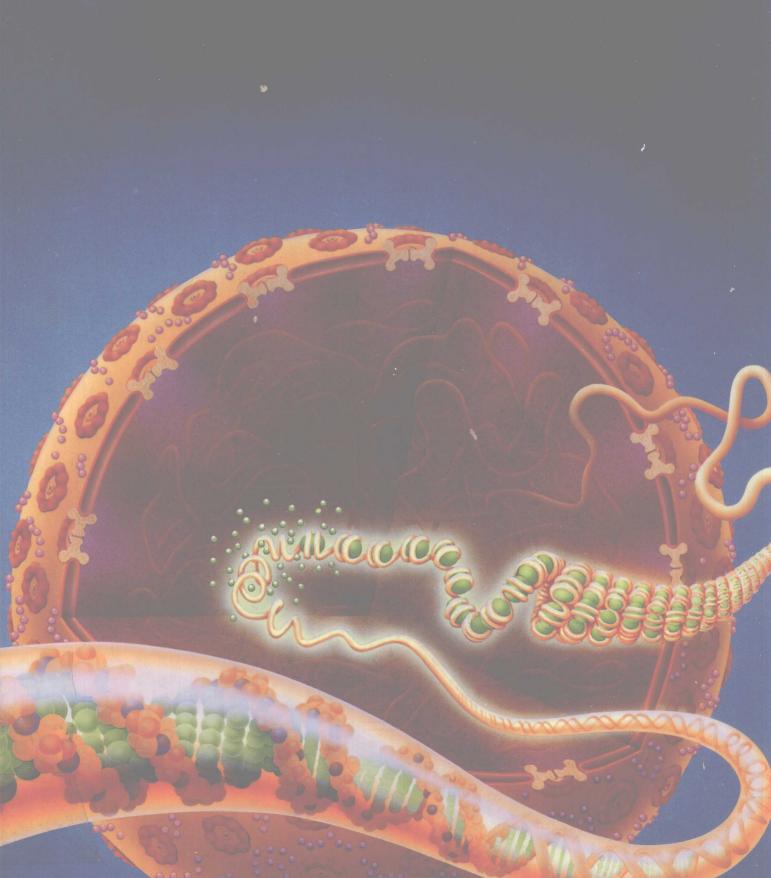
MOLECULAR AND CELLULAR BIOLOGY Stephen L.Wolfe



MOLECULAR AND CELLULAR BIOLOGY

STEPHEN L. WOLFE

University of California at Davis

Wadsworth Publishing Company
Belmont, California
A Division of Wadsworth, Inc.

To my son, Christopher

BIOLOGY PUBLISHER: Jack C. Carey EDITORIAL ASSISTANTS: Kathryn Shea, Laura Jersild DEVELOPMENTAL EDITOR: Mary Arbogast PRODUCTION AND ART SUPERVISION: Hal Lockwood, Christi Payne Fryday/Bookman Productions PRODUCTION COORDINATOR: Harold Humphrey PRINT BUYER: Karen Hunt PERMISSIONS EDITOR: Robert Kauser COPY EDITOR: Elizabeth F. Gehman PAGE MAKE-UP: Judith Levinson TECHNICAL ILLUSTRATORS: Darwen and Vally Hennings, John and Judy Waller, Illustrious, Inc., Academy ArtWorks, Inc., Precision Graphics CHAPTER-OPENING ILLUSTRATORS: Chapters 1, 4, 5, 7, 9, 10, 12, 13, 14, 18, 20, 21, 24, 25, and 27 designed and rendered by Darwen and Vally Hennings; Chapters 6, 8, 11, 19, 22, and 26 designed by Darwen Hennings and rendered by Teresa Snyder; Chapters 2, 3, 15, 16, 17, and 23 designed and rendered by John and Judy Waller COMPOSITOR: Syntax International COLOR SEPARATOR: H&S Graphics, Inc. PRINTER: R. R. Donnelley and Sons Company/Willard COVER DESIGN: Stephen Rapley COVER ILLUSTRATION: Tomo Narashima, based on a design by Darwen and Vally Hennings

© 1993 by Wadsworth, Inc. All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transcribed, in any form or by any means, without the prior written permission of the publisher, Wadsworth Publishing Company, Belmont, California 94002.

45678910 969594

Library of Congress Cataloging-in-Publication Data

Wolfe, Stephen L.

Molecular and cellular biology / Stephen L. Wolfe. p. cm.
Includes bibliographical references and index

ISBN 0-534-12408-9

1. Cytology. 2. Molecular biology. I. Title. QH581.2.W646 1993 92–38636

BOOKS IN THE WADSWORTH BIOLOGY SERIES

Environmental Science, 3rd, Miller

Resource Conservation and Management, Miller

Biology: Concepts and Applications, Starr

Biology: The Unity and Diversity of Life, 6th, Starr and Taggart

Biology of the Cell, 2nd, Wolfe

Cell Ultrastructure, Wolfe

Dimensions of Cancer, Kupchella

Evolution: Process and Product, 3rd, Dodson and Dodson

Introduction to Cell Biology, Wolfe

Oceanography: An Introduction, 4th, Ingmanson and Wallace

Plant Physiology, 4th, Devlin and Witham

Exercises in Plant Physiology, Witham et al.

Plant Physiology, 4th, Salisbury and Ross

Plant Physiology Laboratory Manual, Ross

Plants: An Evolutionary Survey, 2nd, Scagel et al.

Psychobiology: The Neuron and Behavior, Hoyenga and Hoyenga

Sex, Evolution, and Behavior, 2nd, Daly and Wilson

PREFACE

The aim of this book is to integrate molecular biology, biochemistry, and cell biology into a unified course of study. Until now, molecular biology texts have concentrated on gene structure and activity to the near exclusion of more "traditional" biochemistry and cell biology. This reflects a past tendency to throw out the baby with the bath water-to regard many of the findings and conclusions developed by traditional biochemical and cell biological investigations as unreliable and uninteresting because the research was conducted before the molecular revolution. However, the emphasis in molecular biology has now shifted from a concentration on genes for their own sake to the application of molecular genetic studies to all areas of cell biology and biochemistry. As a result, more traditional research areas—ion transport and the control of cell division come to mind as two examples—have become the subjects of highly productive investigations in which the powerful methods of molecular biology are producing many new findings and pushing these areas to the forefront of research.

With these developments in mind, this book offers a balanced view of contemporary molecular biology, biochemistry, and cell biology not currently available in other texts. The central topics of molecular biology are included, among them DNA structure, messenger RNA gene structure and activity, and the molecular methods for studying these genes. However, the molecular biology of genes encoding the other major RNA types is also emphasized, along with their regulation and the biochemistry of their transcription. These topics are integrated with the cell biology of the nucleus, including the structure of nucleosomes, chromatin, the nucleolus, and the nuclear envelope; the changes occurring in nucleosomes and chromatin during the shift between inactivity and gene transcription; and the role of the nuclear envelope in transport between the nucleus and cytoplasm. The organization of genes into genomes and the techniques of genetic engineering are also included in this integration. Similarly, the coverage of cell division includes an integrated view of the molecular biology and biochemistry of cell cycle genes and their regulation, DNA replication and repair, and the changes in nucleosome and chromatin structure that accompany cell division. The molecules, forces, and structures separating the chromosomes during mitotic cell division are also considered, as well as the molecular biology, biochemistry, and cell biology of genetic recombination during meiosis, the formation of gametes, and their functions in fertilization.

Among the more traditional topics of cell biology and biochemistry that are integrated with molecular biology and emphasized in this text are protein synthesis and the modification and distribution of newly synthesized proteins by the endoplasmic reticulum and Golgi complex; membrane structure and transport, and the role of ion transport in the generation and conduction of nerve impulses; the activities of mitochondria and chloroplasts in cellular metabolism, and the mechanisms governing genetic inheritance in these organelles; the roles of microtubules and microfilaments in the generation of motility and, with intermediate filaments, in cytoskeletal support; and the structure and function of the cell surface and extracellular matrix, along with the cellular regulatory mechanisms linked to receptors at the cell surface.

These topics are distributed between the chapters of text, which cover subjects that are central to most or all courses, and chapter Supplements, which add important specialized or peripheral information. This distribution keeps the chapters as direct and to the point as possible. Also included in the chapters are Information Boxes, presenting short but essential items of background information. By selecting among the material presented in the chapters and Supplements, an instructor can tailor the text to suit the aims of the course and its students.

To make the book as clear and graphic as possible, every topic that merits explanation by pictures as well as words is illustrated by a diagram or micrograph. The many light and electron micrographs, in particular, emphasize that molecular and biochemical processes have a structural basis and help relate these processes to cell biology. In addition, each chapter opens with a major piece of art that focuses interest and illustrates a central topic explained in that chapter.

Where possible, molecular and cellular biology and biochemistry are brought home to students by integrating examples from human biology, especially medicine. These examples show that the topics described in the book, as well as having scientific and academic importance, touch directly on human affairs. The regulation of cell division, for example, is illus-

trated by a description of the cell cycle controls that go awry in the transformation of normal cells into cancer cells; the organization of DNA sequences in genomes is elaborated by a discussion of genetic engineering and the genetic rearrangements underlying the production of antibodies, the immune response, and the rejection of transplanted tissues.

The findings, conclusions, and principles described in the text are presented in terms of experimental evidence drawn from work with prokaryotes, fungi, animals, and plants. Controversies as well as conclusions are presented in order to show that the body of scientific information in molecular and cellular biology is not fixed, and that many significant questions remain to be answered. This experimental foundation is bolstered by essays contributed by original investigators, presenting their experiences in the conception and execution of classic and contemporary experiments that have produced key contributions in molecular biology, biochemistry, and cell biology. The essays add a personal element to the information presented in the book and emphasize that this information is the result of experimental work by individual scientists. The essayists include both established researchers, who are essentially household names among biologists, and relative newcomers, who are likely to be among the next generation of famous names. Hopefully, in addition to piquing students' interest, the essays may help turn them toward the possibility of research as a career.

The spate of multiple-author textbooks in molecular and cellular biology has led to a belief that the infor-

mation in this field has become so extensive that it lies beyond the reach of a single author. I hope this book will make it apparent that the opposite is true, and that coverage by a single author brings a degree of organization, integration, and unity that lies beyond even the most carefully prepared multiple-author text.

Although this is a single-author book, I must acknowledge my debt to the many people who have contributed to it. The book could not have been written without the help of colleagues and friends who reviewed the text and offered suggestions for improving its accuracy and content. I am also deeply indebted to the many investigators who generously supplied micrographs, diagrams, and tables. I must also acknowledge my debt to my biology editor, Jack Carey, who provided endless encouragement and guidance in the preparation of the text; to my developmental editor, Mary Arbogast, who provided expert editorial help and unscrambled more glitches than I care to remember; and to Hal Humphrey, Hal Lockwood, and Christi Fryday, who coordinated production at Wadsworth and Bookman Productions, and solved the innumerable problems attending publication of a book of this size and complexity. I am also indebted to Darwen and Vally Hennings, and John and Judy Waller, who prepared original art and redrew many of the diagrams sent by other authors. Darwen Hennings in particular was instrumental in the design and preparation of the more complex illustrations in the book, including the majority of drawings that serve as chapter openers.

REVIEWERS

DAVID ASAL Purdue University

AIMEE BAKKEN, University of Washington

STEPHEN C. BENSON, California State University, Hayward COLIN F. BLAKE, Oxford University DOUGLAS C. BRAATEN, Washington University B. R. BRINKLEY, University of Alabama IAIN L. CARTWRIGHT, University of Cincinnati G. P. CHAPMAN, University of London SUSAN CONRAD, Michigan State University DAVID T. DENHARDT, University of Western Ontario WILLIAM DENTLER, University of Kansas WILLIAM DUNPHY, University of California, San Diego DOUGLAS EASTON, State University College of Buffalo D. J. FINNEGAN, University of Edinburgh CHRISTINE H. FOYER. The University of Sheffield CARL FRANKEL, Pennsylvania State University MICHAEL FREELING, University of California, Berkeley DAVID FROMSON, California State University, Fullerton LEAH T. HAIMO, University of California, Riverside ELIZABETH D. HAY, Harvard University IOHN HERSHEY, University of California, Davis PETER HEYWOOD, Brown University RICKY R. HIRSCHHORN, University of Kentucky J. KENNETH HOOBER, Temple University YASUO HOTTA, Nagoya University LON KAUFMAN, University of Illinois CHARLES C. LAMBERT, California State University, Fullerton ELIAS LAZARIDES, California Institute of Technology MARY LEE LEDBETTER, College of the Holy Cross JOHN LYE, University of Colorado THOMAS LYNCH, University of Arkansas, Little Rock ROBERT MACDONALD, Northwestern University S. K. MALHOTRA, University of Alberta PETER B. MOENS, York University STEPHEN M. MOUNT, Columbia University DOMINIC POCCIA, Amherst College P. ELAINE ROBERTS, Colorado State University THOMAS ROBERTS, Florida State University ENRIQUE RODRIGUEZ-BOULAN, Cornell University JOHN RUFFOLO, Indiana State University ROBERT SAVAGE, Swarthmore College DAVID SAXON, Morehead State University R. L. SEALE, Scripps Clinic Research Foundation BARBARA SEARS, Michigan State University JOEL SHEFFIELD, Temple University SHELDON S. SHEN, Iowa State University ROGER SLOBODA, Dartmouth College GARY STEIN, University of Florida HERBERT STERN, University of California, San Diego ALBERT P. TORZILLI, George Mason University FRED WARNER, Syracuse University RUTH WELTI, Kansas State University LARRY WILLIAMS, Kansas State University JOHN G. WISE, University of California, Los Angeles WILLIAM L. WISSINGER, St. Bonaventure University DEBRA J. WOLGEMUTH, Columbia University D. L. WORCESTER, University of Missouri CHARLES F. YOCUM, University of Michigan, Ann Arbor

THE EXPERIMENTAL PROCESS ESSAYISTS

- KEVAN M. SHOKAT and PETER G. SCHULTZ, Catalytic Antibodies: Tailor-Made Catalysts (Ch. 3)
- KARY B. MULLIS, The Unlikely Origin of the Polymerase Chain Reaction (Ch. 4)
- S. J. SINGER, How Do Ions Get Across Membranes? (Ch. 5)
- HALI A. HARTMANN and ARTHUR M. BROWN, Identification of the Amino Acids Forming the Conduction Pathway in Potassium Channels (Ch. 6)
- LOWELL E. HOKIN, The Road to the Phosphoinositide-Generated Second Messengers (Ch. 7)
- Peter Goodwin, Cell/Cell Communication in Plants: An Open and Shut Case (Ch. 8)
- RICHARD E. MCCARTY, Divide and Conquer: Deciphering the Functions of a Polypeptide of the Chloroplast ATP Synthase (Ch. 9)
- MARSHALL D. HATCH, The C_4 Pathway: A Surprise Option for Photosynthetic Carbon Assimilation (Ch. 10)
- MICHAEL P. SHEETZ, Identification of Microtubule Motors Involved in Axonal Transport (Ch. 11)
- YOSHIE HARADA and TOSHIO YANAGIDA, Do the Two Heads of the Myosin Molecule Function Independently or Cooperatively? (Ch. 12)
- ELAINE V. FUCHS, Of Mice and Men: Genetic Skin Diseases Arising from Defects in Keratin Filaments (Ch. 13)
- ALEXANDER RICH, The Discovery of Left-Handed ZDNA (Ch. 14)
- Myeong-Sok Lee and William T. Garrard, What Happens When RNA Polymerase Encounters a Nucleosome (Ch. 15)
- ALINA C. LOPO, When Sperm Meets Egg: Initiation Factors and Translational Control at Fertilization (Ch. 16)
- GEORGE C. PRENDERGAST, Sequential Clues to the DNA Binding Specificity of Myc (Ch. 17)
- ROY J. BRITTEN, Repeated DNA Sequences and Mobile Elements (Ch. 18)
- GORDON ADA, Instruction or Selection: The Role of Antigen in Antibody Production (Ch. 19)
- VISHWANATH R. LINGAPPA, Redirecting Protein Traffic from Cytoplasm to the Secretory Pathway: A Test of the Signal Hypothesis (Ch. 20)
- Peter E. Thorsness, Escape of DNA from Mitochondria to the Nucleus Dunng Evolution and in Real Time (Ch. 21)
- TIM HUNT, Finding Cyclins and After (Ch. 22)
- CAROL W. GREIDER, Telomerase: The Search for a Hypothetical Enzyme (Ch. 23)
- W. ZACHEUS CANDE, Spindle Elongation in vitro: The Relative Roles of Microtubule Sliding and Tubulin Polymerization (Ch. 24)
- HERBERT STERN, m-rec: An Enzyme That Affects Genetic Recombination in Meiotic Cells (Ch. 25)
- LAURINDA A. JAFFE, The Electrical Polyspermy Block (Ch. 26)
- NOAM LAHAV and SHERWOOD CHANG, How Were Peptides Formed in the Prebiotic Era? (Ch. 27)

BRIEF CONTENTS

1	Introduction to Cell and Molecular Biology 1	14	The Nucleus and Its Molecular Constituents 524
2	Chemical Bonds and Biological Molecules 38	15	RNA Transcription and Processing 573
3	Energy, Enzymes, and Biological Reactions 79	16	The Interaction of mRNA, rRNA, and tRNA in Protein Synthesis 649
4	Major Investigative Methods of Cell and Molecular Biology 107	17	Transcriptional and Translational Regulation 693
5	The Structure of Cellular Membranes 152		Regulation 675
6	The Functions of Membranes in Ionic and Molecular Transport 189	18	Organization of the Genome and Genetic Rearrangements 739
7	The Cell Surface and Intercellular Communication 232	19	Programmed Genetic Rearrangements in the Production of Antibodies 789
8	The Extracellular Matrix of Eukaryotic Cells 273	20	Protein Sorting, Distribution, Secretion, and Endocytosis 824
0		21	Cytoplasmic Genetic Systems 876
9	Energy for Cell Activities: Cellular Oxidations and the Mitochondrion 309	22	The Cell Cycle, Cell Cycle Regulation, and Cancer 909
10	Photosynthesis and the Chloroplast 366	22	DVA D
1	Microtubules and Microtubule-Based	23	DNA Replication and Repair 951
	Cell Motility 416	24	Nuclear and Cytoplasmic Division 999
12	Microfilaments and Microfilament-Based Cell Motility 451	25	Meiosis and Genetic Recombination 1048
		26	Gametogenesis and Fertilization 1090
13	Microtubules, Microfilaments, and Intermediate Filaments in the Cytoskeleton 495	27	The Origins of Cellular Life 1121

DETAILED CONTENTS

CHAPTER 1

INTRODUCTION TO CELL AND MOLECULAR BIOLOGY

Introduction to Cell Structure 2

Prokaryotic Cells 6

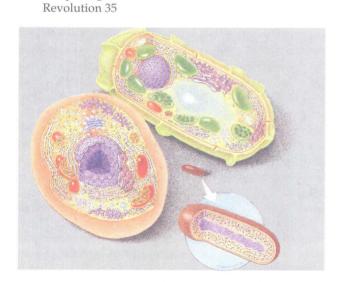
Eukaryotic Cells 8

The Nucleus 10 The Cytoplasm 12 Specialized Cytoplasmic Structures of Plant Cells 19

Viruses 23

Viral Structure 23 Viral Infective Cycles 24 Origin and Significance of Viruses 28 Viroids and Prions 28

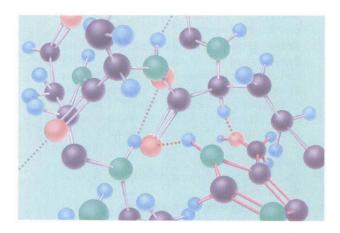
Historical Origins of Cell and Molecular Biology 29 The Discovery of Cells 29 Development of the Cell Theory 30 Early Chemical Investigations in Cell Biology 32 Integration of Chemical and Morphological Studies 32 The Molecular



CHAPTER 2

CHEMICAL BONDS AND BIOLOGICAL MOLECULES

Chemical Bonds Important in Biological Molecules 39
Ionic Bonds 40 Covalent Bonds 40 Unequal
Electron Sharing, Hydrogen Bonds, and Water 42
van der Waals Forces 43



Functional Groups in Biological Molecules 44

The Hydroxyl Group and Alcohols 44 The Carbonyl Group, Aldehydes, and Ketones 45 INFORMATION BOX 2-1: Condensation and Hydrolysis 46

The Carboxyl Group and Organic Acids 46 The Amino Group 46 The Phosphate Group 47 The Sulfhydryl Group 47

Major Classes of Biological Molecules: Carbohydrates 47

Carbohydrate Structure 47
INFORMATION BOX 2-2: Acids, Bases, and pH 48
INFORMATION BOX 2-3: Stereoisomers 51
Linkage of Monosaccharides into Disaccharides and Polysaccharides 51

Lipids 53

Neutral Lipids 53 Phospholipids 55 Steroids 57 Glycolipids 58

Proteins 59

The Amino Acid Subunits of Proteins 59
Primary Structure: The Linkage of Amino Acids to Form Proteins 62 Secondary Structure:
Restrictions on the Movement of Polypeptide Chains 62 Tertiary Structure: The Three-Dimensional Structure of Polypeptide Chains 65 Quaternary Structure: Associations of Multiple Polypeptide Chains 67 Combination of Proteins with Other Substances 68
INFORMATION BOX 2-4: The Coiled Coil 69

Nucleotides and Nucleic Acids 70

The Nucleotides 71 DNA and RNA 72 SUPPLEMENT 2-1: Atomic Structure 77
The Atomic Nucleus 77 The Electrons of an Atom 78

CHAPTER 3

ENERGY, ENZYMES, AND BIOLOGICAL REACTIONS

Chemical and Physical Laws Governing Reactions 80

Spontaneous Reactions and the Laws of Thermodynamics 80 Using the First and Second Laws to Predict the Rate and Direction of Chemical Reactions 81 Reversible Reactions 82 How Living Organisms Push Reversible Reactions Uphill 82 The Standard Free Energy Change 83

The Role of Enzymes in Biological Reactions 85

Enzymes and Enzymatic Catalysis 85 Enzymes and Activation Energy 85 Characteristics of Protein-Based Enzymes 86 Enzyme-Substrate Complexes and the Active Site 88 Catalytic Mechanisms at the Active Site 89 Mechanisms Contributing to the Transition State 91

THE EXPERIMENTAL PROCESS: Catalytic Antibodies: Tailor-Made Catalysts 92

The Active Site, Transition State, and Reversible Reactions 95

Factors Affecting Enzyme Activity 95

The Effects of Substrate Concentration on Enzyme Activity 95 Enzyme Regulation by Allostery and Covalent Modification 98

RNA-Based Catalysts 101

SUPPLEMENT 3-1: A Quantitative Treatment of Enzyme Saturation and Inhibition: The Michaelis—Menten Equation 105

CHAPTER 4

MAJOR INVESTIGATIVE METHODS OF CELL AND MOLECULAR BIOLOGY

Light and Electron Microscopy 108

The Light Microscope and Its Applications 108 Electron Microscopy 112

Preparation of Specimens for Microscopy 117

Specimen Preparation for Light Microscopy 117 Specimen Preparation for Electron Microscopy 119

Cell Culture 121

Culturing Cells from Higher Eukaryotes 121 Cell Fusion 123

Fractionation and Purification of Cell Structures and Molecules 123

Centrifugation 124 Gel Electrophoresis 126 Chromatography 130

Methods for Characterizing Individual Cell Molecules 132

X-Ray Diffraction 132 Obtaining DNA Sequences in Quantity 134

INFORMATION BOX 4-1: DNA Melting and Reannealing 135

THE EXPERIMENTAL PROCESS: The Unlikely Origin of the Polymerase Chain Reaction 140

DNA Sequencing 142 Deducing Protein Sequences from DNA Sequences 145 SUPPLEMENT 4-1: Lenses and Image Formation in the Light Microscope 149

CHAPTER 5

THE STRUCTURE OF CELLULAR MEMBRANES

Lipid and Protein Molecules of Biological Membranes 153

Membrane Lipids 154

INFORMATION BOX 5-1: Phosphoglyceride Structure 156

INFORMATION BOX **5-2:** Sphingolipid Structure 158 Membrane Proteins 159

INFORMATION BOX 5-3: Hopanoids 160

Phospholipid Bilayers and Biological Membranes 163

Physical Characteristics of Artificial and Natural Bilayers 163 Effects of Cholesterol on the Phase Transition 164 Regulation of the Phase Transition in Living Organisms 165

The Fluid Mosaic Model and Its Supporting Evidence 166

Features of the Fluid Mosaic Model 166 Evidence Supporting the Fluid Mosaic Model 169

THE EXPERIMENTAL PROCESS: How Do Ions Get Across Membranes? 174

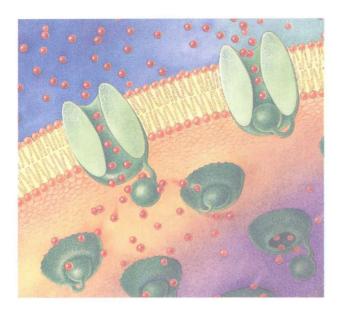


Membrane Biogenesis 179

Lipid Biogenesis and Membrane Asymmetry 179 Origins of Membrane Proteins 180

Historical Developments Leading to the Fluid Mosaic Model 181

The Discovery of Membrane Lipids and Bilayers 181 Membrane Proteins and the Danielli–Davson Model 182 Final Steps Toward the Fluid Mosaic Model 183 SUPPLEMENT 5-1: Membrane Fusion 186



CHAPTER 6

THE FUNCTIONS OF MEMBRANES IN IONIC AND MOLECULAR TRANSPORT

Passive Transport 190

Diffusion as the Basis for Passive Transport 190 The Effects of Semipermeable Membranes on Diffusion 191 Biological Membranes and Passive Transport: Facilitated Diffusion 193 Transporter Types Operating in Facilitated Diffusion 196

THE EXPERIMENTAL PROCESS: Identification of the Amino Acids Forming the Conduction Pathway in Potassium Channels 198

The Passive Transport of Water Through Lipid Bilayers 204

Active Transport 205

Characteristics of Active Transport 205 P-Type Direct Active Transport Pumps 206 V-Type Direct Active Transport Pumps 210 Indirect Active Transport Pumps 211

Active Transport, Membrane Potentials, and Nerve Conduction 212

Membrane Potentials 212 Voltage-Gated Membrane Channels and the Action Potential 213

INFORMATION BOX 6-1: Coordination of Passive and Active Transport in the Maintenance of Cellular Ion Concentrations 214

INFORMATION BOX 6-2: Quantitative Estimations of Membrane Potential: The Nernst Equation 216 INFORMATION BOX 6-3: The Voltage Clamp 217

Movement of Impulses Along an Axon: Propagation of the Action Potential 218 Saltatory Conduction in Myelinated Neurons 219 Ligand-Gated Membrane Channels and the Conduction of Nerve Impulses Between Neurons and Other Cells 220

SUPPLEMENT 6-1: Transport in Bacteria 227

Porins and Transport Through the Outer Membrane of Gram-Negative Bacteria 227 Transport Through the Bacterial Plasma Membrane 228

CHAPTER 7

THE CELL SURFACE AND INTERCELLULAR COMMUNICATION

Molecules of the Cell Surface 233

Membrane Glycolipids and Glycoproteins in Cell Recognition 234

Major Histocompatibility Complex (MHC) Molecules 234 Blood Groups and Other Surface Markers 235

Surface Receptors and Receptor-Response Mechanisms 236

Receptors Binding Peptide Hormones, Growth Factors, and Neurotransmitters 238 Receptors with Integral Protein Kinase Activity 239 Receptors with Separate Protein Kinase Activity 241 Operation of the cAMP and InsP₃/DAG Receptor-Response Pathways 245 THE EXPERIMENTAL PROCESS: The Road to the Phosphoinositide-Generated Second Messengers 248

INFORMATION BOX **7-1:** Calmodulin **251**Integration, Regulation, and Termination of Receptor-Response Activity **252**

Cell Adhesion 253

Characteristics of Cell Adhesion 254 Types of Cell Adhesion Molecules 254

Developmental Interactions of Cell Adhesion Molecules 255

Cell Junctions 256

Adhesive Junctions 256 Tight Junctions 260 Gap Junctions 262

SUPPLEMENT 7-1: Recognition, Reception, and Cell Adhesion in Bacteria 270

Bacterial Recognition and Adhesion 270 Bacterial Receptors Inducing Internal Responses 271

CHAPTER 8

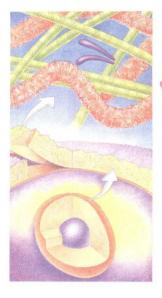
THE EXTRACELLULAR MATRIX OF EUKARYOTIC CELLS

Animal Extracellular Structures 274

The Primary Fiber: Collagens 274 The Network Surrounding Collagen Fibers: Proteoglycans 279 Linkage of the Collagen-Proteoglycan Complex to the Cell Surface 282

Extracellular Structures of Plants: Cell Walls 287

Molecular Constituents of Plant Cell Walls 287 Cell Wall Structure 292 Cell Wall Growth in Higher Plants 293 Plasmodesmata: Communicating Junctions of Plant Cells 295





THE EXPERIMENTAL PROCESS: Cell/Cell Communication in Plants: An Open and Shut Case 296

Cell Wall Structure in Gram-Positive and Gram-Negative Bacteria 302 Molecular Constituents of Prokaryotic Cell Walls 303 The Capsule 308 Cell Wall Structure in Cyanobacteria 308

CHAPTER 9

ENERGY FOR CELL ACTIVITIES: CELLULAR OXIDATIONS AND THE MITOCHONDRION

Pathways Producing ATP: An Overview 310

The Source of Electrons at Elevated Energy Levels 311 The Electron Transport System 311 ATP Synthesis 311

Oxidative Reactions Supplying Electrons for Electron Transport 311

INFORMATION BOX 9-1: Oxidation and Reduction 312

The First Major Stage: Glycolysis 312 INFORMATION BOX 9-2: The Pentose Phosphate Pathway 319

The Second Major Stage: Pyruvate Oxidation and the Citric Acid Cycle 320 The Products of Complete Glucose Oxidation 327 Funneling Fats and Proteins into Oxidative Pathways 327

The Mitochondrial Electron Transport System 327 Carrier Groups and Their Sequence in Electron Transport 328

INFORMATION BOX 9-3: Placing Electron Carriers in Sequence 332

Organization of Prosthetic Groups with Proteins in Electron Transport 333 H⁺ Pumping by the Electron Transport System 335 Discovery of Mitochondrial Electron Transport 337

The Mitochondrial F_oF_1 ATPase and ATP Synthesis 337

Structure of F₀F₁ ATPase 337

THE EXPERIMENTAL PROCESS: Divide and Conquer: Deciphering the Functions of a Polypeptide of the Chloroplast ATP Synthase 338

The Mechanism of ATP Synthesis by F_oF₁ ATPase 343 Evidence That an H⁺ Gradient Drives ATP Synthesis 344 Total ATP Production from Glucose Oxidation 344

Some Details of Mitochondrial Structure and Occurrence 345

Mitochondrial Inclusions 345 Isolation of Mitochondrial Components 345 Occurrence of Mitochondria 347

Mitochondrial Transport 347

INFORMATION BOX 9-4: Mitochondria and Heat Generation 348

Porins and Transport Through the Outer Membrane 349 Transport Carriers of the Inner Membrane 350

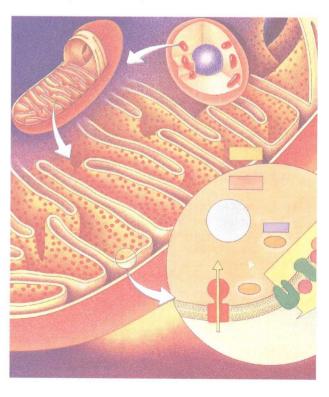
Oxidative Reactions in Microbodies: Peroxisomes and Glyoxisomes 351

Microbodies and Fatty Acid Oxidation 353
Microbodies and the Glyoxylate Cycle:
Glyoxisomes 354 The Glycolate Pathway 355
Other Biochemical Pathways in
Microbodies 355 Microbodies and the Cellular
Economy 356

SUPPLEMENT 9-1: FATTY ACID OXIDATION 360 Bacterial F_oF_1 ATPase 361

SUPPLEMENT 9-2: Oxidative Metabolism in Prokaryotes 362

Electron Transport in Bacteria 362 Oxidations and ATP Synthesis in Cyanobacteria 364



CHAPTER 10

PHOTOSYNTHESIS AND THE CHLOROPLAST

Pathways of Photosynthesis: An Overview 367

The First Major Step: The Light Reactions 367 The Second Major Step: The Dark Reactions 368 Location of the Light and Dark Reactions in Eukaryotes: The Chloroplast 369

The Light Reactions 370

Light and Light Absorption 370 The Structure of Chlorophyll and Other Light-Absorbing Molecules 371 The Organization of Photosynthetic Pigments in Chloroplasts 373 The Electron Transport System Linking the Photosystems 374 Cyclic Electron Flow Within the Z Pathway 379 H⁺ Pumping by the Z Pathway 380 Using the H⁺ Gradient to Synthesize ATP 380 Organization of Light-Reaction Components in Thylakoid Membranes 381 The Light Reactions and the Chemiosmotic Mechanism 382 INFORMATION BOX 10-1: Balancing the Photosystems 384

The Dark Reactions 385

Tracing the Pathways of the Dark Reactions 385 The C_3 Cycle 386

INFORMATION BOX 10-2: The Oxygenase Activity of RuBP Carboxylase and Photorespiration: An Evolutionary Fly in the Photosynthetic Ointment 390

The C₄ Cycle 391

THE EXPERIMENTAL PROCESS: The C_4 Pathway: A Surprise Option for Photosynthetic Carbon Assimilation 392

Some Details of Chloroplast Structure and Occurrence 394

Chloroplast Transport 396

SUPPLEMENT 10-1: The C₄ Cycle and CAM Metabolism 402

The C_4 Cycle 402 The CAM Pathway 404 Supplement 10-2: Plastids and Plastid Development 404

SUPPLEMENT 10-3: Photosynthesis in Prokaryotes 407
The Photosynthetic Bacteria 407

CHAPTER 11

MICROTUBULES AND MICROTUBULE-BASED CELL MOTILITY

Microtubule Structure and Biochemistry 418

Microtubule Structure 418 The Tubulins 419
Polymerization of Tubulins into Microtubules 421
Microtubule Polarity, Dynamic Instability, and
Treadmilling 421 Cellular Factors Regulating
Microtubule Polymerization 425 Cellular Centers
of Microtubule Polymerization: MTOCs 426
Microtubule Poisons 427

The Motile Functions of Microtubules 429

Dynein and Microtubule Sliding 429 Motile Systems Based on Microtubule Sliding: Flagella and Cilia 432 Cytoplasmic Dynein and Kinesin in Fast Axonal Transport 440

THE EXPERIMENTAL PROCESS: Identification of Microtubule Motors Involved in Axonal Transport 442

Motile Systems Based on Microtubule Growth 444 Other Microtubule-Powered Mechanisms 444

SUPPLEMENT 11-1: Bacterial Flagella 447
Structure 447 The Flagellar Motor 448 Flagellar Rotation and Bacterial Swimming Behavior 450



CHAPTER 12

MICROFILAMENTS AND MICROFILAMENT-BASED CELL MOTILITY

Structure and Biochemistry of Microfilaments 452

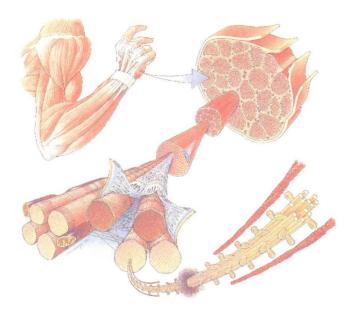
Microfilament Structure 452 Actins 454
Assembly of Actin Molecules into
Microfilaments 455 Microfilament Polarity
and Treadmilling 455 Factors Modifying
Microfilament Polymerization 457 Parallels in
the Biochemistry of Microfilaments and
Microtubules 460

Myosin and Microfilament-Based Motility 460

Myosin Structure 460 Myosin II
Superstructures: Thick Filaments 463
The Myosin Crossbridge Cycle 463
THE EXPERIMENTAL PROCESS: Do the Two Heads of the Myosin Molecule Function Independently or Cooperatively? 464

Regulation of the Crossbridging Cycle 467

Microfilament-Based Motile Systems 471 Striated Muscle Cells 471



INFORMATION BOX 12-1: A Primer of Muscle Cell Terms 474

INFORMATION BOX 12-2: Storage and Supply of ATP in Striated Muscle Cells 478

Smooth Muscle Cells 480 Microfilament-Based Motility in Nonmuscle Cells 482 Movements Generated by Microfilament Growth 486

SUPPLEMENT 12-1: Motility Without Microtubules or Microfilaments 491

Motile Systems Based on Ca²⁺-Sensitive Contractile Proteins 491

CHAPTER 13

MICROTUBULES, MICROFILAMENTS, AND INTERMEDIATE FILAMENTS IN THE CYTOSKELETON

Structure and Biochemistry of Intermediate Filaments 496

Intermediate Filament Structure 497
THE EXPERIMENTAL PROCESS: Of Mice and Men:
Genetic Skin Diseases Arising from Defects in
Keratin Filaments 498

Individual Intermediate Filament Types 502 Intermediate Filament Assembly 504 Intermediate Filament-Associated Proteins (IFAPs) 505 Intermediate Filament Regulation 505

Cytoskeletal Roles of Intermediate Filaments, Microtubules, and Microfilaments 506

Functions of Microtubules, Microfilaments, and Intermediate Filaments 506 Interlinkers and Integration of the Cytoskeleton 507 Other Cytoskeletal Elements 508

Major Domains and Subdivisions of the Cytoskeleton 508

Cytoskeletal Systems Associated with the Plasma Membrane 509 The Cytoskeleton of the Cell Cortex 514 Cytoskeletal Structures of the Inner Cytoplasm 514

Dynamic Changes in the Cytoskeleton 517

Cytoskeletal Alterations Related to Motility 517 Cytoskeletal Alterations in Developing Cells 517 Cytoskeletal Rearrangements During Cell Division 518 Cytoskeletal Abnormalities and Disease 520

CHAPTER 14

THE NUCLEUS AND ITS MOLECULAR CONSTITUENTS

DNA Structure 525

The Watson—Crick Model 525
INFORMATION BOX 14-1: DNA Melting and
Renaturation 528

Refinements to the Model: B-DNA and Other DNA Conformations 529

THE EXPERIMENTAL PROCESS: The Discovery of Left-Handed Z-DNA 532

INFORMATION BOX 14-2: Inverted Sequences (Palindromes) 534

Cruciform DNA 535 Chemical Modification of DNA 536 DNA Supercoiling 537

Proteins Associated with DNA in Eukaryotic Nuclei 538

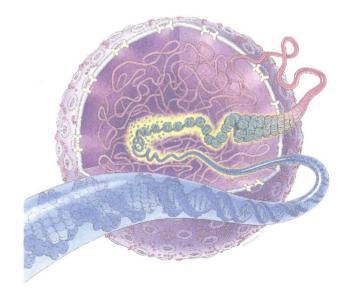
Histones 539 Nonhistone Proteins 541 Information box 14-3: *Histone Modifications* 542

Combination of DNA and Histones in Nucleosomes 546

Experiments Leading to the Discovery of Nucleosomes 547

INFORMATION BOX 14-4: DNA Endonucleases and Nucleosome Periodicity 548

Nucleosome Assembly 549 Nucleosome Phasing 550 Nucleosome Alteration During Transcription and Replication 551 Higher-Order Structure in Chromatin 551



Organization of Chromatin in the Cell Nucleus 553

Heterochromatin and Euchromatin 553 Scaffolds and Domains 554 The Nuclear Matrix 555

The Boundary of the Eukaryotic Nucleus: The Nuclear Envelope 556

Nuclear Envelope Structure 557 Transport Through the Nuclear Envelope 561 SUPPLEMENT 14-1: Nuclear Structure in

Prokaryotes 567

Bacterial Nucleoid Structure 567 Bacterial Nucleoid Proteins 568 Nucleoid Structure in Cyanobacteria 569 Dinoflagellates: Eukaryotes with Prokaryotic DNA Organization 569 SUPPLEMENT 14-2: Annulate Lamellae 571

CHAPTER 15

RNA TRANSCRIPTION AND PROCESSING

General Features of RNA Structure, Transcription, and Processing 574

RNA Structure 574 RNA Transcription 575 INFORMATION BOX **15-1:** Footprinting 578 RNA Processing 581

Transcription and Processing of Messenger RNA 582 mRNA Structure 582 Isolation of mRNAs 585 Pre-mRNAs 585 Structure of mRNA Genes 587 Transcription of Pre-mRNAs 596 Processing Pre-mRNAs 596

Transcription and Processing of Ribosomal RNA 602 The rRNAs 602

INFORMATION BOX 15-2: Determining Secondary Structures in RNAs 604

rRNA Gene Structure, Transcription and Processing 607 5S rRNA Genes, Transcription, and Processing 613 rRNA and the Nucleolus 616

Transcription and Processing of Transfer RNA 618 tRNA Structure 619 tRNA Genes 621 tRNA Transcription and Processing 622

Other RNAs: Small Nuclear and Small Cytoplasmic RNAs 626

snRNAs: Genes, Transcription, and Processing 626 scRNAs 628

Alterations in Chromatin Structure During Transcription 628

Alterations in Regions Containing mRNA Genes 628 Alterations in Regions Containing rRNA Genes 629

THE EXPERIMENTAL PROCESS: What Happens When RNA Polymerase Encounters a Nucleosome? 630 SUPPLEMENT 15-1: RNA Transcription and Processing in Prokaryotes 639

Bacterial RNA Polymerase 639 Bacterial Genes and Their Promoters 640 Sequence Elements Indicating Termination of Bacterial Transcription 641 Bacterial Transcription 642 mRNA Genes,

Transcription, and Processing in Bacteria 642

Bacterial rRNAs and Their Genes, Transcription, and Processing 643 Bacterial tRNA Genes, Transcription, and Processing 645

CHAPTER 16

THE INTERACTION OF mRNA, rRNA, AND tRNA IN PROTEIN SYNTHESIS

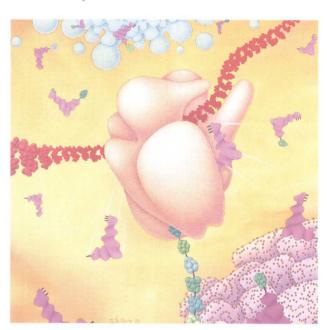
The Assembly of Polypeptides on Ribosomes 650 Initiation 652

THE EXPERIMENTAL PROCESS: When Sperm Meets Egg: Initiation Factors and Translational Control at Fertilization 656

Elongation 658 Termination 663

Ribosome Structure 665

Major Structural Features of Ribosomes 665 Isolating and Identifying Ribosomal Proteins 667 Locating Individual Proteins, rRNAs, and Functions in Ribosomes 668 Assembly of Ribosomes in the Test Tube 669



INFORMATION BOX 16-1: Locating Ribosomal Proteins and rRNAs 670

The Genetic Code for Protein Synthesis 671 Solving the Code 671 Features of the Genetic Code 672 Degeneracy and the Wobble Hypothesis 673 Exceptions to the Universality of the Genetic Code 674 Preferences in Codon Usage 675

Amino Acid Activation 676

The Reactions of Amino Acid Activation 676 The Aminoacyl-tRNA Synthetase Enzymes 677

Posttranslational Modification and Folding of Newly Assembled Polypeptides 680

Chemical Modifications 680 Polypeptide Folding 682

SUPPLEMENT 16-1: Protein Synthesis in
Prokaryotes 687
Polypeptide Assembly in Bacteria 687 Bacterial
Ribosomal Proteins 690
SUPPLEMENT 16-2: Nonribosomal Mechanisms of
Protein Synthesis 691

CHAPTER 17

TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION

Transcriptional Regulation in Eukaryotes 694

THE EXPERIMENTAL PROCESS: Sequential Clues to the DNA Binding Specificity of Myc 696

Regulation by Two Interacting Proteins: The GAL4/80 System of *Saccharomyces* 699
Regulation by a Heterodimer: Gene Control by AP-1 in Mammals 701 Cooperative Regulation of the Serum Albumin Gene 701 Genes Controlled in Groups and Networks by Regulatory Proteins 702

Mechanisms Modifying Transcriptional Control by Regulatory Proteins 708

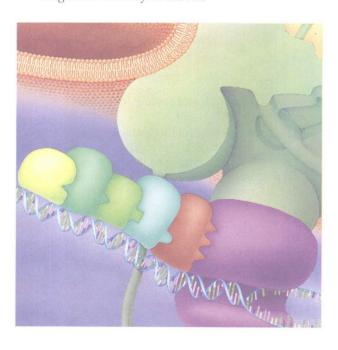
DNA Methylation and Genetic Regulation 708 Histone Modifications and Genetic Regulation 710 Other Mechanisms Affecting Transcription 713

Specialized Mechanisms Regulating rRNA Genes 714 Posttranscriptional Regulation 715

Posttranscriptional Regulation During mRNA Processing 715 Posttranscriptional Regulation of mRNA Transport and Stability 716

Translational Regulation 717

Translational Controls Regulating Steps in Initiation 717 Translational Controls at Later Stages of Protein Synthesis 720



Posttranslational Regulation 720

SUPPLEMENT 17-1: Regulation in Prokaryotes 727
Transcriptional Regulation in Bacteria 727
Translational Regulation in Bacteria 734
SUPPLEMENT 17-2: mRNA Transcription and
Regulation in Polytene Chromosomes 736
Development and Structure of Polytene
Chromosomes 736 Active Sites on Polytene
Chromosomes: Bands and Puffs 737



CHAPTER 18

ORGANIZATION OF THE GENOME AND GENETIC REARRANGEMENTS

Organization of Eukaryotic Genomes: An Overview 740

The Organization of Coding Sequences 741 mRNA Genes 741 rRNA, tRNA, and sn/scRNA Genes 746

Repetitive Sequences in Eukaryotic Genomes 750 Moderately Repeated Sequences 752 Highly Repeated Sequences 753

INFORMATION BOX 18-1: DNA Fingerprinting 754
THE EXPERIMENTAL PROCESS: Repeated DNA
Sequences and Mobile Elements 756

INFORMATION BOX 18-2: Alu and LINE Sequences 758

Genetic Rearrangements 760

INFORMATION BOX 18-3: Sequencing Human and Other Genomes 762

Genetic Rearrangements by Movement of Transposable Elements 763 Examples of Eukaryotic Transposable Elements 765 Transposable Elements and Processed Pseudogenes 768 Genetic Rearrangements by Unequal Crossing Over 768 Genetic Rearrangements by Translocations 769 DNA Transfers Between Species 770 Movement of DNA Segments Between Organelle and Nuclear Genomes 770

SUPPLEMENT 18-1: Genome Structure and Genetic Rearrangement in Prokaryotes 774

Prokaryotic Genomes 774 Genetic Rearrangements in Bacteria 776

SUPPLEMENT 18-2: Genetic Engineering 782
Genetic Engineering in Animals 782 Genetic
Engineering in Plants 786 Engineering Bacteria for
Protein Production 787 The Ethics of Genetic
Engineering 788

CHAPTER 19

PROGRAMMED GENETIC REARRANGEMENTS IN THE PRODUCTION OF ANTIBODIES

The Production of Antibodies 790

Antibody Structure 791 The Antibody Gene Groups 793 The Genetic Rearrangements Assembling Antibody Genes 798 Stimulation of B Cells Producing an Antibody Type: Clonal Selection 803

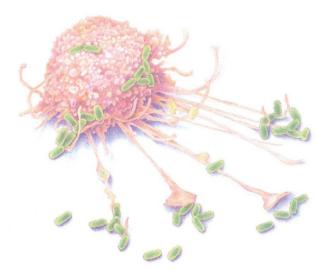
THE EXPERIMENTAL PROCESS: Instruction or Selection: The Role of Antigen in Antibody Production 804

The Immune Response 806

MHC and the Immune Response 807 MHC Binding by T Cells 809 B Cells and the Secretion of Antibodies to the Body Exterior 814

Malfunctions of the Immune System 815

Autoimmune Diseases 815 Infective Agents That Defeat or Sidestep the Immune Response 816



Antibodies in Research 816

INFORMATION BOX 19-1: The Production of Variable Surface Groups in Trypanosoma Bruceii 817

SUPPLEMENT 19-1: AIDS 820

HIV Infection 820 Symptoms and Progress of AIDS 821 Transmission and Prevention of AIDS 822 Possible Origins of the Disease 822 Research Toward Protection or Cure 823

CHAPTER 20

PROTEIN SORTING, DISTRIBUTION, SECRETION, AND ENDOCYTOSIS

Primary Way Stations in Protein Distribution: The ER and Golgi Complex 825

Structure and Molecular Constituents of the System 825 Evidence That Proteins Move Sequentially Through the System 831 Coordination of the ER and Golgi Complex in Processing Reactions 834

The Mechanisms Sorting and Distributing Proteins 835

Signals Directing Proteins to Initial
Destinations 835 Signals Directing Proteins
Past the Entry Membrane 835 Identifying and
Characterizing Signals 835

Mechanisms Targeting Proteins to the ER 836

The Signal Hypothesis 836 Characteristics of ER-Directing Signals 839

THE EXPERIMENTAL PROCESS: Redirecting Protein Traffic from Cytoplasm to the Secretory Pathway: A Test of the Signal Hypothesis 840

The Signal Peptidase 842 Stop-Transfer Signals 842 Postinsertion Signals and Further Destinations for ER-Directed Proteins 844

Distribution of Proteins to Locations Outside the ER-Golgi Pathway 849

Distribution to Mitochondria and Chloroplasts 849 Signals Directing Proteins to the Nuclear Interior 854 Distribution to Microbodies 854

Exocytosis and Endocytosis 855

Exocytosis 855 Endocytosis 857 Endocytosis, Exocytosis, and Membrane Recycling 866
SUPPLEMENT 20-1: Protein Sorting, Distribution, and Secretion in Prokaryotes 871
SUPPLEMENT 20-2: Reactions Adding Sugar Units to Proteins in the ER and Golgi Complex 873

CHAPTER 21

CYTOPLASMIC GENETIC SYSTEMS

The Discovery of DNA and Genes in Mitochondria and Chloroplasts 877

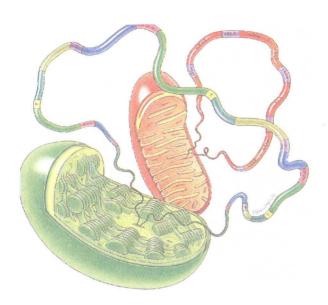
Cytoplasmic Inheritance of Mutations Affecting the Organelles 877 The Search for DNA in Organelles 879

The Organelle DNAs 881

Isolation of the DNA 881 The DNA Circles of Mitochondria 881 Chloroplast DNA 882

Genes and Genome Structure in the Organelles 883 Mitochondrial Genes 883 Chloroplast Genes and Genomes 889

Transcription and Translation in Mitochondria and Chloroplasts 892



Transcription and Processing in
Mitochondria 893 Transcription and
Processing in Chloroplasts 895 Polypeptide
Assembly in Mitochondria and
Chloroplasts 896 Transcriptional and
Translational Regulation in the Organelles 899

Origins of the Organelle Genomes 900 Genetic Information in Centrioles 901

THE EXPERIMENTAL PROCESS: Escape of DNA from Mitochondria to the Nucleus During Evolution and in Real Time 902

CHAPTER 22

THE CELL CYCLE, CELL CYCLE REGULATION, AND CANCER

An Overview of the Mitotic Cell Cycle 910 Interphase 910 Mitotic Cell Division 911 Alterations in the Cell Cycle 915

Cell Cycle Regulation: Factors Modifying the Cell Cycle 917

Regulation of Growth and Division Through Surface Receptors 917 Alterations in Ion Transport 919 pH and the Cell Cycle 920 Histone and Nonhistone Modifications 920 Polyamines and Cell Cycle Modification 922 Modification by Cytoplasmic Volume 923

Genes Regulating the Cell Cycle 923

Cell Cycle Genes Discovered Through the Study of Mutants 924 Equivalents of the cdc2/28 and cdc13 Genes in Higher Eukaryotes 926 Cell Cycle Genes Identified Through the Study of Oncogenes 929

THE EXPERIMENTAL PROCESS: Finding Cyclins and After 930

Alterations Changing Proto-Oncogenes into Oncogenes 935

Cancer: Characteristics and Causes 939

The Characteristics of Cancer 939 Establishing the Genetic Basis of Cancer 940 Viruses and Cancer 941 Multistep Progression from Initiation to Malignancy 942 The Role of Chemicals and Radiation in Multistep Progression 944

CHAPTER 23

DNA REPLICATION AND REPAIR

Semiconservative DNA Replication 952

The Reactions of DNA Replication 955

An Overview of Replication 955 The Specialized Mechanisms of DNA

Replication 959

Replication 959
INFORMATION BOX 23-1: Tautomers and Base
Mismatches During Replication 961
The Enzymes and Factors of DNA
Replication 962 Telomere Replication:
Replacing the Primer of the Leading Chain 971
THE EXPERIMENTAL PROCESS: Telomerase: The

Initiation of Replication: Replication Origins and Replicons 975

Replication Origins 975 Replicons 978 Replicons and DNA Amplification 979

Search for a Hypothetical Enzyme 972

DNA Repair 981

Mismatch Repair 981 Repair of DNA Alterations Arising from Chemicals or Radiation 982

INFORMATION BOX 23-2: Mutations and Their Effects 983

Duplication of Chromosomal Proteins in Eukaryotes 984

Synthesis of Chromosomal Proteins 984 Nucleosomes and DNA Replication 986 SUPPLEMENT 23-1: The Taylor Experiment Demonstrating That DNA Replication Is Semiconservative in Eukaryotes 992

