456

Assembly of microtubules 459

MAPs and the organization of microtubules 461

14.4 Microtubule Motors and Movement 462

Microtubule motor proteins 463

Key Experiment The Isolation of Kinesin 464

Cargo transport and intracellular organization 466

Cilia and flagella 468

Microtubules during mitosis 470

14.5 Intermediate Filaments 473

Intermediate filament proteins 473

Assembly of intermediate filaments 474

Intracellular organization of intermediate filaments 475

Key Experiment Function of Intermediate Filaments 477**DATA ANALYSIS PROBLEM 479****15 The Plasma Membrane 483****15.1 Structure of the Plasma Membrane 483**

The lipid bilayer 483

Plasma membrane proteins 487

Plasma membrane domains 492

15.2 Transport of Small Molecules 495

Facilitated diffusion and carrier proteins 495

Ion channels 497

Active transport driven by ATP hydrolysis 503

Molecular Medicine Cystic Fibrosis 506

Active transport driven by ion gradients 507

15.3 Endocytosis 508

Phagocytosis 508

Clathrin-mediated endocytosis 510

Key Experiment The LDL Receptor 512

Transport to lysosomes and receptor recycling 514

DATA ANALYSIS PROBLEM 515**16 Cell Walls, the Extracellular Matrix, and Cell Interactions 519****16.1 Cell Walls 519**

Bacterial cell walls 519

Eukaryotic cell walls 521

16.2 The Extracellular Matrix and Cell–Matrix Interactions 524

Matrix structural proteins 524

Matrix polysaccharides 526

Adhesion proteins 527

Cell–matrix interactions 528

Key Experiment The Characterization of Integrin 530**16.3 Cell–Cell Interactions 532**

Adhesion junctions 532

Tight junctions 535

Gap junctions 536

Plasmodesmata 536

Molecular Medicine Gap Junction Diseases 537**DATA ANALYSIS PROBLEM 539**

Part IV Cell Regulation 541

17 Cell Signaling 543

17.1 Signaling Molecules and Their Receptors 543

Modes of cell–cell signaling 544

Steroid hormones and the nuclear receptor superfamily 545

Signaling by other small molecules 547

Peptide hormones and growth factors 548

17.2 G Proteins and Cyclic AMP 550

G proteins and G protein-coupled receptors 550

Key Experiment G Protein-Coupled Receptors and Odor Detection 551

The cAMP pathway: Second messengers and protein phosphorylation 554

17.3 Tyrosine Kinases and Signaling by the MAP Kinase and PI 3-Kinase Pathways 557

Receptor tyrosine kinases 557

Nonreceptor tyrosine kinases 559

MAP kinase pathways 561

Molecular Medicine Cancer: Signal Transduction and the *ras* Oncogenes 563

The PI 3-kinase/Akt and mTOR pathways 566

17.4 Receptors Coupled to Transcription Factors 570

The TGF- β /Smad pathway 570

NF- κ B signaling 571

The Wnt and Notch pathways 571

17.5 Signaling Dynamics and Networks 573

Feedback loops and signaling dynamics 573

Networks and crosstalk 574

DATA ANALYSIS PROBLEM 575

18 The Cell Cycle 579

18.1 The Eukaryotic Cell Cycle 579

Phases of the cell cycle 580

Regulation of the cell cycle by cell growth and extracellular signals 582

Cell cycle checkpoints 584

18.2 Regulators of Cell Cycle Progression 585

Protein kinases and cell cycle regulation 586

Key Experiment The Discovery of MPF 587

Key Experiment The Identification of Cyclin 589

Families of cyclins and cyclin-dependent kinases 591

Growth factors and the regulation of G₁ Cdk's 593

S phase and regulation of DNA replication 594

DNA damage checkpoints 596

18.3 The Events of M Phase 598

Stages of mitosis 598

Entry into mitosis 601

The spindle assembly checkpoint and progression to anaphase 604

Cytokinesis 606

DATA ANALYSIS PROBLEM 607

19 Cell Renewal and Cell Death 611

19.1 Stem Cells and the Maintenance of Adult Tissues 611

- Proliferation of differentiated cells 612
- Stem cells 614
- Medical applications of adult stem cells 619

19.2 Pluripotent Stem Cells, Cellular Reprogramming, and Regenerative Medicine 621

- Embryonic stem cells 621
- Key Experiment** Culture of Embryonic Stem Cells 623
- Somatic cell nuclear transfer 624
- Induced pluripotent stem cells 626

Transdifferentiation of somatic cells 628

19.3 Programmed Cell Death 628

- The events of apoptosis 629
- Caspases: The executioners of apoptosis 631
- Key Experiment** Identification of Genes Required for Programmed Cell Death 632
- Central regulators of apoptosis: The Bcl-2 family 634
- Signaling pathways that regulate apoptosis 635
- Alternative pathways of programmed cell death 638

DATA ANALYSIS PROBLEM 640

20 Cancer 643

20.1 The Development and Causes of Cancer 643

- Types of cancer 644
- The development of cancer 645
- Properties of cancer cells 646
- Causes of cancer 649

20.2 Oncogenes 651

- Retroviral oncogenes 651
- Proto-oncogenes 652
- Oncogenes in human cancer 654
- Key Experiment** The Discovery of Proto-Oncogenes 655
- Functions of oncogene products 658

20.3 Tumor Suppressor Genes 664

- Identification of tumor suppressor genes 664
- Functions of tumor suppressor gene products 667
- Cancer genomics 671

20.4 Molecular Approaches to Cancer Treatment 672

- Prevention and early detection 673
- Oncogene-targeted drugs 674
- Molecular Medicine** Imatinib: Cancer Treatment Targeted against the *bcr/abl* Oncogene 677
- Immunotherapy 678

DATA ANALYSIS PROBLEM 679

Answers to Questions 683

Glossary 697

Illustration Credits 715

Index 717