Cover Design: Wiley
Cover Image: Janet Iwasa, in collaboration with the Innovative Genomics Institute

www.wiley.com

WILEY

Wiley Loose-Leaf Print Edition

ISBN 978-1-119-59824-4

WILEY

Karp's Cell and Molecular Biology

Concepts and Experiments

Ninth Edition

JANET IWASA
University of Utah

WALLACE MARSHALL
University of California, San Francisco

WILEY

VICE PRESIDENT & DIRECTOR	Laurie Rosatone
SENIOR EDITOR	Maria Guarascio
MARKETING MANAGER	Michael Olsen
SENIOR INSTRUCTIONAL DESIGNER	Linda Muriello
EDITORIAL ASSISTANT	Georgia Larsen
SENIOR CONTENT MANAGER	Svetlana Barskaya
SENIOR PRODUCTION EDITOR	Trish McFadden
ART DIRECTOR	Jonathon Boylan
SENIOR DESIGNER	Wendy Lai
PRODUCTION MANAGEMENT SERVICES	Lumina Datamatics
COVER PHOTO CREDIT	Janet Iwasa and the Innovative Genomics Institute

This book was set in 9.5/12 SourceSansPro by Lumina Datamatics and printed and bound by Quad Graphics.

Founded in 1807, John Wiley & Sons, Inc. has been a valued source of knowledge and understanding for more than 200 years, helping people around the world meet their needs and fulfill their aspirations. Our company is built on a foundation of principles that include responsibility to the communities we serve and where we live and work. In 2008, we launched a Corporate Citizenship Initiative, a global effort to address the environmental, social, economic, and ethical challenges we face in our business. Among the issues we are addressing are carbon impact, paper specifications and procurement, ethical conduct within our business and among our vendors, and community and charitable support. For more information, please visit our website:www.wiley.com/go/citizenship.

The paper in this book was manufactured by a mill whose forest management programs include sustained yield-harvesting of its timberlands. Sustained yield harvesting principles ensure that the number of trees cut each year does not exceed the amount of new growth.

EPUB ISBN: 978-1-119-59816-9

Library of Congress Cataloging-in-Publication Data

Names: Iwasa, Janet, author. | Marshall, Wallace F., author. | Karp, Gerald. Cell and molecular biology.
Title: Karp's cell and molecular biology / Janet Iwasa, University of Utah, Wallace Marshall, University of California, San Francisco.
Other titles: Cell and molecular biology
Description: Ninth edition. | Hoboken : Wiley, 2020. | Includes index.
Identifiers: LCCN 2019047994 (print) | LCCN 2019047995 (ebook) | ISBN 9781119598244 (paperback) | ISBN 9781119598169 (epub)
Subjects: LCSH: Cytology. | Molecular biology.
Classification: LCC QH581.2 .K369 2020 (print) | LCC QH581.2 (ebook) | DDC 571.6—dc23
LC record available at https://lccn.loc.gov/2019047994
LC ebook record available at https://lccn.loc.gov/2019047995

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

The inside back cover will contain printing identification and country of origin if omitted from this page. In addition, if the ISBN on the back cover differs from the ISBN on this page, the one on the back cover is correct.

About the Authors

JANET IWASA is an Assistant Professor in the Biochemistry Department at the University of Utah. She received her bachelor's degree from Williams College and a Ph.D. in Cell Biology from the University of California, San Francisco, where she first became interested in the visualization of biological processes. As a postdoctoral fellow, she was awarded a fellowship from the National Science Foundation to create a multimedia exhibit with Nobel Laureate Jack Szostak (Harvard University) and the Museum of Science, Boston. She later joined Harvard Medical School as a faculty member in the Department of Cell Biology, where she utilized visualization tools to aid in scientific communication, exploration, and outreach. Janet's award-winning illustrations and animations have appeared in scientific journals including *Nature*, *Science*, and *Cell*, as well as in the *New York Times*.

She currently runs a group, called the Animation Lab, that focuses on the innovative use of visualization tools for molecular biology research, education and outreach.

WALLACE MARSHALL is Professor of Biochemistry and Biophysics at the University of California San Francisco and an ASCB Fellow. A native Long-Islander, he received his bachelor's degrees in Electrical Engineering and Biochemistry from the State University of New York at Stony Brook, and his Ph.D. in Biochemistry from UC San Francisco, where he studied organization of chromosomes within the nucleus with John Sedat. He then moved to Yale University for postdoctoral studies with Joel Rosenbaum, where he became interested in questions of organelle size control and cell organization, using cilia, flagella, and centrioles as model systems. In 2003, he joined the faculty at UCSF where he continues to study questions of cellular organization in a variety of model organisms including green algae, yeast, ciliates, and mammalian cells. In addition to his cell biology research, he teaches Human Metabolism for the UCSF School of Pharmacy, Cell Biology for the UCSF Graduate Division, and a two-week lab course on cell behavior. In 2014, he served as Program Committee Chair organizing the annual meeting of the American Society for Cell Biology. From 2014 to 2018 he served as co-director of the Physiology summer course at the Marine Biological Laboratory in Woods Hole, Massachusetts. He currently directs the Center for Cellular Construction, a National Science Foundation Science and Technology Center devoted to engineering cells.

Both authors are Council members of the American Society for Cell Biology.

Nobel Prizes Awarded for Research in Cell and Molecular Biology Since 1958

Year	Recipient*	Prize	Area of Research	Pages in Text
2018	Frances Arnold George Smith Gregory Winter	Chemistry	Directed evolution of enzymes and antibodies	99, 811
2018	James Allison Tasuku Honjo	Physiology**	Cancer Immunotherapy	808–809
2017	Jacques Dubochet Joachim Frank Richard Henderson	Chemistry	Cryo-electron microscopy for high resolution structure determination	39, 811
2017	Jeffrey Hall Michael Rosbash Michael Young	Physiology	Mechanism of the circadian rhythm	134
2016	Yoshinori Ohsumi	Physiology	Mechanism of autophagy	349–350
2015	Tomas Lindahl Paul Modrich Aziz Sancar	Chemistry	Mechanisms of DNA repair	532
2014	Eric Betzig W. E. Moerner Stefan Hell	Chemistry	Development of super-resolved fluorescence microscopy	699–700
2013	James E. Rothman Randy W. Schekman Thomas C. Südhof	Physiology	Discoveries of machinery regulating vesicle traffic	263, 279
2012	John B. Gurdon	Physiology	Animal cloning, nuclear reprogramming	483
	Shinya Yamanaka		Cell reprogramming	20, 489
	Brian K. Kobilka Robert J. Lefkowitz	Chemistry	G protein-coupled receptors	588
2011	Bruce A. Beutler Jules A. Hoffmann	Physiology	Innate immunity	664
	Ralph M. Steinman		Dendritic cells and Adaptive immunity	676
2009	Venkatraman Ramakrishnan Thomas A. Steitz Ada E. Yonath	Chemistry	Ribosome structure and function	453
	Eliazbeth H. Blackburn Carol W. Greider Jack W. Szostak	Physiology	Telomeres and telomerase	475
2008	Francoise Barré-Sinoussi Luc Montagnier	Physiology	Discovery of HIV	23
	Harald zur Hausen		Role of HPV in cancer	631
	Martin Chalfie Osamu Shimomura Roger Tsien	Chemistry	Discovery and development of GFP	260, 697
2007	Mario R. Capecchi Martin J. Evans Oliver Smithies	Physiology	Development of techniques for knockout mice	735
2006	Andrew Z. Fire Craig C. Mello	Physiology	RNA Interference	430
	Roger D. Kornberg	Chemistry	Transcription in eukaryotes	412, 465
2004	Richard Axel Linda B. Buck	Physiology	Olfactory receptors	603
	Aaron Ciechanover	Chemistry	Ubiquitin and proteasomes	509

Year	Recipient*	Prize	Area of Research	Pages in Text
	Avram Hershko			
	Irwin Rose			
2003	Peter Agre	Chemistry	Structure of membrane channels	143, 144
	Roderick MacKinnon			
2002	Sydney Brenner	Physiology	Introduction of *C. elegans* as a model organism	16
	John Sulston			
	H. Robert Horvitz		Apoptosis in *C. elegans*	622
	John B. Fenn	Chemistry	Electrospray ionization in MS	717
	Koichi Tanaka		MALDI in MS	717
	Kurt Wüthrich		NMR analysis of proteins	55
2001	Leland H. Hartwell	Physiology	Control of the cell cycle	547, 550
	Tim Hunt			
	Paul Nurse			
2000	Arvid Carlsson	Physiology	Synaptic transmission and signal transduction	164
	Paul Greengard			614
	Eric Kandel			
1999	Günter Blobel	Physiology	Protein trafficking	268
1998	Robert Furchgott	Physiology	NO as intercellular messenger	620
	Louis Ignarro			
	Ferid Murad			
1997	Jens C. Skou	Chemistry	Na^+/K^+-ATPase	152
	Paul Boyer		Mechanism of ATP synthesis	189, 190
	John Walker			
	Stanley B. Prusiner	Physiology	Protein nature of prions	63
1996	Rolf M. Zinkernagel	Physiology	Recognition of virus-infected cells by the immune system	672
	Peter C. Doherty			
1995	Edward B. Lewis	Physiology	Genetic control of embryonic development	15
	Christiane Nüsslein-Volhard			
	Eric Wieschaus			
1994	Alfred Gilman	Physiology	Structure and function of GTP-binding (G) proteins	593
	Martin Rodbell			
1993	Kary Mullis	Chemistry	Polymerase chain reaction (PCR)	726
	Michael Smith		Site-directed mutagenesis (SDM)	735
	Richard J. Roberts	Physiology	Intervening sequences	420
	Phillip A. Sharp			
1992	Edmond Fischer	Physiology	Alteration of enzyme activity by phosphorylation/dephosphorylation	109, 600
	Edwin Krebs			
1991	Erwin Neher	Physiology	Measurement of ion flux by patch-clamp recording	143
	Bert Sakmann			
1990	Joseph E. Murray	Physiology	Organ and cell transplantation in human disease	684
	E. Donnall Thomas			
1989	J. Michael Bishop	Physiology	Cellular genes capable of causing malignant transformation	633
	Harold Varmus			425, 450
	Thomas R. Cech	Chemistry	Ability of RNA to catalyze reactions	
	Sidney Altman			207
1988	Johann Deisenhofer	Chemistry	Bacterial photosynthetic reaction center	
	Robert Huber			
	Hartmut Michel			
1987	Susumu Tonegawa	Physiology	DNA rearrangements responsible for antibody diversity	681
1986	Rita Levi-Montalcini	Physiology	Factors that affect nerve outgrowth	379
	Stanley Cohen			
1985	Michael S. Brown	Physiology	Regulation of cholesterol metabolism and endocytosis	319
	Joseph L. Goldstein			
1984	Georges Köhler	Physiology	Monoclonal antibodies	738, 739
	Cesar Milstein			
	Niels K. Jerne		Antibody formation	666

Year	Recipient*	Prize	Area of Research	Pages in Text
1983	Barbara McClintock	Physiology	Mobile elements in the genome	391, 392, 394
1982	Aaron Klug	Chemistry	Structure of nucleic acid–protein complexes	55
1980	Paul Berg	Chemistry	Recombinant DNA technology	692, 723
	Walter Gilbert		DNA sequencing technology	728
	Frederick Sanger			
	Baruj Bennacerraf	Physiology	Major histocompatibility complex	684
	Jean Dausset			
	George D. Snell			
1978	Werner Arber	Physiology	Restriction endonuclease technology	723
	Daniel Nathans			
	Hamilton O. Smith			
	Peter Mitchell	Chemistry	Chemiosmotic mechanism of oxidative phosphorylation	176
1976	D. Carleton Gajdusek	Physiology	Prion-based diseases	63
1975	David Baltimore	Physiology	Reverse transcriptase and tumor virus activity	633
	Renato Dulbecco			
	Howasrd M. Temin			
1974	Albert Claude	Physiology	Structure and function of internal components of cells	262
	Christian de Duve			
	George E. Palade			
1972	Gerald Edelman	Physiology	Immunoglobulin structure	678
	Rodney R. Porter			
	Christian B. Anfinsen	Chemistry	Relationship between primary and tertiary structure of proteins	60
1971	Earl W. Sutherland	Physiology	Mechanism of hormone action and cyclic AMP	590, 595, 596
1970	Bernard Katz	Physiology	Nerve impulse propagation and transmission	160
	Ulf von Euler			
	Luis F. Leloir	Chemistry	Role of sugar nucleotides in carbohydrate synthesis	273
1969	Max Delbrück	Physiology	Genetic structure of viruses	23, 380
	Alfred D. Hershey			
	Salvador E. Luria			
1968	H. Gobind Khorana	Physiology	Genetic code	722–723
	Marshall W. Nirenberg			
	Robert W. Holley		Transfer RNA structure	439
1966	Peyton Rous	Physiology	Tumor viruses	632
1965	Francois Jacob	Physiology	Bacterial operons wand messenger RNA	406, 456
	Andre M. Lwoff			
	Jacques L. Monod			
1964	Dorothy C. Hodgkin	Chemistry	X-ray structure of complex biological molecules	717
1963	John C. Eccles	Physiology	Ionic basis of nerve membrane potentials	160
	Alan L. Hodgkin			
	Andrew F. Huxley			
1962	Francis H. C. Crick	Physiology	Three-dimensional structure of DNA	374–377
	James D. Watson			
	Maurice H. F. Wilkins			
	John C. Kendrew	Chemistry	Three-dimensional structure of globular proteins	56
	Max F. Perutz			
1961	Melvin Calvin	Chemistry	Biochemistry of CO_2 assimilation during photosynthesis	213, 214–215
1960	F. MacFarlane Burnet	Physiology	Clonal selection theory of antibody formation	666
	Peter B. Medawar			
1959	Arthur Kornberg	Physiology	Synthesis of DNA and RNA	518, 523
	Severo Ochoa			
1958	George W. Beadle	Physiology	Gene expression	405–406
	Joshua Lederberg			
	Edward L. Tatum			
	Frederick Sanger	Chemistry	Primary structure of proteins	53

*In a few cases, corecipients whose research was in an area outside of cell and molecular biology have been omitted from this list.

**Physiology denotes the Nobel Prize in Physiology or Medicine

Topics of Human Interest

NOTE: An f after a page denotes a figure; t denotes a table; fn denotes a footnote; HP denotes a Human Perspective; EP denotes an Experimental Pathway.

Acquired immun deficiency syndrome. *See* AIDS
Acute lymphoblastic leukemia (ALL), 755, 765, 766, Chapter 16
Acute myeloid leukemia (AML), 759, 764, 765, 766, Chapter 16
Adaptive (acquired) immune response, 777, 779, 780, 781, 789, Chapter 17
Adenoviruses, 22HP, 27, 28f, 187HP, 495, 497, 741, 753, Chapter 1, Chapter 4, Chapter 11, Chapter 16
Adrenoleukodystrophy (ALD), 236HP, Chapter 5
African populations, genomes of, 454-455, 473HP, Chapter 10
Agammaglobulinemia, 780, Chapter 17
Aging
- Down syndrome (trisomy 21) and, 680HPf, 681HP, Chapter 14
- mitochondrial disorders and, 235HP, Chapter 5
- premature (progeria), 544, 635, Chapter 12, Chapter 13
- radicals and, 42-43HP, Chapter 2
- telomeres and, 553, 562, 562f, 563, 564f, 564, 565, 565fn, 566, 604, Chapter 12

AIDS (acquired immune deficiency syndrome)
- helper T cells and, 786, 789fn, 796, 801, 806, Chapter 17
- resistance, 720, 777f, 781, 797, 810, Chapter 15, Chapter 17
- resistance to drugs, 74, 101–102HP, Chapter 2, Chapter 3
- therapies for, 433HP, Chapter 11

ALD (adrenoleukodystrophy), 236HP, Chapter 5
ALL (Acute lymphoblastic leukemia), 755, 765, 766, Chapter 16
Alzheimer's disease (AD), 76HP, 78f, 98, 197, Chapter 2, Chapter 4
AML (Acute myeloid leukemia), 759, 764f, 765, 766f, Chapter 16
Anesthetics, 193, 196, Chapter 4
Aneuploidy, 666, 680HP, 755f, 757, 763f, 740fn, Chapter 14, Chapter 16
Antacid medications, 154, Chapter 4
Antibiotics, 118–121HP
- modes of, in clinical use, 119HPt, Chapter 3
- penicillin, 120, 120f, 121, Chapter 3

Antibiotic resistance, 118, Chapter 3
Antidepressants, 197, Chapter 4
Anti-inflammatory drugs, and cancer, 742, Chapter 16
Antioxidants, 43HP, Chapter 2
Appetite, 132, 197, Chapter 3, Chapter 4
Arthritis, rheumatoid, 785–786HP, Chapter 17
Artificial fertilizer, 52, Chapter 2
Atherosclerosis, 361, Chapter 8
Autoimmune diseases, 782, 784–786HP, 790, 805, Chapter 17
- Graves' disease and thyroiditis, 785HP, Chapter 17
- inflammatory bowel diseases (IBDs), 785HP, Chapter 17
- multiple sclerosis (MS), 784HP, Chapter 17
- rheumatoid arthritis, 785HP, Chapter 17
- systemic lupus erythematosus (SLE), 785HP, Chapter 17
- treatment of, 785–786HP, Chapter 17
- type 1 diabetes (T1D), 785HP, Chapter 17

Bacterial toxins, 695HP, 697, Chapter 15
Bacteriophage therapy, 27, 29f, Chapter 1
Benign tumors, 748, 765, Chapter 16
Biofilms, 14, 186f, 685, Chapter 1, Chapter 4, Chapter 15
Blistering diseases, 284, 402, 402fn, Chapter 7, Chapter 9
Blood-brain barrier, 296, Chapter 7
Blood cell differentiation, 780f, Chapter 17
Blood clots, 57, 60, 276, 277, 280, 280f, 281, 337, 361, 362, 689, 462, Chapter 2, Chapter 7, Chapter 8, Chapter 10, Chapter 15
Blood glucose, 704, 709, 717, 718, 720, Chapter 15
Blood-group antigens, 122–123, 123f, 398, 797, Chapter 4, Chapter 10, Chapter 17
Bone marrow, 18HP–22HP, 236HP, Chapter 1, Chapter 5, Chapter 7, Chapter 8, Chapter 10, Chapter 12, Chapter 17
Bone marrow transplantation, 18HP, 236HP, 288HP, Chapter 1, Chapter 5, Chapter 7
Booster shots, 787, Chapter 17
Breast cancer
- *BRCA1* and, 605, 605f, 631, 751t, 751fn, 757, 757f, 764f, 772, 773, Chapter 13, Chapter 16
- cause of, 757, Chapter 16
- gene-expression analysis, 763, Chapter 16
- genetic mutations in, 747, 761, 763f, Chapter 16
- genetics and, 755, Chapter 16
- immunotherapy for, 768, 769, 770, 774, Chapter 16
- karyotype of cell from, 740F, 751, 757, Chapter 16
- new cases and deaths in US in 2019, 739f, Chapter 16
- PI3K pathway in, 759fn, 763, Chapter 16
- Preventive mastectomy, 512, Chapter 13
- protein-inhibiting drugs for, 770, Chapter 16
- PSA determinations for detecting, 774, Chapter 16
- research efforts, 738, Chapter 16
- tumor-suppressor genes in, 749, 749f, 750, 751t, 753, 755, 756, 757, 761, 765, Chapter 16
- tyrosine phosphorylation in cells, 688, 689f, 689, 712, 718, 719, Chapter 15

Burkitt's lymphoma, 741, 759, Chapter 16

Calorie-restricted diet, life span and, 742, Chapter 16, 132–133HP, Chapter 3
Cancer, 737–774, Chapter 16
- cancer genome, 761–763, Chapter 16
- causes of, 741–746, Chapter 16
- cells, properties of, 738–741, Chapter 16
  - aneuploidy, 740, 740fn, 750f, 755f, 757, 763, Chapter 16
  - cells of origin of malignant tumors, 748f, Chapter 16
  - effects of serum deprivation on growth of, 740f, Chapter 16
  - growth rate, 629, 629f, Chapter 16
  - metastasis, 738, Chapter 16
- combating, strategies for, 768–774, Chapter 16
  - angiogenesis, 773–774, Chapter 16
  - cancer stem cells, 773, 773fn, Chapter 16
  - chemotherapy, 738, 747, 755, 760, 765, 767, 768, 769, 773, Chapter 16
  - immunotherapy, 774, 768–770, Chapter 16
  - inhibiting activity of cancer-promoting proteins, 770–773, Chapter 16
  - targeted therapies, 773, 738, 768, 771t, 772, Chapter 16
- diet and, 742, Chapter 16
- gene-expression analysis, 763–765, Chapter 16
- as genetic disorder, 747–767, Chapter 16
- microRNAs, 761, Chapter 16
- multiple myeloma, 791, 792, Chapter 17
- mutator phenotype, 760–761, Chapter 16
- new cases and deaths in US in 2019, 739f, Chapter 16
- oncogenes, 761, 762, 765, 770, 772, 742–746EP, Chapter 16
  - activation of proto-oncogene to, 749, 750f, Chapter 16
  - discovery of, 742–745EP, Chapter 16
  - overview of, 749–750, 749f, Chapter 16
- research efforts, 738, Chapter 16
- tumor-suppressor genes, 749–765, 638f, 640t, Chapter 16
  - *APC* genes, 756, 761–762, Chapter 16
  - *BRCA1/BRCA2* genes, 605, 605f, 631, 757, 757f, 764, 772, 773, Chapter 13, Chapter 16

overview of, 738–741, Chapter 16
PTEN gene, 757, Chapter 16
RB gene, 750–753, 752f, Chapter 16
TP53 gene, 753–756, 754f, Chapter 16
Carcinogens, 741, 742EP, 754, Chapter 16
Cell-mediated immunity, 779, 780, 784HP, Chapter 17
Cell replacement therapy, 18–22HP Chapter 1
adult stem cells, 19HP, 19HPf, Chapter 1
direct cell reprogramming, 21–22HP, Chapter 1
embryonic stem cells, 19–20HP, 20HPf, Chapter 1
induced pluripotent stem cells, 21HP, Chapter 1
Cervical cancer, 631, 632, 637, 637f, 660, Chapter 17
Chemotherapy, 738, 747, 755, 760, 765, 767–769, 773, 774, Chapter 16
Cholera, 695EP, 695EPf, 696EP, 697, 736, Chapter 15
Cholesterol
in cell membranes, 59, Chapter 2
in membrane lipids, 142, 143f, Chapter 4
in phospholipids, 144, 146f, Chapter 4
simple sugars and, 54, Chapter 2
as steroid, importance of, 49f, 59, Chapter 2
structure of, 49, 49f, 60, Chapter 2
Chromosomes
aberrations and human disorders, 560HP, Chapter 12
as carriers of genetic information, 436, Chapter 10
compaction, 642–643EP, 651, 653, 654, 662, 677, Chapter 14
premature, 640, Chapter 14
defined, 435, Chapter 10
discovery of, 434f, 435, Chapter 10
fertilization, process of, 435, 436, 439, 441EP, Chapter 10
giant, 439, 440, 440f, Chapter 10
heterochromatin, 553, 554, 555f, 556, Chapter 12
homologous, 434f, 437–438, 437f–439f, 441, 458–460, 469, 475, Chapter 10
mitotic, structure of, 553, 553f, 554, 558, 559f, 564, 565, 565f, Chapter 12
polytene, 440, 440f, 441, Chapter 10
X chromosome inactivation, 554–556, 554fn, 591, Chapter 12
Chronic lymphocytic leukemia (CLL), 761, Chapter 16
Chronic myelogenous leukemia (CML), 90, 91, 91f, 761, 770, Chapter 2, Chapter 16
Ciliopathies, 393HP, 394HP, Chapter 9
Climate change, 260, Chapter 6
CLL (chronic lymphocytic leukemia), 761, Chapter 16
Clonal selection theory applied to B cells, 781, Chapter 17
Cloning of animals, 570f, Chapter 12
CML (chronic myelogenous leukemia), 90, 91, 91f, 761, 770, Chapter 2, Chapter 16
Cochlear implants, 199, Chapter 4
Cockayne syndrome (CS), 634HP, Chapter 13
Collagen, diseases of, 268f, 271–273, 270f–273f, 275f, 277, 278, 279f, 279t, 280, 282f, 283, 284, 284f, 289, 306, Chapter 7
Colon cancer
cause of, 742f, 763f, 756-757, Chapter 16
genetic mutations in, 763f, 658, Chapter 16
NSAIDs and, 742, Chapter 16
research efforts, 762f, Chapter 16
tumor-suppressor genes, 754t, Chapter 16
Color blindness, 555, 698HPt, Chapter 12, Chapter 15
Congenital Disorders of Glycosylation (CDGs), 326, Chapter 8
Creutzfeld-Jakob disease (CJD), 76HP, 76HPfn, 77HP, 98, Chapter 2
Cystic fibrosis (CF), 184–187, 185t, 186f, 328, 532, Chapter 4, Chapter 8, Chapter 11

Deafness, myosin mutations and, 410, Chapter 9
Diabetes
congenital nephrogenic diabetes insipidus, 170, Chapter 4
Hemoglobin A1c and, 54, Chapter 2
type 1, 720, 732, 785HP, Chapter 15, Chapter 17
type 2, 720, 721, Chapter 15
Diarrhea, 169, 695EP, Chapter 4, Chapter 15
Diet, cancer and, 742, Chapter 16
DNA fingerprinting, 454, 454f, 474, Chapter 10
DNA repair, 605, 606, 610, 617, 625, 628, 629, 630f, 634HP, 635HP, Chapter 13
Down syndrome (trisomy 21), 680HPf, 681HP, Chapter 14
Drug development, 91f, 509HP, Chapter 2, Chapter 3, Chapter 11
Dwarfism, 273, 634HP, Chapter 7, Chapter 13

Embryonic development
cadherins in, 289HP, 291, 293, Chapter 7, Chapter 9
changes in cell shape during, 291, 429
Embryonic stem cells, 19HP, 20HP, 20HPf, 21HP, 861, Chapter 1, Chapter 18
Enzyme replacement therapy, 342HP, 343HP, Chapter 8
Epstein-Barr virus, 741, 759, Chapter 16
Exercise, 203, 213HP, 214HP, 234HP, Chapter 5

Fabry's disease, 343HPt, Chapter 8
Fragile X syndrome, 455, 455HPf, 456, Chapter 10
Free radicals, aging and, 42, 42HP, 43HP, Chapter 2

Gamma knife, 44, Chapter 2
Gaucher's disease, 342HP, 343HPt, 343, Chapter 8
Gene number, 459, 460f, 465, 467, Chapter 10
Gene therapy, 29, 187HP, 236HP, 858, Chapter 1, Chapter 4, Chapter 5, Chapter 18
Genome analysis, 459, Chapter 10
Gleevec, 90, 91, 91f, 92, 122, 770, 771t, 773, 774, Chapter 2, Chapter 3, Chapter 16
Glycolipids, diseases of, 141f, 143f, 144, 145t, 146, 147, 324, 330, 342HPf, Chapter 4, Chapter 8
Graft rejection, 797, Chapter 17
Graves' disease and thyroiditis, 785HP, Chapter 17

Heart attack/heart disease, 19HP, 133HP, 277, 280, 287HP, 355EP, 361, 362, 362f, 544, Chapter 1, Chapter 3, Chapter 7, Chapter 8, Chapter 12
Heartburn, 21HP, 184, 184f, 472HP, Chapter 1, Chapter 4, Chapter 10
Heart muscle, 92, 214HP, 300, 730, Chapter 2, Chapter 5, Chapter 7, Chapter 15
Hemolytic anemias, 166, Chapter 4
Hemophilia, 462, Chapter 10
Herceptin, 769, Chapter 16
Herpes virus (HHV-8), 741, Chapter 16
HIV (human immunodeficiency virus), 25f, 27, 28, 28f, 29f, 121, 122, Chapter1, Chapter 3, Chapter 11
azidothymidine (AZT) and, 621, Chapter 13
T_H cells and, 789, Chapter 17
Human Genome Project, 470, 473HP, 853, 855, Chapter 10, Chapter 18
Human immunodeficiency virus. *see* HIV (human immunodeficiency virus)
Human papilloma virus (HPV), 741, 753, Chapter 16
Humoral immunity, 779, 780, Chapter 17
Huntington's disease, 455, 455HP, 471HP, Chapter 10
Hydrocephalus, 289, 393HP, Chapter 7, Chapter 9
Hypertension, 121, Chapter 3

I-cell disease, 342HP, 367, Chapter 8
Immune response, 775–777, 777f, 778–783, 784HP–786HP, 789, 791t, 802EP, 806, Chapter 17
antibodies, modular structure of, 790, Chapter 17
autoimmune diseases, 782, 784HP–786HP, 789fn, 790, 805, Chapter 17
clonal selection theory applied to B cells, 781, Chapter 17
distinguishing self from nonself, 805, Chapter 17
DNA rearrangements producing genes encoding B- and T-cell antigen receptors, 793, Chapter 17
lymphocyte activation by cell-surface signals, 790, 806, Chapter 17
major histocompatibility complex, 790, 797, 801EP, Chapter 17
membrane-bound antigen receptor complexes, 790, 796, Chapter 17
overview of, 776, Chapter 17
adaptive immune responses, 777f, 779–781, 789, Chapter 17
immune system, 776–781, 784HP–786HP, 787, 788, 790, 797, 798, 801EP, 805, 806, 808, Chapter 17
immunity, 775, 776, 777f, 778, 779, 779f, 780, 781, 782, 782f, 783, 785HP, 787, 790, 793, 798, Chapter 17
innate immune responses, 775, 777, 780, Chapter 17
signal transduction pathways in lymphocyte activation, 809, Chapter 17
T lymphocyte activation and mechanism of action, 787, Chapter 17
vaccination, 783, Chapter 17
Immune system, 776–781, 784HP–786HP, 787, 788, 790, 797, 798, 801EP, 805, 806, 808, Chapter 17
Immunization, 783, 787, Chapter 17
Immunotherapy, 768–770, 774, Chapter 16

Inborn errors of metabolism, 478, Chapter 11
Induced pluripotent stem cells (iPS cells), 21HP, 22HPf, 577, 577fn, Chapter 1, Chapter 12
Infections
adaptive immune responses, 665, Chapter 17
cancer-causing, 699HP, 716, 734fn, Chapter 15
innate immune responses, 775, 777, 780, Chapter 17
lytic, 28, Chapter 1
protective mechansims, 777, Chapter 17
resistant bacterial, 121HP, Chapter 3
viral, 27–28, Chapter 1
Inflammation
cell adhesion in, 277, 284–285, 286f, 287–289HP, Chapter 7
as innate responses to invading pathogens, 779, Chapter 17
Inflammatory bowel diseases (IBDs), 785HP, Chapter 17
Influenza, 26–28, 38, 89, 90f, Chapter 1, Chapter 2
Innate immune responses, 775, 777, 780, Chapter 17
Insulin signaling, 719–720, Chapter 15
Interferons (IFNs), 775, 779, 789, 810, Chapter 17
Interleukins (ILs), 789, Chapter 17

Kaposi's sarcoma, 699HP, 741, Chapter 15, Chapter 16
Karyotypes, 559f, 560, 740f, 751, 757, Chapter 12, Chapter 16
Kidneys
cancer, 754f, 751t, Chapter 16
failure from diabetes, 269, Chapter 7
polycystic kidney diseases, 393HP, 393HPf, Chapter 9
Klinefelter syndrome, 681HP, Chapter 14
Krabbe's disease, 343HPt, Chapter 8

Lactose tolerance, 473HP, Chapter 10
Leukemias
acute lymphoblastic, 755, 765, 766f, Chapter 16
acute myeloid, 759, 764f, 765, 766f, Chapter 16
cancer genome and, 761, 763, Chapter 16
cancer stem cells, 773, Chapter 16
chronic lymphocytic, 761, Chapter 16
chronic myelogenous, 770, Chapter 16
gene-expression analysis, 763, Chapter 16
genetic mutations in, 761, 763f, Chapter 16
genetics and, 755, 761, 763, Chapter 16
immunotherapy for, 769, Chapter 16
microRNAs and, 761, Chapter 16
new cases and deaths in US in 2019, 739f, Chapter 16
oncogenes and, 742EP, 744–746EP, 749-750, Chapter 16
protein-inhibiting drugs for, 770, Chapter 16
tumor-suppressor genes and, 749, Chapter 16
Leukocyte adhesion deficiency (LAD), 288HP, Chapter 7
***Listeria monocytogenes*,** 363, 422EP, 422EPf, 424EP, Chapter 8, Chapter 9
Longevity, 99, 132–133HP, 564, 566, 571f, 599–600, 602, Chapter 3, Chapter 12
Lysosomal storage disorders, 342HP, 342HPf, Chapter 8

Macular degeneration, 20–21HP, 471HP, Chapter 1, Chapter 10, Chapter 11
Mad cow disease, 76HP, Chapter 2
Malaria, 164, 741, 767, 776, 797, Chapter 4, Chapter 16, Chapter 17
Marijuana, 197, 255, Chapter 4, Chapter 6
Marker chromosome, 565–566, Chapter 12
Melanoma
BRAF genes in, 761, Chapter 16
genetic mutations in, 761, 763f, Chapter 16
immunotherapy for, 768–770, Chapter 16
new cases and deaths in US in 2019, 739f, Chapter 16
protein-inhibiting drugs for, 770, 658, Chapter 16
tumor-suppressor genes, 751t, Chapter 16
ultraviolet radiation and, 633, 634–635HP, Chapter 13, Chapter 16
Metabolism, 99, 103, 106–108, 113, 120HP, 123, 124f, 125–126, 129, 130fn, 133HP, 134f, 133–136, Chapter 3
capture and utilization of energy, 124, Chapter 3
defined, 130, Chapter 3
enzymes of, 16, 33EP, Chapter 1
inborn errors of, 478, Chapter 11
oxidation–reduction (redox) reactions, 123, Chapter 3
photosynthetic, 243, 245, Chapter 6
stages of, 124f, Chapter 3
Metastasis, 738, 749–750, 758fn, 758f, 761, Chapter 16
cell adhesion in, 287–288HP, Chapter 7
cell-surface properties of, 288HP, Chapter 7
defined, 288HP, Chapter 7
gap Junctions in, 298–299EP, 299EPf, Chapter 7
miRNAs in, 759, Chapter 16
oncogenes in, 749–750, Chapter 16
spread of, steps in, 287–288HPf, 288HP, Chapter 7
Methicillin resistant Staph aureus (MRSA), 120HP, Chapter 3.
Microbiome, 16, Chapter 1
Mitochondrial diseases, 234-235HP, 235HPf, Chapter 5
Multiple sclerosis (MS), 194, 784HP, 786HP, Chapter 4, Chapter 17
Muscle fiber, 410, 411f, 412, 415, 415f, 416, Chapter 9
Muscular dystrophy, 166, 402fn, 420, 532, 544, Chapter 4, Chapter 9, Chapter 11, Chapter 12
Mutagenic agents, 439, Chapter 10
Mutations, 433, 437f, 438, 439, 441, 449, 455–456HP, 459–460, 461f, 466, 468–470, 473HP, Chapter 10
altering structure of signaling proteins, 698HP, Chapter 15
in DNA, 21–22EP, Chapter 1
gain-of-function, 456HP, Chapter 10
genetic
in breast cancer, 738, 740f, 742, 747, 751t, 754f, 757, 765, 767f, 769, Chapter 16
colon cancer in, 738, 741, 742f, 754–757, 762–763, 763f, 772, 774, Chapter 16
in leukemias, 773, Chapter 16
in melanoma, 739, 754, 758, 761, 771f, 771–772, 739f, 751t, Chapter 16
in prostate cancer, 738, 742, 747, 754f, 755, 770, Chapter 16
in inherited disorders, 75, Chapter 2
in iPS cells, 21HP, Chapter 1
J. D., 357EP, Chapter 8
molecular structure of genes and, 6, Chapter 1
myosin, deafness and, 410, Chapter 9
nonsense, 517f, 531, Chapter 11
somatic, 699HP, Chapter 15
spontaneous rate of, 619, Chapter 13
viral, 27, Chapter 1

Nerve cells (neurons)
function of, 159, Chapter 4
postsynaptic, 194, 195f, 196–198, 196f, 176–177EP, Chapter 4
presynaptic, 194, 195f, 196f, 196–198, Chapter 4
structure of, 190f, Chapter 4
synaptic transmission, 195, 196f, 197, Chapter 4
Nerve gas, 118, 137, 197, Chapter 3, Chapter 4
Nervous system disorders, 195–197HP, 338, 387–389HP, 784HP, Chapter 5, Chapter 9, Chapter 10, Chapter 17
Neurofibrillary tangles (NFTs), 78HPf, 80HP, Chapter 2
Nicotine addiction, 176EP, Chapter 4
Niemann-Pick type C disease, 343HP, 361, Chapter 8
Non-Hodgkin's B-cell lymphoma, 769, Chapter 16
Nonself, distinguishing from self, 777, 790, 805, Chapter 17
Nonsteroidal anti-inflammatory drugs (NSAIDs), 742, Chapter 16

Oxygen monitoring, 224, Chapter 5
Ovarian cancer, 754, 757, 768, 772, Chapter 16

Pap smear, 748, 748f, Chapter 16
Parkinson's disease (PD), 235HP, Chapter 5
PET scan, 135, Chapter 3
Periodontal disease, 289HP, Chapter 7
Polycystic kidney diseases (PKD), 393HP, 393HPf, Chapter 9
Prader-Willi syndrome (PWS), 590, Chapter 12
Precocious puberty, 698HPt, 699HP, Chapter 15
Pregnancy, IgG-based immunity, 793, Chapter 17
Prilosec, 184, 184f, Chapter 4
Prions, 76HP, 302, Chapter 2, Chapter 7
Prostate cancer
cause of, 740fn, 741–742, 746, Chapter 16
genetics and, 755, Chapter 16
immunotherapy, 768–770, Chapter 16
internal radiation therapy, 44, Chapter 2 engineering linkage
new cases and deaths in US in 2019, 629f, Chapter 16
protein-inhibiting drugs for, 770, Chapter 16

PSA test for, 86, Chapter 2
RB mutations, 752, Chapter 16
research efforts, 738, Chapter 16
TP53 mutations in, 753, 763f, Chapter 16
Prozac, 197, Chapter 4

Radiation, as carcinogen, 439, 452, 605, 628, 631–634, 633f, 634–635HP, 649, 650f, Chapter 10, Chapter 13, Chapter 14
Retinoblastoma, 751–753, 751t, 752f, 765, Chapter 16
Retroviruses, 462, 714, 741, 858, Chapter 10, Chapter 15, Chapter 16, Chapter 18
Rheumatoid arthritis, 785–786HP, Chapter 17
Rheumatoid arthritis, 865, Chapter 18
RNA interference (RNAi), 316, 317f, 477, 507, 509, 509–510HP, Chapter 8, Chapter 11, Chapter 18
clinical applications of, 509–510, Chapter 11

Sandhoff's disease, 343HPt, Chapter 8
Scurvy, 271, Chapter 7
Self, distinguishing from nonself, 777, 782, 784HP, 805, Chapter 17
Sex chromosomes, 452, 554, 680–681HP, Chapter 10, Chapter 12, Chapter 14
Sexual arousal, 730, Chapter 15
Sickle cell anemia, 66, 66f, 97, 514, 536, Chapter 2, Chapter 11
Skin
blistering diseases, 284, 402fn, 783, Chapter 7, Chapter 9, Chapter 17
cancer (*see* melanoma)
grafts, 797, Chapter 17
tight junctions, 267, 292f, 294, 295f, 295f, 296, 298EP, Chapter 7
Smell (olfaction), 691, 707–708 707fn, Chapter 15
Smoking, 176EP, 471HP, 741, Chapter 4, Chapter 10, Chapter 16
Snake venom, 280, Chapter 7
Speech and language disorders, 468, Chapter 10
Sphingolipid storage diseases, 343HPt, Chapter 8
Spongiform encephalopathy, 76HP, Chapter 2
Statin drugs, 362, Chapter 8
Stem cells
adult, 19HP, 19HPf, 21HP, 24HPf, Chapter 1
cancer, 747, 748f, 773, 773fn, 774, Chapter 16
defined, 19HP, Chapter 1
embryonic, 19–21HP, 20HPf, Chapter 1
hematopoietic, 780HP, 780f, 786HP, Chapter 17
induced pluripotent, 21HP, 22HPf, 20HPf, Chapter 1
mesenchymal, 19HP, Chapter 1
Stroke, 280, 287HP, Chapter 7
Sulfatide lipidosis, 343HPt, Chapter 8
Systemic lupus erythematosus (SLE), 785HP, Chapter 17

Taste (gustation), 708, Chapter 15
Tay-Sachs disease, 342HP, 343HPt, Chapter 8
Testosterone, 49f, 59, 60f, 699f, 732, Chapter 2, Chapter 15
Thymus, 276f, 776, 776f, 780, 780f, 784HP, 786HP, 805, 805f, 806, Chapter 7, Chapter 17
Tolerance, immunologic, 782, 785HP, 786HP, 787, 806, 807, Chapter 17
Trans fats, 59, Chapter 2
Transplant rejection, 797, Chapter 17
Treatment of, 786HP, 802EP, 808, Chapter 17
Tuberculosis, 118HP, Chapter 3
Tumor necrosis factor (TNF), 732, Chapter 15
Type 1 diabetes (T1D), 785HP, Chapter 17

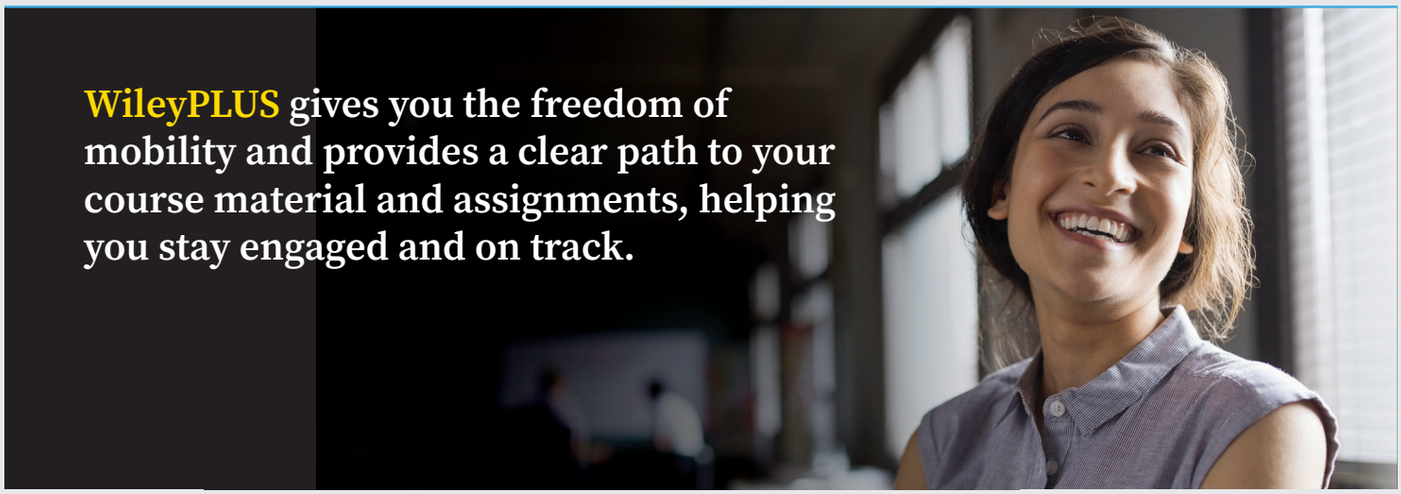
Ultraviolet (UV) radiation, DNA damage from, 628, 633, 634HP, 635HP, Chapter 13

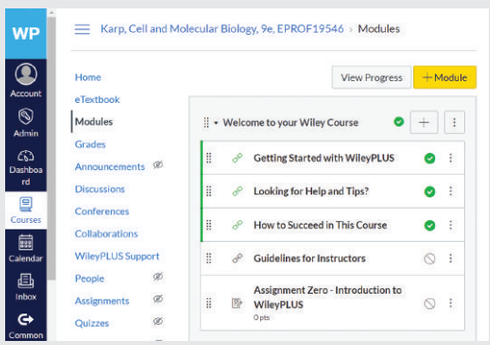
Vaccination, 783, Chapter 17
Vancomycin, 119HPt, 120HP, Chapter 3
Viagra, 730, Chapter 15
Viruses, 21HP, 22HPf, 22HP, 26–27, 29, 37–38, Chapter 1
benefits of, 29, Chapter 1
capsids of, 26, 27f, 27, Chapter 1
DNA replication in, 621, Chapter 13
host-cell specificity of, change in, 27, Chapter 1
host range of, 27, Chapter 1
innate immune response to, 777, 780, Chapter 17
oncogenes and, 742EP, 745EP, 746, 749, 749f, 750, 757–760, 762, 765, Chapter 16
properties of, 26, 27, 29, Chapter 1
protein on surface of, 27, Chapter 1
resistance to, interferon and, 775, 777f, 779, 781, 787, 789, 797, 810, Chapter 17
treatment with RNAi, 509–510HP, Chapter 11
viral infections, types of, 28, Chapter 1
Vision, 705, 707, 709, Chapter 15
Vitamin C deficiency, 271, Chapter 7

X chromosome inactivation, 554fn, 554–556, 591, Chapter 12
Xeroderma pigmentosum (XP), 633, 634HP, Chapter 13

Zellweger syndrome (ZS), 236HP, Chapter 5

WileyPLUS





When course materials are presented in an organized way, you are more likely to stay focused, develop mastery, and participate in class. WileyPLUS provides you a clear path through the course material.

Starting with Wiley's quality curated content and adaptive practice questions, you can quickly access material most relevant to your course and understand topics that are most challenging to you. This easy-to-use, intuitive interface saves you time, helps you stay on track, and keeps you motivated throughout your course.

Customized Content

Your coursehas been customized with videos, documents, pages, and relevant links to keep you motivated.

Interactive eTextbook

You can easily search content, highlight and take notes, access instructor's notes and highlights, and read offline.

Adaptive Practice Questions

Quickly identify areas that are most challenging for you and then focus on material most relevant to your needs.

Linear Design and Organization

Course modules organized by learning objective include eTextbook content, videos, animations, interactives, and practice questions.

Calendar

The course calendar syncs with other features—like assignments and syllabus—so any updates from your instructor will immediately appear.

Student App

You can view due dates, submit assignments, read material, and communicate with your instructor all from your phone.

www.wileyplus.com/student-register

WILEY

Preface to the Ninth Edition

For over two decades, Dr. Gerald Karp wrote, and continuously revised, *Cell and Molecular Biology: Concepts and Experiments*. During this time, he maintained a consistent focus on combining rigor with accessibility, so that even students without prior training in cell biology, molecular biology, or biochemistry have been able to learn cell biology not just as a collection of facts but as a process of discovery. The value of this approach is that the lessons learned extend far beyond the field of cell biology, and provide a way for students to learn how science works, how new experiments can overturn previous dogmas, and how new techniques can lead to groundbreaking discovery. This approach makes cell biology come alive.

After seven editions, Dr. Karp was ready to move on to other adventures. We were excited to take on the challenge of continuing Dr. Karp's unique approach to teaching cell biology, while continuing to put students first. Our goal has been to build upon Karp's hallmark experimental approach by bringing in our own unique perspectives and harnessing today's technology. With our new ***Experimental Walkthrough*** feature, available in WileyPLUS Learning Space, students can see first-hand how key experimental techniques are performed in the lab. These offer a mix of video, which show how researchers carry out experiments, and 3D animations that show a molecular-level view of how the experiments work. These Walkthroughs provide context and a visual explanation that helps make these important experimental techniques more concrete.

One key feature of the past editions was to highlight how cell biology impacts our daily lives, in terms of medicine and other areas of society. The ***Human Perspectives*** sections highlight human interest stories to reinforce and review basic cell biology, and also provide examples of how fundamental discoveries have progressed into clinical practice. We have expanded this feature so that now every chapter has at least one Human Perspectives section. As part of this feature we report on the latest clinical trials for various cell biology-based therapies and drugs, a feature that we hope will inspire students who are pursuing careers in health sciences fields. In addition to the full Human Perspectives sections, each chapter is now introduced with a short "chapter opener" designed to generate enthusiasm about the science in each chapter through provocative issues or questions. We hope that this will give our readers the opportunity to think more about the links between science, society, and our place in the universe.

In the ninth edition we have introduced two new sections for each chapter. ***Green Cells*** sections highlight important features of plant cell biology that underscore central concepts from each chapter and illustrate how the study of plants has informed our understanding of cells in general. ***Engineering Linkages*** sections address the interface between cell biology and biomedical engineering and are intended to make the cell biology material more accessible for engineering students while introducing biology students to important trends in bioengineering.

Working on the 9th edition has given us renewed admiration for Dr. Karp's writing and his ability to keep track of the cutting edge in the full range of topics that comprise cell and molecular biology. In this and future editions of *Karp's Cell and Molecular Biology: Concepts and Experiments*, we are dedicated to carrying out Dr. Karp's original mission of providing an interesting, modern and readable text that is grounded in the experimental approach. We welcome your ideas and feedback as we continue our work on this text, so please feel free to get in touch.

Janet Iwasa (jiwasa@biochem.utah.edu)
Wallace Marshall (Wallace.Marshall@ucsf.edu)

WileyPLUS

WileyPLUS connects the text to carefully selected video, animations, and diagrams, and provides students many opportunities for self-study and practice. Instructors can customize their course content for students, create online homework and quizzes, and have insight into student activity through data analytics and reporting features. To try it, visit www.wileyplus.com. Here are some of the resources available in WileyPLUS:

- Experimental Walkthrough Videos
- Quantitative Tutorial Videos
- Cell View Animations
- Video Library
- Biology Basics Animations
- Instructor's Manual
- Clicker Questions
- Lecture PowerPoint Presentations
- Testbank and Answer Key

Acknowledgments

Gerald Karp, who dedicated many years to carefully and thoughtfully writing and editing this text, has left a remarkable legacy that we are grateful to inherit. In putting together this edition, we continue to be thankful for his insight, wisdom, and advice that was always cheerfully and generously provided to us when we first took on the project.

We are grateful to many individuals at John Wiley & Sons who made this edition possible. Kevin Witt brought us on board at the beginning stages and infused us with his enthusiasm for the project. For the ninth edition, we are incredibly appreciative of all of the assistance we have received from our Wiley team, especially Maria Guarascio, Georgia Larsen, Lauren Elfers, and Alan Halfen. We thank Karen Trost for her sharp eyes and careful editing of the text and Tristann Jones from Lumina Datamatics for coordinating the text production.

In preparing the 9th edition we are grateful for the instructors and students who have pointed out errors and omissions, or suggested new points of interest. In writing the Green Cells and Engineering Linkage sections, we benefited from helpful discussions with Liz Haswell (Washington University), Magdalena Bezanilla (Dartmouth University), and Hongmin Qin (Texas A&M).

Janet Iwasa thanks Rob Savage, Dyche Mullins, and Jack Szostak, for inspiring and guiding her along the path toward becoming a biologist. Janet is particularly grateful for the support of her family, Adam, Aki, and Kenzo, and the lifelong encouragement of her parents, Kuni and Mieko.

Wallace Marshall thanks his scientific mentors, Rolf Sternglanz, John Sedat, and Joel Rosenbaum, for launching him in the direction that he went. He thanks his parents, Clifford and Adele Marshall, for making him who he is. And he thanks his family, Jennifer and Wyeth, for continued inspiration and support.

We also wish to thank all reviewers of this and previous editions:

STEVE ALAS
California State Polytechnic University, Pomona
RAVI ALLADA
Northwestern University
LINDA AMOS
MRC Laboratory of Molecular Biology
DEREK APPLEWHITE
Reed College
KARL J. AUFDERHEIDE
Texas A&M University
GERALD T. BABCOCK
Michigan State University
KENNETH J. BALAZOVICH
University of Michigan
WILLIAM E. BALCH
The Scripps Research Institute
JAMES BARBER
Imperial College of Science—Wolfson Laboratories
JOHN D. BELL
Brigham Young University
WENDY A. BICKMORE
Medical Research Council, United Kingdom
ASHOK BIDWAI
West Virginia University
ALLAN BLAKE
Seton Hall University
MARTIN BOOTMAN
Babraham Institute
DAVID BOURGAIZE
Whittier College
DANIEL BRANTON
Harvard University
THOMAS R. BREEN
Southern Illinois University
DOUGLAS J. BRIANT
University of Victoria
SHARON K. BULLOCK
Virginia Commonwealth University
MICHAEL B. BURNS
Loyola University Chicago
RODERICK A. CAPALDI
University of Oregon
GORDON G. CARMICHAEL
University of Connecticut Health Center
RATNA CHAKRABARTI
University of Central Florida
KENT D. CHAPMAN
University of North Texas
K. H. ANDY CHOO
Royal Children's Hospitals—The Murdoch Institute
DENNIS O. CLEGG
University of California—Santa Barbara
GARY COOMBS
Waldorf University
KATE COOPER
Loras College
RONALD H. COOPER
University of California—Los Angeles
PHILIPPA D. DARBRE
University of Reading
ROGER W. DAVENPORT
University of Maryland
RICHARD E. DEARBORN
Albany College of Pharmacy
SUSAN DESIMONE
Middlebury College
LINDA DEVEAUX
Idaho State University
BARRY J. DICKSON
Research Institute of Molecular Pathology
DAVID DOE
Westfield State College
ROBERT S. DOTSON
Tulane University
JENNIFER A. DOUDNA
Yale University
MICHAEL EDIDIN
Johns Hopkins University
EVAN E. EICHLER
University of Washington
ARRI EISEN
Emory University
ROBERT FILLINGAME
University of Wisconsin Medical School
ORNA COHEN-FIX
National Institute of Health, Laboratory of Molecular and Cellular Biology
JACEK GAERTIG
University of Georgia
BENJAMIN GLICK
The University of Chicago
BREE GRILLO-HILL
San Jose State University
REGINALD HALABY
Montclair State University
MICHAEL HAMPSEY
University of Medicine and Dentistry of New Jersey
MICHAEL HARRINGTON
University of Alberta
MARCIA HARRISON
Marshall University
R. SCOTT HAWLEY
Stowers Institute
REBECCA HEALD
University of California, Berkeley
ROBERT HELLING
University of Michigan
ANNE HEMSLEY
Antelope Valley College
MARK HENS
University of North Carolina, Greensboro
ARTHUR HORWICH
Yale University School of Medicine
JEN-CHIH HSIEH
State University of New York at Stony Brook
JOEL A. HUBERMAN
Roswell Park Cancer Institute
GREGORY D. D. HURST
University College London
KEN JACOBSON
University of North Carolina
MARIE JANICKE
University at Buffalo—SUNY
MICHAEL JONZ
University of Ottawa
ROLAND KAUNAS
Texas A&M University
HAIG H. KAZAZIAN, JR.
University of Pennsylvania
LAURA R. KELLER
Florida State University
TOM KELLER
Florida State University
REBECCA KELLUM
University of Kentucky
GREG M. KELLY
University of Western Ontario
NEMAT O. KEYHANI
University of Florida
KIM KIRBY
University of Guelph
NANCY KLECKNER
Harvard University
WERNER KÜHLBRANDT
Max-Planck-Institut für Biophysik
JAMES LAKE
University of California—Los Angeles
MELINDA LARSEN
University at Albany SUNY
AMANDA LEE
Kapiolani Community College
CLAIRE M. LEONARD
William Paterson University
ROBERT C. LIDDINGTON
Burnham Institute

FAITH LIEBL
Southern Illinois University, Edwardsville
VISHWANATH R. LINGAPPA
University of California—San Francisco
JEANNETTE M. LOUTSCH
Arkansas State University
JON LOWRANCE
Lipscomb University
MARGARET LYNCH
Tufts University
CHARLES MALLERY
University of Miami
MICHAEL A. MCALEAR
Wesleyan University
ARDYTHE A. MCCRACKEN
University of Nevada—Reno
THOMAS MCKNIGHT
Texas A&M University
JOANN MEERSCHAERT
St. Cloud State University
JOHN MENNINGER
University of Iowa
KIRSTEN MONSEN
Montclair State University
MICHELLE MORITZ
University of California—San Francisco
ROBERT MORRIS
Wheaton College
ANGELA NELSON
Western Governors University
ANDREW NEWMAN
Cambridge University
ALAN NIGHORN
University of Arizona
ROBERT M. NISSEN
California State University, Los Angeles
JONATHAN NUGENT
University of London
VERONICA C. NWOSU
North Carolina Central University
MIKE O'DONNELL
Rockefeller University
GREG ODORIZZI
University of Colorado, Boulder
LEOCADIA PALIULIS
Bucknell University
ANN PATERSON
Williams Baptist College
JAMES G. PATTON
Vanderbilt University
HUGH R. B. PELHAM
MRC Laboratory of Molecular Biology
JONATHON PINES
Institute of Cancer Research
JOEL B. PIPERBERG
Millersville University
DEBRA PIRES
University of California—Los Angeles
MITCH PRICE
Pennsylvania State University
CHARLES PUTNAM
University of Arizona
DAVID REISMAN
University of South Carolina
DONNA RITCH
University of Wisconsin—Green Bay
JOEL L. ROSENBAUM
Yale University
EDMUND B. RUCKER III
University of Kentucky
WOLFRAM SAENGER
Freie Universitat Berlin
SHIVENDRA V. SAHI
Western Kentucky University
JOSHUA SANDQUIST
Grinnell College
JAMIE SANFORD
Ohio Northern University
PRASANNA SATPUTE-KRISHNAN
National Institute of Health
INDER M. SAXENA
University of Texas, Austin
RANDY SCHEKMAN
University of California—Berkeley
SANDRA SCHMID
The Scripps Research Institute
TRINA SCHROER
Johns Hopkins University
TIM SCHUH
St. Cloud State University
DAVID SCHULTZ
University of Louisville
ROD SCOTT
Wheaton College
ROBERT M. SEISER
Roosevelt University
KATIE SHANNON
University of North Carolina—Chapel Hill
JOEL B. SHEFFIELD
Temple University
ERIC SHELDEN
Washington State University
JINGSHI SHEN
University of Colorado Boulder
DENNIS SHEVLIN
College of New Jersey
JEFF SINGER
Portland State University
ROGER D. SLOBODA
Dartmouth College
HARRIETT E. SMITH-SOMERVILLE
University of Alabama
SHANNON STEVENSON
University of Minnesota Duluth
BRUCE STILLMAN
Cold Spring Harbor Laboratory
ADRIANA STOICA
Georgetown University
ANN STURTEVANT
University of Michigan-Flint
COLLEEN TALBOT
California State Univerity, San Bernardino
WILLIAM TERZAGHI
Wilkes University
GISELLE THIBAUDEAU
Mississippi State University
JEFFREY L. TRAVIS
University at Albany—SUNY
PAUL TWIGG
University of Nebraska-Kearney
NIGEL UNWIN
MRC Laboratory of Molecular Biology
AJIT VARKI
University of California—San Diego
JOSE VAZQUEZ
New York University
CLAIRE E. WALCZAK
Indiana University
JULIE WALTON
American National University
PAUL E. WANDA
Southern Illinois University, Edwardsville
JENNIFER WATERS
Harvard University
CHRIS WATTERS
Middlebury College
ANDREW WEBBER
Arizona State University
BEVERLY WENDLAND
Johns Hopkins University
GARY M. WESSEL
Brown University
MICHELLE WILSON
The University of Texas at Dallas
ERIC V. WONG
University of Louisville
ANDREW WOOD
Southern Illinois University
GARY YELLEN
Harvard Medical School
MASASUKE YOSHIDA
Tokyo Institute of Technology
DANIELA ZARNESCU
University of Arizona
JIANZHI ZHANG
University of Michigan
ROBERT A. ZIMMERMAN
University of Massachusetts

Contents

1 Introduction to the Study of Cell and Molecular Biology 1

1.1 The Discovery of Cells 2
Microscopy 2
Cell Theory 3
1.2 Basic Properties of Cells 3
Cells Are Highly Complex and Organized 3
Cells Possess a Genetic Program and the Means to Use It 6
Cells Are Capable of Producing More of Themselves 6
Cells Acquire and Utilize Energy 6
Cells Carry Out a Variety of Chemical Reactions 6
Cells Engage in Mechanical Activities 7
Cells Are Able to Respond to Stimuli 7
Cells Are Capable of Self-Regulation 7
Cells Evolve 8
1.3 Two Fundamentally Different Classes of Cells 8
Characteristics That Distinguish Prokaryotic and Eukaryotic Cells 10
Types of Prokaryotic Cells 15
Types of Eukaryotic Cells 17
The Human Perspective: The Prospect of Cell Replacement Therapy 18
The Sizes of Cells and Their Components 23
1.4 Viruses and Viroids 26
Experimental Pathways: The Origin of Eukaryotic Cells 30
1.5 Green Cells: *Volvox*, an Experiment in Multicellularity 35
1.6 Engineering Linkage: Tissue Engineering 36

2 The Chemical Basis of Life 39

2.1 Covalent Bonds 40
Polar and Nonpolar Molecules 40
Ionization 42
The Human Perspective: Do Free Radicals Cause Aging? 42
2.2 Engineering Linkage: Radionuclides for Imaging and Treatment 43
2.3 Noncovalent Bonds 44
Ionic Bonds: Attractions between Charged Atoms 44
Hydrogen Bonds 45
Hydrophobic Interactions and van der Waals Forces 45
The Life-Supporting Properties of Water 47
2.4 Acids, Bases, and Buffers 48
2.5 The Nature of Biological Molecules 49
Functional Groups 50
A Classification of Biological Molecules by Function 50
2.6 Green Cells: Chemical Fertilizers 52
2.7 Four Types of Biological Molecules 53
Carbohydrates 53
Lipids 58
Building Blocks of Proteins 60
Primary and Secondary Structures of Proteins 65
Tertiary Structure of Proteins 67
Quaternary Structure of Proteins 72
Protein Folding 74
The Human Perspective: Protein Misfolding Can Have Deadly Consequences 76
Experimental Pathways: Chaperones—Helping Proteins Reach Their Proper Folded State 82
Proteomics and Interactomics 86
Protein Engineering 88
Protein Adaptation and Evolution 92
Nucleic Acids 93
2.8 The Formation of Complex Macromolecular Structures 95
The Assembly of Tobacco Mosaic Virus Particles 95
The Assembly of Ribosomal Subunits 96
Phase-Separated Compartments 96

3 Bioenergetics, Enzymes, and Metabolism 99

3.1 Bioenergetics 100
The Laws of Thermodynamics 100
Free Energy 103
3.2 Enzymes as Biological Catalysts 108
The Properties of Enzymes 109
Overcoming the Activation Energy Barrier 109
The Active Site 111
Mechanisms of Enzyme Catalysis 112
Enzyme Kinetics 117
The Human Perspective: The Growing Problem of Antibiotic Resistance 118
3.3 Metabolism 123
An Overview of Metabolism 123
Oxidation and Reduction: A Matter of Electrons 123
The Capture and Utilization of Energy 124
Metabolic Regulation 130

The Human Perspective: Caloric Restriction and Longevity 132
3.4 Green Cells: Regulation of Metabolism by the Light/Dark Cycle 134
3.5 Engineering Linkage: Using Metabolism to Image Tumors 135

4 The Structure and Function of the Plasma Membrane 137

4.1 Introduction to the Plasma Membrane 138
An Overview of Membrane Functions 138
A Brief History of Studies on Plasma Membrane Structure 140
4.2 The Chemical Composition of Membranes 142
Membrane Lipids 142
The Nature and Importance of the Lipid Bilayer 144
The Asymmetry of Membrane Lipids 146
Membrane Carbohydrates 146
4.3 Membrane Proteins 148
Integral Membrane Proteins 148
Peripheral Membrane Proteins 149
Lipid-Anchored Membrane Proteins 150
Studying the Structure and Properties of Integral Membrane Proteins 151
4.4 Membrane Lipids and Membrane Fluidity 156
The Importance of Membrane Fluidity 157
Maintaining Membrane Fluidity 157
Lipid Rafts 157
4.5 The Dynamic Nature of the Plasma Membrane 159
The Diffusion of Membrane Proteins after Cell Fusion 159
Restrictions on Protein and Lipid Mobility 159
The Red Blood Cell: An Example of Plasma Membrane Structure 163
4.6 The Movement of Substances across Cell Membranes 166
The Energetics of Solute Movement 166
Formation of an Electrochemical Gradient 167
Diffusion of Substances through Membranes 168
Experimental Pathways: The Acetylcholine Receptor 176
Facilitated Diffusion 180
Active Transport 181
The Human Perspective: Defects in Ion Channels and Transporters as a Cause of Inherited Disease 184
4.7 Membrane Potentials and Nerve Impulses 189
The Resting Potential 190
The Action Potential 191
Propagation of Action Potentials as an Impulse 193
Neurotransmission: Jumping the Synaptic Cleft 194
4.8 Green Cells: Electrical Signaling in Plants 198
4.9 Engineering Linkage: Neurotechnology 199

5 Aerobic Respiration and the Mitochondrion 203

5.1 Mitochondrial Structure and Function 204
Mitochondrial Membranes 206
The Mitochondrial Matrix 206
5.2 Aerobic Metabolism in the Mitochondrion 209
The Tricarboxylic Acid (TCA) Cycle 209
The Importance of Reduced Coenzymes in the Formation of ATP 209
The Human Perspective: The Role of Anaerobic and Aerobic Metabolism in Exercise 213
5.3 The Role of Mitochondria in the Formation of ATP 215
Oxidation–Reduction Potentials 215
Electron Transport 216
Types of Electron Carriers 217
5.4 Engineering Linkage: Measuring Blood Oxygen 224
5.5 Establishment of a Proton-Motive Force 225
5.6 The Machinery for ATP Formation 226
The Structure of ATP Synthase 227
The Binding Change Mechanism of ATP Formation 227
Other Roles for the Proton-Motive Force in Addition to ATP Synthesis 233
5.7 Peroxisomes 233
The Human Perspective: Diseases That Result from Abnormal Mitochondrial or Peroxisomal Function 234
5.8 Green Cells: Glyoxysomes 237

6 Photosynthesis and the Chloroplast 239

6.1 The Origin of Photosynthesis 240
6.2 Chloroplast Structure 241
6.3 An Overview of Photosynthetic Metabolism 243
6.4 The Absorption of Light 244
6.5 Green Cells: Chromoplasts 246
6.6 Photosynthetic Units and Reaction Centers 247
Oxygen Formation: Coordinating the Action of Two Different Photosynthetic Systems 248
The Operations of Photosystem II and Photosystem I 248
6.7 Photophosphorylation 255
6.8 Carbon Dioxide Fixation and the Synthesis of Carbohydrate 256
Carbohydrate Synthesis in C_3 Plants 256

The Human Perspective: Global Warming and Carbon Sequestration 260
Carbohydrate Synthesis in C_4 and CAM Plants 262
6.9 **Engineering Linkage: Photodynamic Therapy** 264

7 Interactions Between Cells and Their Environment 267

7.1 **Extracellular Interactions** 268
The Extracellular Matrix 268
Dynamic Properties of the Extracellular Matrix 277
7.2 **Engineering Linkage: Organoids** 277
7.3 **Interactions of Cells with Extracellular Materials** 278
Integrins 278
Focal Adhesions 281
Hemidesmosomes 283
7.4 **Interactions of Cells with Other Cells** 284
Selectins 286
The Human Perspective: The Role of Cell Adhesion in Inflammation and Metastasis 287
The Immunoglobulin Superfamily 289
Cadherins 290
Adherens Junctions and Desmosomes 292
7.5 **Tight Junctions: Sealing the Extracellular Space** 294
7.6 **Intercellular Communication** 296
Gap Junctions 296
Experimental Pathway: The Role of Gap Junctions in Intercellular Communication 298
Plasmodesmata 301
Long-Range Intercellular Communication 301
7.7 **Cell Walls** 303
7.8 **Green Cells: Cell Walls and Plant Terrestrialization** 306

8 Cytoplasmic Membrane Systems: Structure, Function, and Membrane Trafficking 307

8.1 **An Overview of the Endomembrane System** 308
8.2 **A Few Approaches to the Study of Endomembranes** 311
Insights Gained from Autoradiography 311
Insights Gained from the Use of Fluorescent Proteins 312
Insights Gained from the Analysis of Subcellular Fractions 313
Insights Gained from the Use of Cell-Free Systems 314
Insights Gained from the Study of Mutant Phenotypes 315
8.3 **The Endoplasmic Reticulum** 317
The Smooth Endoplasmic Reticulum 318
The Rough Endoplasmic Reticulum 319
ER to Golgi Vesicular Transport 329
8.4 **The Golgi Complex** 330
Glycosylation in the Golgi Complex 330
The Movement of Materials through the Golgi Complex 332
8.5 **Types of Vesicle Transport** 335
COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex 337
COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER 338
Beyond the Golgi Complex: Sorting Proteins at the TGN 340
The Human Perspective: Disorders Resulting from Defects in Lysosomal Function 342
Targeting Vesicles to a Particular Compartment 344
8.6 **Engineering Linkage: Extracellular Vesicles for Drug Delivery** 347
8.7 **Lysosomes** 348
8.8 **Green Cells: Plant Cell Vacuoles** 350
8.9 **The Endocytic Pathway: Moving Membrane and Materials into the Cell Interior** 351
Endocytosis 351
Experimental Pathways: Receptor-Mediated Endocytosis 355
Phagocytosis 362
8.10 **Posttranslational Uptake of Proteins by Peroxisomes, Mitochondria, and Chloroplasts** 363
Uptake of Proteins into Peroxisomes 364
Uptake of Proteins into Mitochondria 364
Uptake of Proteins into Chloroplasts 366

9 The Cytoskeleton and Cell Motility 369

9.1 **Overview of the Major Functions of the Cytoskeleton** 370
9.2 **Structure and Function of Microtubules** 372
Structure and Composition of Microtubules 372
Microtubule-Associated Proteins 372
Microtubules as Structural Supports and Organizers 372
Microtubules as Agents of Intracellular Motility 375
9.3 **Green Cells: Why the Woodbine Twineth** 376
9.4 **Motor Proteins: Kinesins and Dyneins** 377
Motor Proteins Traverse the Microtubular Cytoskeleton 377
Kinesins 378
Experimental Pathway: The Step Size of Kinesin 379
Cytoplasmic Dynein 382

9.5 Microtubule-Organizing Centers (MTOCs) 383
Centrosomes **383**
Basal Bodies and Other MTOCs **385**
Microtubule Nucleation **385**
The Dynamic Properties of Microtubules **385**
The Underlying Basis of Microtubule Dynamics **387**
9.6 Structure and Function of Cilia and Flagella 390
Structure of Cilia and Flagella **392**
The Human Perspective: The Role of Cilia in Development and Disease **393**
Growth by Intraflagellar Transport **396**
The Mechanism of Ciliary and Flagellar Locomotion **396**
9.7 Intermediate Filaments 398
Intermediate Filament Assembly and Disassembly **399**
Types and Functions of Intermediate Filaments **400**
9.8 Actin and Myosin 402
Actin Structure **402**
Actin Filament Assembly and Disassembly **403**
Myosin: The Molecular Motor of Actin **405**
Conventional (Type II) Myosins **405**
Unconventional Myosins **407**
9.9 Muscle Organization and Contraction 410
Organization of Sarcomeres **410**
The Sliding Filament Model of Muscle Contraction **410**
9.10 Engineering Linkage: Muscle Biomechanics 416
9.11 Actin-Binding Proteins 417
9.12 Cellular Motility 420
Experimental Pathway: Studying Actin-Based Motility Without Cells **422**
Actin-Dependent Processes During Development **427**
Axonal Outgrowth **427**
9.13 The Bacterial Cytoskeleton 430

10 The Nature of the Gene and the Genome 433

10.1 The Concept of a Gene as a Unit of Inheritance 434
10.2 The Discovery of Chromosomes 435
10.3 Chromosomes as the Carriers of Genetic Information 436
Genetic Analysis in *Drosophila* **437**
Crossing Over and Recombination **438**
Mutagenesis and Giant Chromosomes **439**
10.4 The Chemical Nature of the Gene 441
The Structure of DNA **441**
Experimental Pathways: The Chemical Nature of the Gene **441**
The Watson–Crick Proposal **447**
The Importance of the Watson–Crick Proposal **447**
DNA Supercoiling **450**
10.5 The Complexity of the Genome 452
DNA Denaturation **452**
DNA Renaturation **452**
The Human Perspective: Diseases That Result from Expansion of Trinucleotide Repeats **455**
10.6 The Stability of the Genome 458
Whole-Genome Duplication (Polyploidization) **458**
Duplication and Modification of DNA Sequences **459**
Evolution of Globin Genes **460**
The Dynamic Nature of the Genome: "Jumping Genes" **461**
10.7 Sequencing Genomes: The Footprints of Biological Evolution 464
Number of Protein-Coding Genes in the Human Genome **465**
Comparative Genomics: "If It's Conserved, It Must Be Important" **466**
10.8 Engineering Linkage: Engineering Genomes 467
10.9 The Genetic Basis of "Being Human" 468
What Genes Are Unique to the Human Lineage? **468**
Genetic Variation within the Human Species Population **470**
The Human Perspective: Application of Genomic Analyses to Medicine **471**
10.10 Green Cells: Gene Transfer by *Agrobacterium tumefaciens* 475

11 The Central Dogma: DNA to RNA to Protein 477

11.1 The Relationships among Genes, Proteins, and RNAs 478
Evidence That DNA Is the Genetic Material **478**
An Overview of the Flow of Information through the Cell **478**
The Role of RNA Polymerases in Transcription **481**
11.2 An Overview of Transcription in Both Prokaryotic and Eukaryotic Cells 483
Transcription in Bacteria **483**
Transcription and RNA Processing in Eukaryotic Cells **485**
11.3 Synthesis and Processing of Eukaryotic Ribosomal and Transfer RNAs 487
Synthesis and Processing of the rRNA Precursor **488**
The Role of snoRNAs in the Processing of Pre-rRNA **488**
Synthesis and Processing of the 5S rRNA **490**
Transfer RNAs **491**

11.4 Synthesis and Structure of Eukaryotic Messenger RNAs 491
The Formation of Heterogeneous Nuclear RNA **491**
The Machinery for mRNA Transcription **492**
The Structure of mRNAs **494**
Split Genes: An Unexpected Finding **495**
The Processing of Eukaryotic Messenger RNAs **498**
Evolutionary Implications of Split Genes and RNA Splicing **503**
Creating New Ribozymes in the Laboratory **506**
11.5 Small Regulatory RNAs and RNA Silencing Pathways 507
The Human Perspective: Clinical Applications of RNA Interference **509**
MicroRNAs: Small RNAs That Regulate Gene Expression **510**
piRNAs: A Class of Small RNAs That Function in Germ Cells **512**
11.6 Green Cells: Long-Range siRNA Movement 512
11.7 CRISPR and Other Noncoding RNAs 513
CRISPR: Noncoding RNA in Bacteria **513**
Other Noncoding RNAs **513**
11.8 Encoding Genetic Information 514
The Properties of the Genetic Code **514**
Identifying the Codons **515**
11.9 Decoding the Codons: The Role of Transfer RNAs 517
The Structure of tRNAs **517**
tRNA Charging **519**
11.10 Translating Genetic Information 520
Initiation **521**
Elongation **524**
Experimental Pathways: The Role of RNA as a Catalyst **526**
Termination **531**
mRNA Surveillance and Quality Control **531**
Polyribosomes **532**
11.11 Engineering Linkage: DNA Origami 534

12 Control of Gene Expression 537

12.1 Control of Gene Expression in Bacteria 538
Organization of Bacterial Genomes **538**
The Bacterial Operon **538**
Riboswitches **542**
12.2 Engineering Linkage: Building Digital Logic with Genes 542
12.3 Structure of the Nuclear Envelope 543
The Nuclear Pore Complex and Its Role in Nucleocytoplasmic Trafficking **546**
RNA Transport **549**
12.4 Chromosomes and Chromatin 550
Nucleosomes: The Lowest Level of Chromosome Organization **550**
Higher Levels of Chromatin Structure **552**
Heterochromatin **553**
X Chromosome Inactivation **554**
The Histone Code and Formation of Heterochromatin **555**
The Structure of a Mitotic Chromosome **558**
The Human Perspective: Chromosomal Aberrations and Human Disorders **560**
Telomeres **562**
Centromeres **565**
Epigenetics: There's More to Inheritance than DNA **566**
12.5 The Nucleus as an Organized Organelle 567
12.6 An Overview of Gene Regulation in Eukaryotes 570
12.7 Transcriptional Control 571
DNA Microarrays **572**
RNA Sequencing **574**
The Role of Transcription Factors in Regulating Gene Expression **575**
The Structure of Transcription Factors **577**
DNA Sites Involved in Regulating Transcription **579**
An Example of Transcriptional Activation: The Glucocorticoid Receptor **582**
Transcriptional Activation: The Role of Enhancers, Promoters, and Coactivators **583**
Transcriptional Repression **587**
12.8 Green Cells: The ABC Model and MADS Domain Transcription Factors 592
12.9 RNA Processing Control 593
12.10 Translational Control 594
Initiation of Translation **596**
Cytoplasmic Localization of mRNAs **597**
The Control of mRNA Stability **599**
The Role of MicroRNAs in Translational Control **600**
12.11 Posttranslational Control: Determining Protein Stability 601

13 DNA Replication and Repair 605

13.1 DNA Replication 606
13.2 DNA Replication in Bacterial Cells 610
Replication Forks and Bidirectional Replication **610**
Unwinding the Duplex and Separating the Strands **611**
The Properties of DNA Polymerases **611**
Semidiscontinuous Replication **613**
The Machinery Operating at the Replication Fork **614**
13.3 The Structure and Functions of DNA Polymerases 617
Exonuclease Activities of DNA Polymerases **617**
Ensuring High Fidelity during DNA Replication **618**

13.4 **Replication in Viruses** 621
13.5 **Engineering Linkage: Storing Data in DNA** 621
13.6 **DNA Replication in Eukaryotic Cells** 622
Initiation of Replication in Eukaryotic Cells 623
Restricting Replication to Once per Cell Cycle 623
The Eukaryotic Replication Fork 625
Replication and Nuclear Structure 626
Chromatin Structure and Replication 626
13.7 **DNA Repair** 628
Nucleotide Excision Repair 629
Base Excision Repair 629
Mismatch Repair 631
Double-Strand Breakage Repair 631
13.8 **Green Cells: Gamma Gardens** 632
13.9 **Between Replication and Repair** 633
The Human Perspective: Consequences of DNA Repair Deficiencies 634

14 Cell Division 637

14.1 **The Cell Cycle** 638
Phases of the Cell Cycle 638
Cell Cycles In Vivo 639
Control of the Cell Cycle 640
Experimental Pathways: The Discovery and Characterization of MPF 641
14.2 **M Phase: Mitosis and Cytokinesis** 651
Prophase 651
Prometaphase 658
Metaphase 661
Anaphase 662
Telophase 667
Cytokinesis 669
14.3 **Engineering Linkage: The Role of Membrane Tension in Cell Division** 672
14.4 **Green Cells: Unique Aspects of Plant Cell Division** 673
14.5 **Meiosis** 673
The Stages of Meiosis 677
The Human Perspective: Meiotic Nondisjunction and Its Consequences 680
Genetic Recombination during Meiosis 682

15 Cell Signaling and Signal Transduction: Communication between Cells 685

15.1 **The Basic Elements of Cell Signaling Systems** 686
15.2 **A Survey of Extracellular Messengers and Their Receptors** 689
15.3 **G Protein-Coupled Receptors and Their Second Messengers** 690
Signal Transduction by G Protein-Coupled Receptors 691
Experimental Pathway: The Discovery and Characterization of GTP-Binding Proteins 693
The Human Perspective: Disorders Associated with G Protein-Coupled Receptors 698
Second Messengers 699
The Specificity of G Protein-Coupled Responses 703
Regulation of Blood Glucose Levels 704
The Role of GPCRs in Sensory Perception 707
15.4 **Engineering Linkage: Biosensors in Medicine and Biology** 709
15.5 **Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction** 710
Receptor Dimerization 710
Protein Kinase Activation 710
Phosphotyrosine-Dependent Protein–Protein Interactions 710
Activation of Downstream Signaling Pathways 712
Ending the Response 713
The Ras-MAP Kinase Pathway 714
Signaling by the Insulin Receptor 717
Signaling Pathways in Plants 720
15.6 **Green Cells: Auxin Signaling** 721
15.7 **The Role of Calcium as an Intracellular Messenger** 721
IP_3 and Voltage-Gated Ca^{2+} Channels 721
Visualizing Cytoplasmic Ca^{2+} Concentration in Living Cells 722
Ca^{2+}-Binding Proteins 723
Regulating Calcium Concentrations in Plant Cells 725
15.8 **Convergence, Divergence, and Cross-Talk among Different Signaling Pathways** 726
15.9 **The Role of NO as an Intracellular Messenger** 729
NO as an Activator of Guanylyl Cyclase 730
Inhibiting Phosphodiesterase 730
15.10 **Apoptosis (Programmed Cell Death)** 731
The Extrinsic Pathway of Apoptosis 732
The Intrinsic Pathway of Apoptosis 734
Necroptosis 735
Signaling Cell Survival 735

16 Cancer 737

16.1 **Basic Properties of a Cancer Cell** 738
16.2 **The Causes of Cancer** 741
Experimental Pathways: The Discovery of Oncogenes 742
16.3 **Cancer: A Genetic Disorder** 747
Tumor-Suppressor Genes and Oncogenes: Brakes and Accelerators 749
The Cancer Genome 761
Gene-Expression Analysis 763
16.4 **Engineering Linkage: Therapeutic Radiation** 767

16.5 Green Cells: Plant-Based Chemotherapies 767
16.6 Strategies for Combating Cancer 768
Immunotherapy 768
Inhibiting the Activity of Cancer-Promoting Proteins 770
The Concept of a Cancer Stem Cell 773
Inhibiting the Formation of New Blood Vessels (Angiogenesis) 773

17 The Immune Response 775

17.1 An Overview of the Immune Response 776
Innate Immune Responses 777
Adaptive Immune Responses 779
17.2 Green Cells: The Plant Immune System 780
17.3 The Clonal Selection Theory as It Applies to B Cells 781
Features of B-cell Clonal Selection 781
Vaccination 783
The Human Perspective: Autoimmune Diseases 784
17.4 T Lymphocytes: Activation and Mechanism of Action 787
17.5 Selected Topics on the Cellular and Molecular Basis of Immunity 790
The Modular Structure of Antibodies 790
DNA Rearrangements That Produce Genes Encoding B- and T-Cell Antigen Receptors 793
Membrane-Bound Antigen Receptor Complexes 796
The Major Histocompatibility Complex 797
Experimental Pathways: The Role of the Major Histocompatibility Complex in Antigen Presentation 801
Distinguishing Self from Nonself 805
Lymphocytes Are Activated by Cell-Surface Signals 806
17.6 Engineering Linkage: Adoptive T-cell Therapy 808
17.7 Signal Transduction Pathways in Lymphocyte Activation 809

18 Techniques in Cell and Molecular Biology 811

18.1 The Light Microscope 812
Resolution 813
Visibility 814
Bright-Field Light Microscopy 815
Phase-Contrast Microscopy 815
Fluorescence Microscopy (and Related Fluorescence-Based Techniques) 816
Laser Scanning Confocal Microscopy 819
Super-Resolution Fluorescence Microscopy 820
Light Sheet Fluorescence Microscopy 821
18.2 Transmission Electron Microscopy 822
The Transmission Electron Microscope 822
Specimen Preparation for Electron Microscopy 824
18.3 Scanning Electron and Atomic Force Microscopy 828
Scanning Electron Microscopy 828
Atomic Force Microscopy 830
18.4 The Use of Radioisotopes 830
18.5 Cell Culture 831
Characteristics of Cell Cultures 832
Studying Cells Using Microfluidics 833
18.6 The Fractionation of a Cell's Contents by Differential Centrifugation 833
18.7 Isolation, Purification, and Fractionation of Proteins 834
Liquid Column Chromatography 834
Characterization of Proteins by Polyacrylamide Gel Electrophoresis 837
Characterization of Proteins by Spectrometry 839
18.8 Determining the Structure of Proteins and Multisubunit Complexes 840
18.9 Fractionation of Nucleic Acids 842
Separation of DNAs by Gel Electrophoresis 843
Separation of Nucleic Acids by Ultracentrifugation 843
18.10 Nucleic Acid Hybridization 845
18.11 Chemical Synthesis of DNA 846
18.12 Recombinant DNA Technology 847
Restriction Endonucleases 847
Formation of Recombinant DNAs 847
DNA Cloning 849
18.13 Enzymatic Amplification of DNA by PCR 851
Process of PCR 851
Applications of PCR 851
18.14 DNA Sequencing 853
18.15 DNA Libraries 855
Genomic Libraries 856
cDNA Libraries 856
18.16 DNA Transfer into Eukaryotic Cells and Mammalian Embryos 857
Transgenic Animals 858
Transgenic Plants 859
18.17 Gene Editing and Silencing 860
In Vitro Mutagenesis 860
Knockout Mice 860
RNA Interference 862
Genome Editing Using Engineered Nucleases 863
18.18 The Use of Antibodies 864

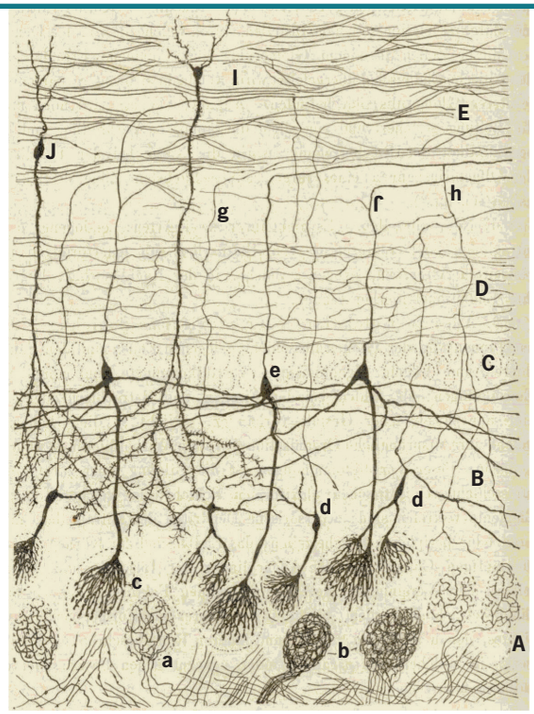
ADDITIONAL READINGS A-1

GLOSSARY G-1

INDEX I-1

CHAPTER 1

Introduction to the Study of Cell and Molecular Biology



Source: Adapted from the works of Ramon y Cajal.

Diagram of nerve cells from the cat brain, hand-drawn by Santiago Ramón y Cajal. Ramón y Cajal was the first to recognize that the brain is made up of huge numbers of individual cells, rather than a continuous connected network as proposed by his competitor, Camillo Golgi. Ramón y Cajal and Golgi fought a protracted battle over this point, but eventually the meticulous detail of Ramón y Cajal's work convinced the world that the brain is indeed a collective of individual cells.

We Are Cells

We are made of cells. Cells make up our skin, our organs, and our muscles. The brain, the seat of our thoughts and desires, is made of cells. Our blood vessels teem with cells. Fertilization is no more or less than a joining of two separate cells to produce a single new cell, which then multiplies to produce the embryo. When we grow from a tiny embryo into a large adult, we do so by adding more and more cells. When we get sick, it is often because our cells have run amok. And when we grow old, it is because our cells gradually give up the ghost. After we die and are buried, soon the only remnants of our existence are bones, teeth, and hair, structures that were sculpted in life by the ceaseless activity of cells. Many medicines work by changing how cells behave, and in recent years cells themselves are being used as medicines to cure sick people. Because all living things are made up of one or more cells, the origin of life corresponds to the origin of cells. Starting with this chapter, we explore what cells are and how they work, themes that will be expanded throughout this book.

CHAPTER OUTLINE

1.1 The Discovery of Cells

1.2 Basic Properties of Cells

1.3 Two Fundamentally Different Classes of Cells

The Human Perspective: The Prospect of Cell Replacement Therapy

1.4 Viruses and Viroids

Experimental Pathways: The Origin of Eukaryotic Cells

1.5 Green Cells: *Volvox*, an Experiment in Multicellularity

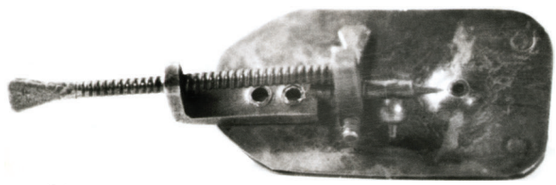
1.6 Engineering Linkage: Tissue Engineering

1.1 The Discovery of Cells

Cells, and the structures they comprise, are too small to be seen, heard, or touched directly. In spite of this tremendous handicap, cells are the subject of hundreds of thousands of publications each year, with virtually every aspect of their minuscule structure coming under scrutiny. In many ways, the study of cell and molecular biology stands as a tribute to human curiosity for seeking discoveries and to human creative intelligence for devising the complex instruments and elaborate techniques that allow these discoveries to be made. This is not to imply that cell and molecular biologists have a monopoly on these noble traits. At one end of the scientific spectrum, astronomers are utilizing an orbiting telescope to capture images of primordial galaxies that are so far from Earth they appear to us today as they existed more than 13 billion years ago, only a few hundred million years after the Big Bang. At the other end of the spectrum, nuclear physicists have forced protons to collide with one another at velocities approaching the speed of light, confirming the existence of a hypothesized particle—the Higgs boson—that is proposed to endow all other subatomic particles with mass. Clearly, our universe consists of worlds within worlds, all aspects of which make for fascinating study.

As will be apparent throughout this book, cell and molecular biology is *reductionist*; it is based on the view that knowledge of the parts of the whole can explain the character of the whole. When viewed in this way, our feeling for the wonder and mystery of life may be replaced by the need to explain everything in terms of the workings of the "machinery" of the living system. Although we may feel less of a sense of wonder, it is hoped that this loss can be replaced by an equally strong appreciation for the beauty and complexity of the mechanisms underlying cellular activity.

(a)



(b)

Source: (*a*) The Granger Collection, New York; inset Science & Society Picture Library/SSPL/Getty Images; (*b*) Bettmann/Getty Images.

FIGURE 1.1 The discovery of cells. **(a)** One of Robert Hooke's more ornate compound (double-lens) microscopes. (Inset) Hooke's drawing of a thin slice of cork, showing the honeycomb-like network of "cells." **(b)** Single-lens microscope used by Antonie van Leeuwenhoek to observe bacteria and other microorganisms. The biconvex lens, which was capable of magnifying an object approximately 270 times and providing a resolution of approximately 1.35 µm, was held between two metal plates.

Microscopy

Because of their small size, cells can only be observed with the aid of a **microscope**, an instrument that provides a magnified image of a tiny object. We do not know when humans first discovered the remarkable ability of curved-glass surfaces to bend light and form images. Spectacles were first made in Europe in the thirteenth century, and the first compound (double-lens) light microscopes were constructed by the end of the sixteenth century. By the mid-1600s, a handful of pioneering scientists had used their handmade microscopes to uncover a world that would never have been revealed to the naked eye. The discovery of cells (**Figure 1.1a**) is generally credited to Robert Hooke, an English microscopist who, at age 27, was awarded the position of curator of the Royal Society of London, England's foremost scientific academy. One of the many questions Hooke attempted to answer was why stoppers made of cork (part of the bark of trees) were so well suited to holding air in a bottle. As he wrote in 1665: "I took a good clear piece of cork, and with a Pen-knife sharpen'd as keen as a Razor, I cut a piece of it off, and . . . then examining it with a *Microscope*, me thought I could perceive it to appear a little porous . . . much like a Honeycomb." Hooke called the pores *cells* because they reminded him of the cells inhabited by monks living in a monastery. In actual fact, Hooke had observed the empty cell walls of dead plant tissue, walls that had originally been produced by the living cells they surrounded.

Meanwhile, Antonie van Leeuwenhoek, a Dutchman who earned a living selling clothes and buttons, was spending his spare time grinding lenses and constructing simple microscopes of remarkable quality (Figure 1.1*b*). For 50 years,

Leeuwenhoek sent letters to the Royal Society of London describing his microscopic observations—along with a rambling discourse on his daily habits and the state of his health. Leeuwenhoek was the first to examine a drop of pond water under the microscope and, to his amazement, observe the teeming microscopic "animalcules" that darted back and forth before his eyes. He was also the first to describe various forms of bacteria, which he obtained from two sources: water in which pepper had been soaked and scrapings from his teeth. His initial letters to the Royal Society describing this previously unseen world were met with such skepticism that the society dispatched its curator, Robert Hooke, to confirm the observations. Hooke did just that, and Leeuwenhoek was soon a worldwide celebrity, receiving visits in Holland from Peter the Great of Russia and the queen of England.

Cell Theory

It was not until the 1830s that the widespread importance of cells was realized. In 1838, Matthias Schleiden, a German lawyer turned botanist, concluded that, despite differences in the structure of various tissues, plants were made of cells and the plant embryo arose from a single cell. In 1839, Theodor Schwann, a German zoologist and colleague of Schleiden's, published a comprehensive report on the cellular basis of animal life. Schwann concluded that the cells of plants and animals are similar structures and proposed these two tenets of **cell theory**:

- All organisms are composed of one or more cells.
- The cell is the structural unit of life.

Schleiden and Schwann's ideas on the *origin* of cells proved to be less insightful; both agreed that cells could arise from noncellular materials. Given the prominence that these two scientists held in the scientific world, it took a number of years before observations by other biologists demonstrating that cells did not arise in this manner any more than organisms arose by spontaneous generation were accepted. By 1855, Rudolf Virchow, a German pathologist, had made a convincing case for the third tenet of cell theory:

- Cells can arise only by division from a preexisting cell.

Since the discovery of DNA as the genetic material, a fourth tenet of cell theory is sometimes added:

- Cells contain genetic information in the form of DNA, and that information is passed from parent to daughter cell.

When you think about the cell as the structural unit of life, you might be tempted to think that cells are small and simple, like LEGO bricks. While most cells are microscopic, some are large enough to see with the human eye (see Section 1.3). As for cells being simple, the rest of this book should convince you otherwise.

Review

1. **When Robert Hooke first described cells, what was he actually looking at?**
2. **What are the three main components of cell theory?**

1.2 Basic Properties of Cells

Just as plants and animals are alive, so too are cells. Life, in fact, is the most basic property of cells, and cells are the smallest units to exhibit this property. Unlike the parts of a cell, which simply deteriorate if isolated, whole cells can be removed from a plant or animal and cultured in a laboratory where they will grow and reproduce for extended periods of time. If mistreated, they may die. Death can also be considered one of the most basic properties of life, because only a living entity faces this prospect. Remarkably, cells within the body generally die "by their own hand"—the victims of an internal program that causes cells that are no longer needed or those that pose a risk of becoming cancerous to eliminate themselves.

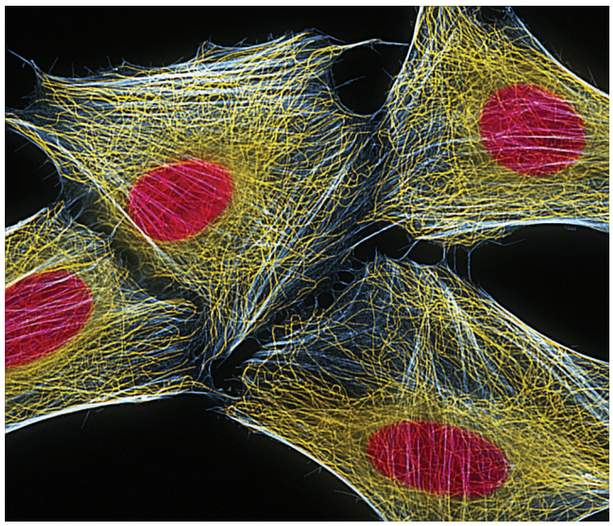
The first culture of human cells was begun by George and Martha Gey of Johns Hopkins University in 1951. The cells were obtained from a malignant tumor and named HeLa cells after the donor, Henrietta Lacks. HeLa cells—descended by cell division from this first cell sample—are still being grown in laboratories around the world today (**Figure 1.2**). Because they are so much simpler to study than cells situated within the body, cells grown **in vitro** (i.e., in culture, outside the body) have become an essential tool of cell and molecular biologists. In fact, much of the information that will be discussed in this book has been obtained using cells grown in laboratory cultures.

We begin our exploration of cells by examining a few of their most fundamental properties.

Cells Are Highly Complex and Organized

Complexity is a property that is evident when encountered, but difficult to describe. For the present, think of complexity in terms of order and consistency. The more complex a structure, the greater the number of parts that must be in their proper place, the less tolerance for errors in the nature and interactions of the parts, and the more regulation or control that must be exerted to maintain the system. Cellular activities can be remarkably precise. DNA duplication, for example, occurs with an error rate of less than one mistake for every ten million nucleotides incorporated—and most of these are quickly corrected by an elaborate repair mechanism that recognizes the defect.

During the course of this book, we will have occasion to consider the complexity of life at several different levels. We will



Source: Dr. Torsten Wittmann/Science Source.

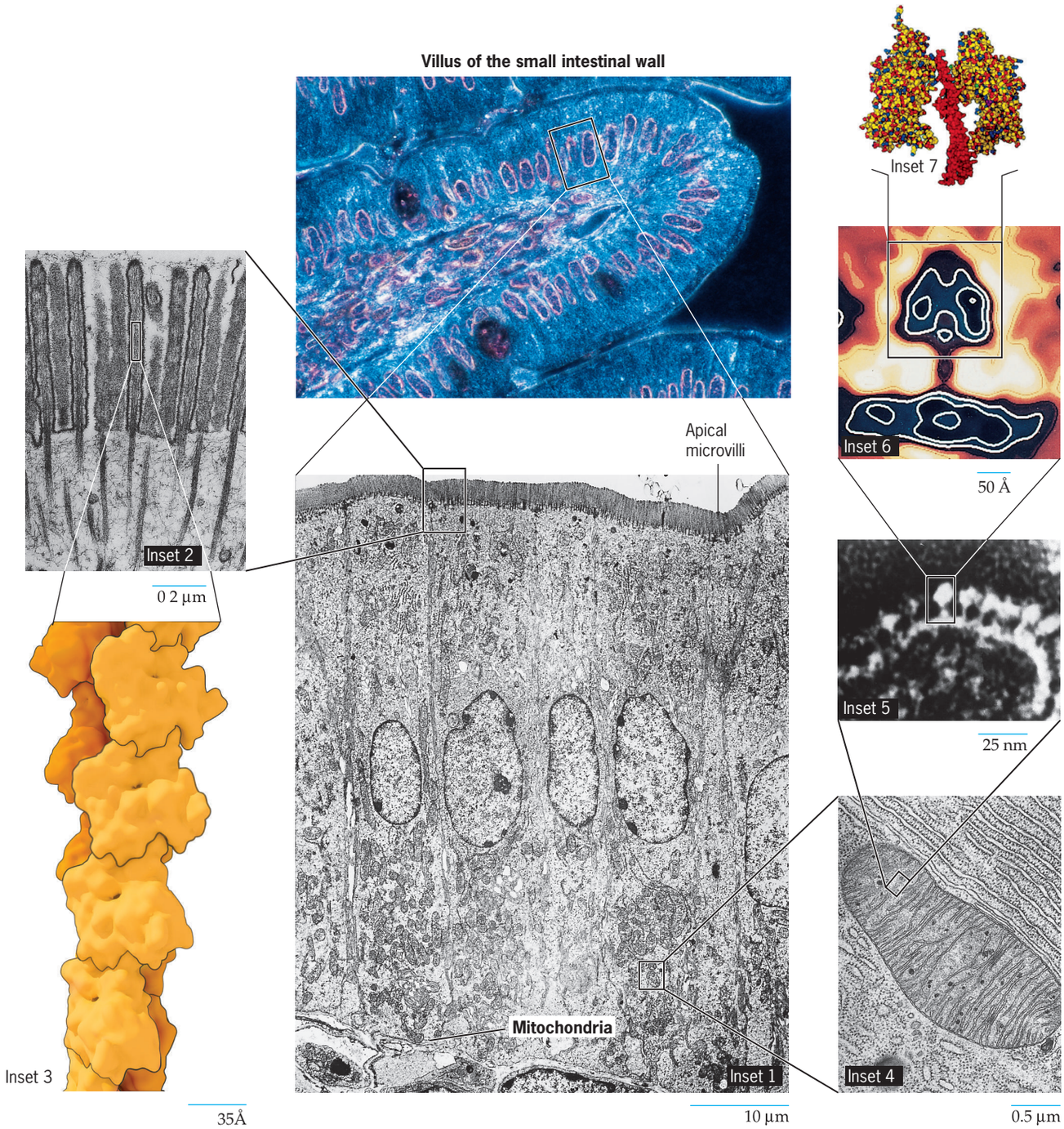
FIGURE 1.2 **HeLa cells,** such as the ones pictured here, were the first human cells to be kept in culture for long periods of time and are still in use today. Unlike normal cells, which have a finite lifetime in culture, these cancerous HeLa cells can be cultured indefinitely as long as conditions are favorable to support cell growth and division.

discuss the organization of atoms into small-sized molecules; the organization of these molecules into giant polymers; and the organization of different types of polymeric molecules into complexes, which in turn are organized into subcellular organelles and finally into cells. As will be apparent, there is a great deal of consistency at every level. Each type of cell has a consistent appearance when viewed under a high-powered electron microscope; that is, its organelles have a particular shape and location, from one individual of a species to another. Similarly, each type of organelle has a consistent composition of macromolecules, which are arranged in a predictable pattern. Consider the cells lining your intestine that are responsible for removing nutrients from your digestive tract. **Figure 1.3** illustrates the many different levels of organization present in such a tissue.

The epithelial cells that line the intestine are tightly connected to each other like bricks in a wall (Figure 1.3 inset 1). The apical ends of these cells, which face the intestinal channel, have long processes (*microvilli*) that facilitate absorption of nutrients (inset 2). The microvilli are able to project outward from the apical cell surface because they contain an internal skeleton made of filaments, which in turn are composed of protein (*actin*) monomers polymerized in a characteristic array (inset 3). At their basal ends, intestinal cells have large numbers of mitochondria (inset 4) that provide the energy required to fuel various membrane transport processes. Each mitochondrion is composed of a defined pattern of internal membranes, which in turn are composed of a consistent array of proteins, including an electrically powered ATP-synthesizing machine that projects from the inner membrane like a ball on a stick (insets 5–7).

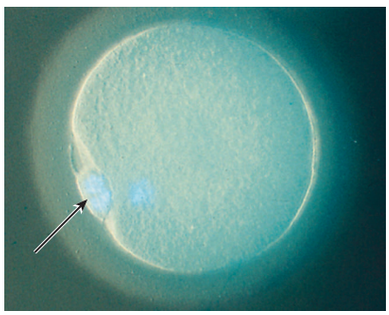
One of the truly fascinating aspects of cells is that they achieve organization at many different scales using physical processes that are essentially random. Even though living cells are highly complex and ordered, it has become increasingly evident in recent years that random (*stochastic*) events play a critical role in all cellular activities. Many of the molecules within living cells are in a constant state of random movement, propelled by thermal energy they acquire from their environment. Cells have evolved the capacity to utilize this movement in highly directed ways. Consider one example of this phenomenon, keeping in mind that many other cases could be described. Proteins are complex molecules often consisting of hundreds of amino acid building blocks and attaining molecular masses over one hundred thousand Daltons. Despite their huge size, proteins consist of a polypeptide chain that must fold into a precisely defined three-dimensional (*native*) structure. If it fails to fold properly, the protein will lack meaningful function. In 1969, Cyrus Levinthal of Columbia University identified certain features of this folding process that became known as Levinthal's paradox. Levinthal noted that, if protein folding depended solely on random molecular movements, it would require a period of time greater than the age of the universe for a protein to fold into its native structure. Compare the time it would take for a protein to fold properly to the period required for a monkey sitting at a piano to compose one of Beethoven's concertos. The paradox inherent in protein folding becomes evident with the knowledge that, despite their enormous complexity, proteins actually acquire their native structures within fractions of a second. How is the paradox resolved? Even though folding of a protein is driven by random thermal motion, the process occurs in stepwise fashion; the protein folds along pathways in which less structured intermediates guide the formation of better formed subsequent intermediates. In other words, the folding pathway allows proteins to rapidly "jump" from one step to the next until the native structure is reached. Returning to the monkey at the piano, it would be as if every time the monkey tapped an appropriate key, that note would be recorded, allowing the monkey to move toward the next note in the concerto. As long as the monkey was an active player, the composition of the concerto could be accomplished quite rapidly. It can be said that these types of events are *biased*. They depend on random activities, but lead to directed outcomes because they select for intermediate stages that lie on the path leading to the desired outcome.

Fortunately for cell and molecular biologists, evolution has moved rather slowly at the levels of biological organization with which they are concerned. Whereas a human and a cat, for example, have very different anatomical features, the cells that make up their tissues, and the organelles that make up their cells, are very similar. The actin filament portrayed in Figure 1.3, inset 3, and the ATP-synthesizing enzyme of inset 6 are virtually identical in such diverse organisms as humans, snails, yeast, and redwood trees. Information obtained by studying cells from one type of organism often has direct application to other forms of life. Many of the most basic processes, such as the synthesis of proteins, the conservation of chemical energy, and the construction of a membrane, are remarkably similar in all living organisms.



Source: Dr. Cecil H. Fox/Science Source; inset 1 Courtesy of Dr. Shakti P. Kapur, Georgetown University Medical Center; inset 2 ©1975 M S Mooseker & L G Tilney. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.67.3.725; inset 3 Courtesy of Kenneth C. Holmes; inset 4 K.R. Porter/Science Source; inset 5 ©1964 H. Fernández-Morán et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.22.1.63; inset 6 Courtesy of Dr. Roderick A. Capaldi; inset 7 from Wolfgang Junge. Protons, Proteins and ATP from Photosynthesis Research, Kluwar Academic, Jan 1, 2004. ©2004. Reprinted with permission from Springer nature.

FIGURE 1.3 Levels of cellular and molecular organization. The brightly colored photograph of a stained section shows the microscopic structure of a villus of the wall of the small intestine, as seen through the light microscope. Inset 1 shows an electron micrograph of the epithelial layer of cells that lines the inner intestinal wall. The apical surface of each cell, which faces the channel of the intestine, contains a large number of microvilli involved in nutrient absorption. The basal region of each cell contains large numbers of mitochondria, where energy is made available to the cell. Inset 2 shows the apical microvilli; each microvillus contains a bundle of actin filaments. Inset 3 shows the actin protein subunits that make up each filament. Inset 4 shows an individual mitochondrion similar to those found in the basal region of the epithelial cells. Inset 5 shows a portion of an inner membrane of a mitochondrion, including the stalked particles that project from the membrane and correspond to the sites where ATP is synthesized. Insets 6 and 7 show molecular models of the ATP-synthesizing machinery, which is discussed at length in Chapter 5.



Source: Courtesy of Jonathan van Blerkom.

FIGURE 1.4 Cell reproduction. This mammalian oocyte has recently undergone a highly unequal cell division in which most of the cytoplasm has been retained within the large oocyte, which has the potential to be fertilized and develop into an embryo. The other cell is a nonfunctional remnant that consists almost totally of nuclear material (indicated by the blue-staining chromosomes, arrow).

Cells Possess a Genetic Program and the Means to Use It

Organisms are built according to information encoded in a collection of genes, which are constructed of DNA. If converted to words, the human genetic program would contain enough information to fill millions of pages of text. Remarkably, this vast amount of information is packaged into a set of chromosomes that occupies the space of a cell nucleus—hundreds of times smaller than the dot on this *i*.

Genes are more than storage lockers for information: They constitute the recipes for constructing cellular structures, the directions for running cellular activities, and the program for making more of themselves. The molecular structure of genes allows for changes in genetic information (mutations) that lead to variation among individuals, which forms the basis of biological evolution. Discovering the mechanisms by which cells use and transmit their genetic information has been one of the greatest achievements of science during the last century.

Cells Are Capable of Producing More of Themselves

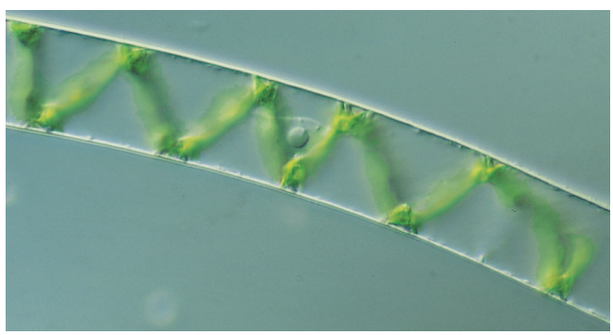
Just as individual organisms are generated by reproduction, so too are individual cells. Cells reproduce by division, a process in which the contents of a *mother* cell are distributed into two *daughter* cells. Prior to division, the genetic material is faithfully duplicated, and each daughter cell receives a complete and equal share of genetic information. In most cases, the two daughter cells have approximately equal volume. In some cases, however, as occurs when a human oocyte undergoes division, one of the cells can retain nearly all of the cytoplasm, even though it receives only half of the genetic material (**Figure 1.4**).

Cells Acquire and Utilize Energy

Every biological process requires the input of energy. Virtually all of the energy utilized by life on the Earth's surface arrives in the form of electromagnetic radiation from the sun. The energy of light is trapped by light-absorbing pigments present in the membranes of photosynthetic cells (**Figure 1.5**). Light energy is converted by photosynthesis into chemical energy that is stored in energy-rich carbohydrates, such as sucrose or starch. For most animal cells, energy arrives prepackaged, often in the form of the sugar glucose. In humans, glucose is released by the liver into the blood, where it circulates through the body delivering chemical energy to all the cells. Once in a cell, the glucose is disassembled in such a way that its energy content can be stored in a readily available form (usually as ATP) that is later put to use in running all of the cell's myriad energy-requiring activities. Cells expend an enormous amount of energy simply breaking down and rebuilding the macromolecules and organelles of which they are made. This continual *turnover* maintains the integrity of cell components in the face of inevitable wear and tear and enables the cell to respond rapidly to changing conditions.

Cells Carry Out a Variety of Chemical Reactions

Cells function like miniaturized chemical plants. Even the simplest bacterial cell is capable of hundreds of different chemical transformations, none of which occurs at any significant rate in the inanimate world. Virtually all chemical changes that take place in cells require *enzymes*—molecules that greatly increase the rate at which a chemical reaction occurs. The sum total of the chemical reactions in a cell represents that cell's **metabolism**.



Source: M. I. Walker/Science Source.

FIGURE 1.5 Acquiring energy. A living cell of the filamentous alga *Spirogyra*. The ribbon-like chloroplast, which is seen to zigzag through the cell, is the site where energy from sunlight is captured and converted to chemical energy during photosynthesis.

Cells Engage in Mechanical Activities

Cells are sites of bustling activity. Materials are transported from place to place, structures are assembled and then rapidly disassembled, and, in many cases, the entire cell moves itself from one site to another. These types of activities are based on dynamic, mechanical changes within cells, many of which are initiated by changes in the shape of *motor proteins*. Motor proteins are just one of many types of molecular "machines" employed by cells to carry out mechanical activities.

Cells Are Able to Respond to Stimuli

Some cells respond to stimuli in obvious ways; a single-celled protist, for example, moves away from an object in its path or moves toward a source of nutrients. Cells within a multicellular plant or animal respond to stimuli less obviously. Most cells are covered with *receptors* that interact with substances in the environment in highly specific ways. Cells possess receptors to hormones, growth factors, and extracellular materials, as well as to substances on the surfaces of other cells. Receptors provide pathways through which external stimuli can evoke specific responses in target cells. Cells may respond to specific stimuli by altering their metabolic activities, moving from one place to another, or even committing suicide.

Cells Are Capable of Self-Regulation

In recent years, a new term has been used to describe cells: *robustness*. Cells are robust, that is, hearty or durable, because they are protected from dangerous fluctuations in composition and behavior. Should such fluctuations occur, specific feedback circuits are activated that serve to return the cell to the appropriate state. In addition to requiring energy, maintaining a complex, ordered state requires constant regulation. The importance of a cell's regulatory mechanisms becomes most evident when they break down. For example, failure of a cell to correct a mistake when it duplicates its DNA may result in a debilitating mutation, or a breakdown in a cell's growth-control safeguards can transform the cell into a cancer cell with the capability of destroying the entire organism. We are gradually learning how a cell controls its activities, but much more is left to discover.

Consider the following experiment conducted in 1891 by Hans Driesch, a German embryologist. Driesch found that he could completely separate the first two or four cells of a sea urchin embryo and each of the isolated cells would proceed to develop into a normal embryo (**Figure 1.6**). How can a cell that is normally destined to form only part of an embryo regulate its own activities and form an entire embryo? How does the isolated cell recognize the absence of its neighbors, and how does this recognition redirect the entire course of the cell's development? How can a part of an embryo have a sense of the whole?

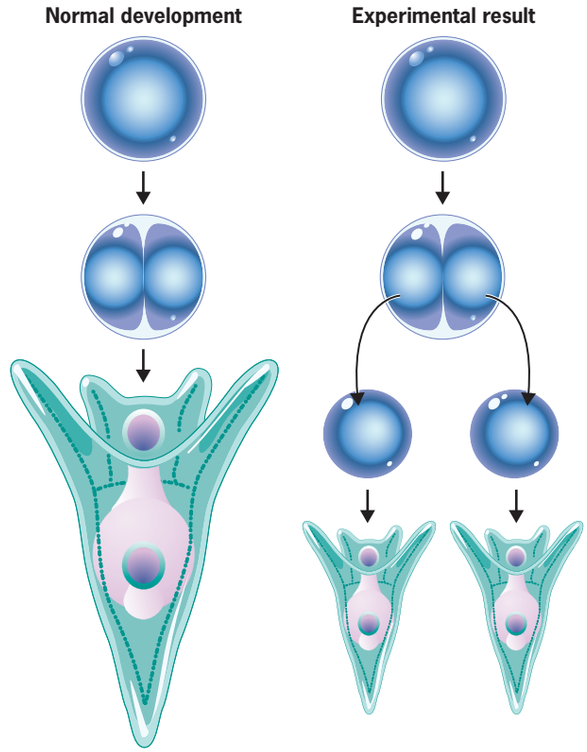
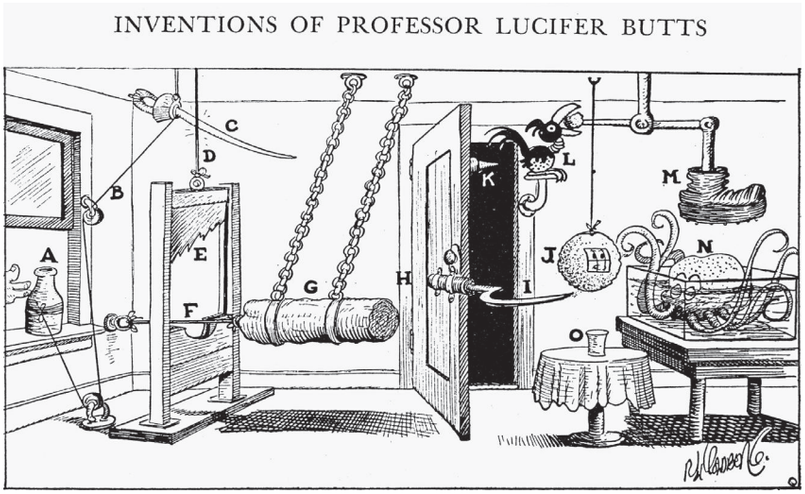


FIGURE 1.6 Self-regulation. The left panel depicts the normal development of a sea urchin in which a fertilized egg gives rise to a single embryo. The right panel depicts an experiment in which the cells of an early embryo are separated after the first division, and each cell is allowed to develop in isolation. Rather than developing into half of an embryo, as it would if left undisturbed, each isolated cell recognizes the absence of its neighbor, regulating its development to form a complete (although smaller) embryo.

We are not able to answer these questions much better today than we were more than a hundred years ago when the experiment was performed.

Throughout this book we will be discussing processes that require a series of ordered steps, much like the assembly-line construction of an automobile in which workers add, remove, or make specific adjustments as the car moves along. In the cell, the information for product design resides in the nucleic acids, and the construction workers are primarily proteins. It is the presence of these two types of macromolecules that, more than any other factor, sets the chemistry of the cell apart from that of the nonliving world. In the cell, the workers must act without the benefit of conscious direction. Each step of a process must occur spontaneously in such a way that the next step is triggered automatically. In many ways, cells operate in a manner analogous to the orange-squeezing contraption discovered by "The Professor" and shown in **Figure 1.7**. Each type of cellular activity requires a unique set of highly complex molecular tools and machines—the products of eons of natural selection and biological evolution. A primary goal of biologists is to understand the molecular structure and role of



Professor Butts steps into an open elevator shaft and when he lands at the bottom he finds a simple orange squeezing machine. Milk man takes empty milk bottle **(A)** pulling string **(B)** which causes sword **(C)** to sever cord **(D)** and allow guillotine blade **(E)** to drop and cut rope **(F)** which releases battering ram **(G)**. Ram bumps against open door **(H)** causing it to close. Grass sickle **(I)** cuts a slice off end of orange **(J)** at the same time spike **(K)** stabs"prune hawk" **(L)** he opens his mouth to yell in agony, thereby releasing prune and allowing diver's boot **(M)** to drop and step on sleeping Octopus **(N)**. Octopus awakens in a rage and seeing diver's face which is painted on orange, attacks it and crushes it with tentacles, thereby causing all the juice in the orange to run into glass **(O)**.

Later on you can use the log to build a log cabin where you can raise your son to be president like Abraham Lincoln.

FIGURE 1.7 **Cellular activities** are often analogous to this *Rube Goldberg machine* (named after a cartoonist famous for drawing this type of complicated machine) in which one event "automatically" triggers the next event in a reaction sequence.

each component involved in a particular activity, the means by which these components interact, and the mechanisms by which these interactions are regulated.

Cells Evolve

How did cells arise? Of all of the major questions posed by biologists, this one may be the least likely ever to be answered. It is presumed that cells evolved from some type of precellular life form, which in turn evolved from nonliving organic materials that were present in the primordial seas. Whereas the origin of cells is shrouded in near-total mystery, the evolution of cells can be studied by examining organisms that are alive today. If you were to observe the features of a bacterial cell living in the human intestinal tract (see Figure 1.18*a*) and a cell that is part of the lining of that tract (Figure 1.3), you would be struck by the differences between the two cells. Yet both of these cells, as well as all other cells that are present in living organisms, share many features, including a common genetic code, a plasma membrane, and ribosomes. According to one of the tenets of modern biology, all living organisms have evolved from a single, common ancestral cell that lived more than three billion years ago. Because it gave rise to all of the living organisms that we know of, this ancient cell is often referred to as the *last universal common ancestor* (or *LUCA*). We examine some of the events that occurred during the evolution of cells in the Experimental Pathways feature in Section 1.4. Future chapters will explore biochemical aspects of the origin of life. Keep in mind that evolution is not simply an event of the past, but an ongoing process that continues to modify the properties of cells that will be present in organisms that have yet to appear. For example, evolution of drug resistance in bacteria is a major health concern and is discussed in The Human Perspective feature in Section 3.2.

Review

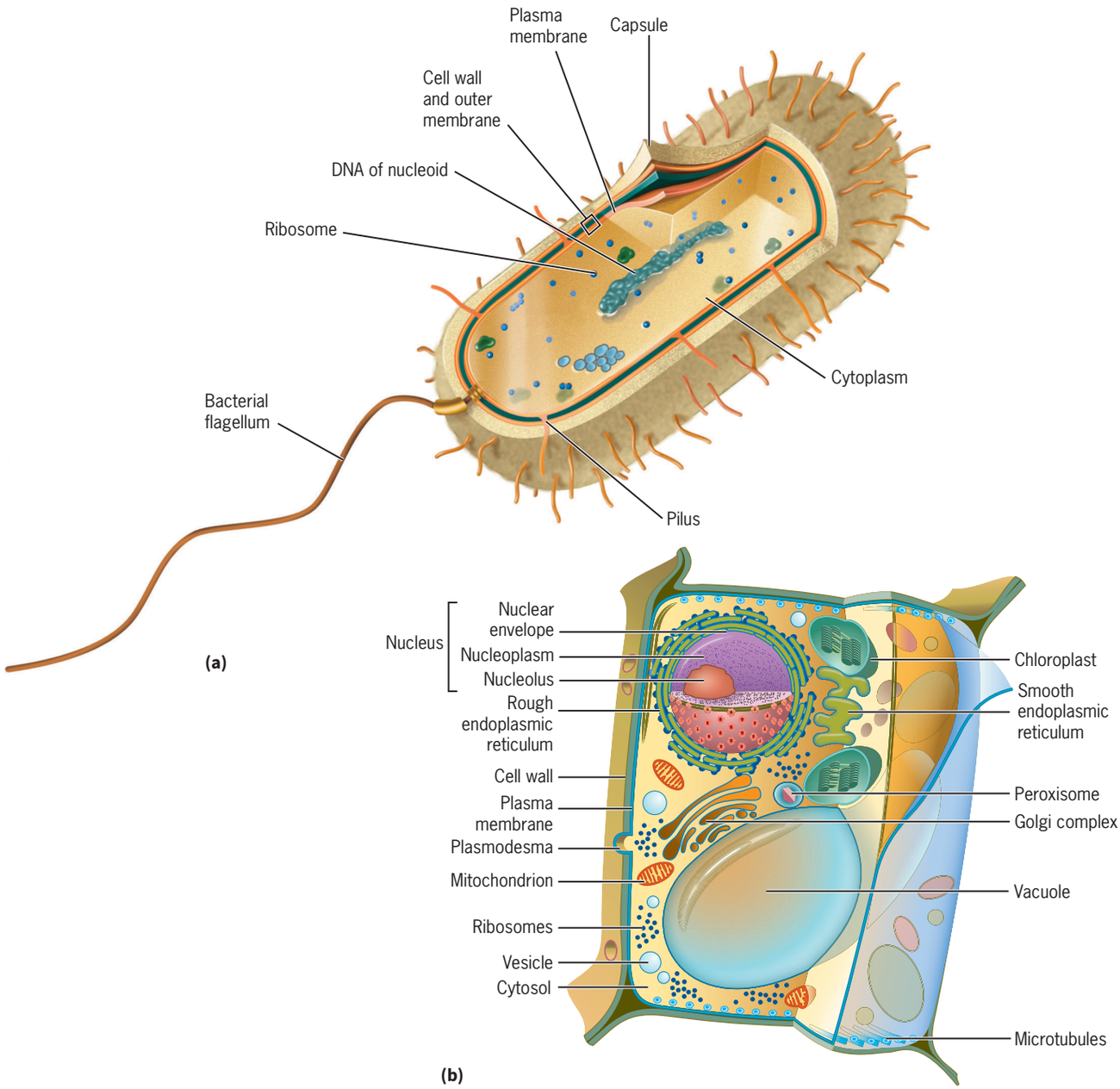
1. **List the fundamental properties shared by all cells. Describe the importance of each of these properties.**
2. **Describe the features of cells suggesting that all living organisms are derived from a common ancestor.**
3. **What is the source of energy that supports life on Earth? How is this energy passed from one organism to the next?**

1.3 Two Fundamentally Different Classes of Cells

Once the electron microscope became widely available, biologists were able to examine the internal structure of a wide variety of cells. It became apparent from these studies

that there were two basic classes of cells—prokaryotic and eukaryotic—distinguished by their size and the types of internal structures, or **organelles**, they contain (**Figure 1.8**). The existence of two distinct classes of cells, without any known intermediates, represents one of the most fundamental evolutionary divisions in the biological world. The structurally simpler **prokaryotic** cells include bacteria, whereas the structurally more complex **eukaryotic** cells include protists, fungi, plants, and animals.[1]

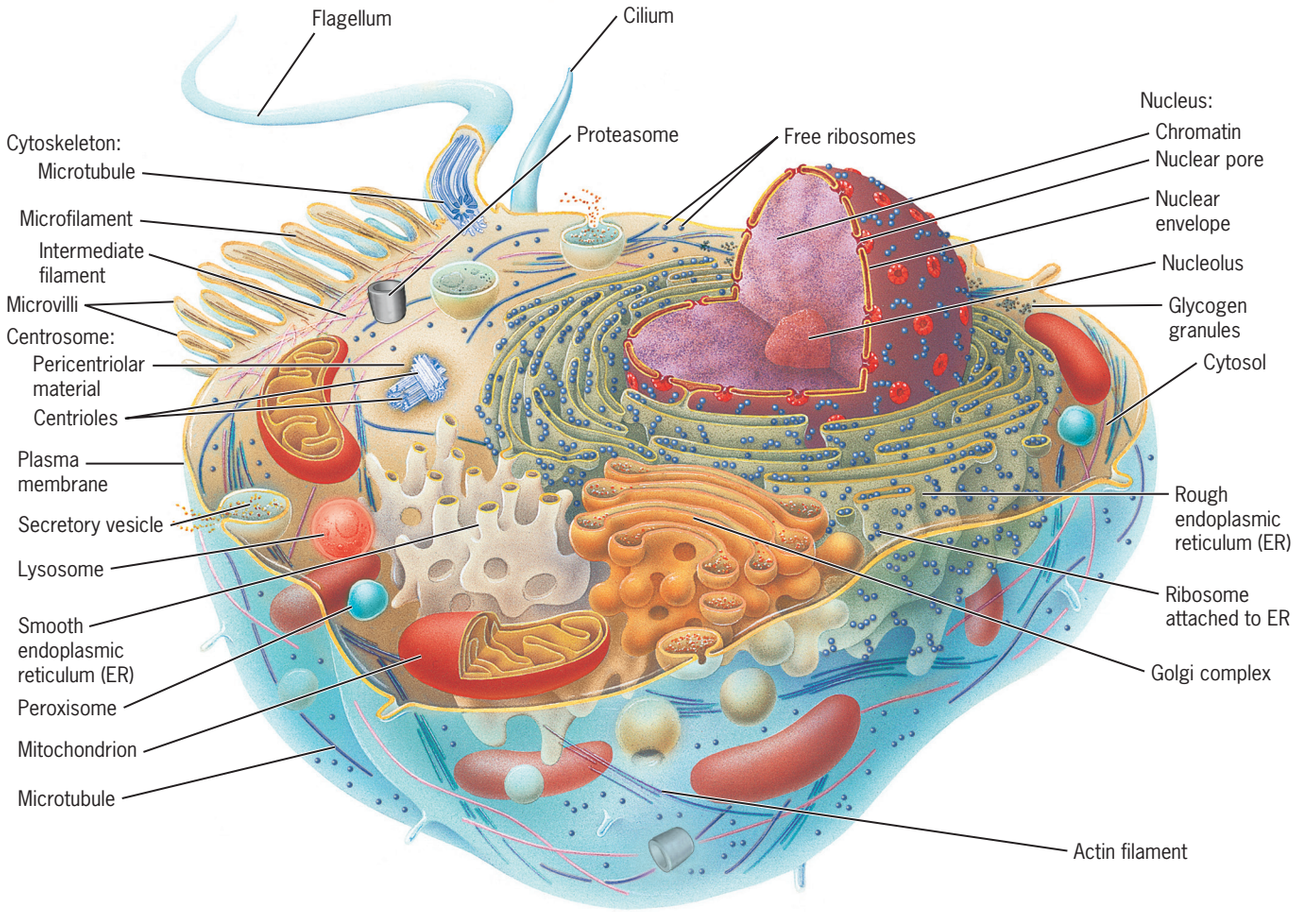
[1]Those interested in examining a proposal to do away with the concept of prokaryotic versus eukaryotic organisms can read a brief essay by N. R. Pace in *Nature* 441:289, 2006.



Source: Adapted from D. J. Des Marais, Science 289:1704, 2001 from AAAS.

FIGURE 1.8 **The structure of cells.** Schematic diagrams of a "generalized" bacterial **(a)**, plant **(b)**, and animal **(c)** cell. Note: Organelles are not drawn to scale.

(*continued*)



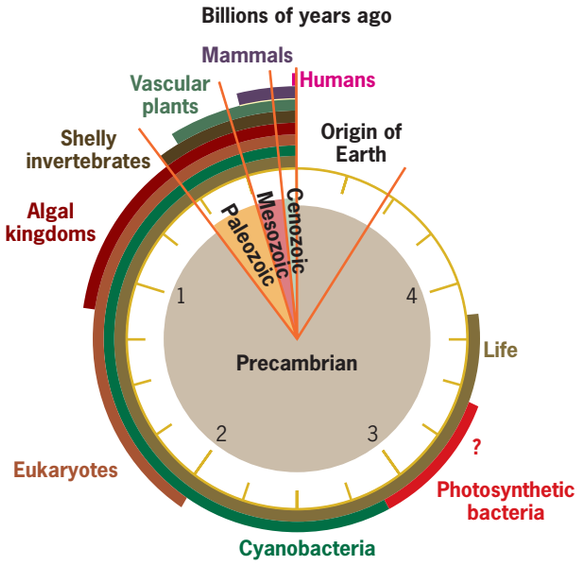
(c)

FIGURE 1.8 (*continued*)

We are not sure when prokaryotic cells first appeared on Earth. Evidence of prokaryotic life has been obtained from rocks approximately 2.7 billion years of age. Not only do these rocks contain what appear to be fossilized microbes, they contain complex organic molecules that are characteristic of particular types of prokaryotic organisms, including cyanobacteria. It is unlikely that such molecules could have been synthesized abiotically, that is, without the involvement of living cells. Cyanobacteria almost certainly appeared by 2.4 billion years ago, because that is when the atmosphere became infused with molecular oxygen (O_2), which is a by-product of the photosynthetic activity of these prokaryotes. The dawn of the age of eukaryotic cells is also shrouded in uncertainty. Complex multicellular animals appear rather suddenly in the fossil record approximately 600 million years ago, but there is considerable evidence that simpler eukaryotic organisms were present on Earth more than one billion years earlier. The estimated time of appearance on Earth of several major groups of organisms is depicted in **Figure 1.9**. Even a superficial examination of Figure 1.9 reveals how "quickly" life arose following the formation of Earth and cooling of its surface and how long it took for the subsequent evolution of complex animals and plants.

Characteristics That Distinguish Prokaryotic and Eukaryotic Cells

The following brief comparison between prokaryotic and eukaryotic cells reveals many basic differences between the two types, as well as many similarities (see Figure 1.8). The similarities and differences between the two types of cells are listed in **Table 1.1**. The shared properties reflect the fact that eukaryotic cells almost certainly evolved from prokaryotic ancestors. Because of their common ancestry, both types of cells share an identical genetic language, a common set of metabolic pathways, and many common structural features. For example, both types of cells are bounded by plasma membranes of similar construction that serve as a selectively permeable barrier between the living and nonliving worlds. Both types of cells may be surrounded by a rigid, nonliving *cell wall* that protects the delicate life form within. Although the cell walls of



Source: Adapted from D. J. Des Marais, *Science* 289:1704, 2001 AAAS.

FIGURE 1.9 Earth's biogeologic clock. A portrait of the past five billion years of Earth's history showing a proposed time of appearance of major groups of organisms. Complex animals (shelly invertebrates) and vascular plants are relatively recent arrivals. The time indicated for the origin of life is speculative. In addition, photosynthetic bacteria may have arisen much earlier, hence the question mark. The geologic eras are indicated in the center of the illustration.

prokaryotes and eukaryotes may have similar functions, their chemical composition is very different.

Internally, eukaryotic cells are much more complex—both structurally and functionally—than prokaryotic cells (Figure 1.8). The difference in structural complexity is evident in the electron micrographs of a bacterial and an animal cell shown in Figure 1.18*a* and **Figure 1.10**, respectively. Both contain a nuclear region that houses the cell's genetic material, surrounded by cytoplasm. The genetic material of a prokaryotic cell is present in a **nucleoid**: a poorly demarcated region of the cell that lacks a boundary membrane to separate it from the surrounding cytoplasm. In contrast, eukaryotic cells possess a **nucleus**: a region bounded by a complex membranous structure called the *nuclear envelope*. This difference in nuclear structure is the basis for the terms *prokaryotic* (*pro* = before, *karyon* = nucleus) and *eukaryotic* (*eu* = true, *karyon* = nucleus). Prokaryotic cells contain relatively small amounts of DNA; the DNA content of bacteria ranges from about 600,000 base pairs to nearly 8 million base pairs and encodes between about 500 and several thousand proteins.[2] Although a "simple" baker's yeast cell has only slightly more DNA (12 million base pairs encoding about 6200 proteins) than the most complex prokaryotes, most eukaryotic cells contain considerably more genetic information. Both prokaryotic and eukaryotic cells have DNA-containing chromosomes. Eukaryotic cells possess a number of separate chromosomes, each containing a single linear molecule of DNA. In contrast, nearly all prokaryotes that have been studied contain a single, circular chromosome. More importantly, the chromosomal DNA of eukaryotes, unlike that of prokaryotes, is tightly associated with proteins to form a complex nucleoprotein material known as **chromatin**.

The cytoplasm of the two types of cells is also very different. The cytoplasm of a eukaryotic cell is filled with a great diversity of structures, as is readily apparent by examining an electron micrograph of nearly any plant or animal cell (Figure 1.10). Even yeast, the simplest eukaryote, is much more complex structurally than an average bacterium, even though these two organisms have a similar number of genes. Eukaryotic cells

[2]Eight million base pairs is equivalent to a DNA molecule nearly 3 mm long.

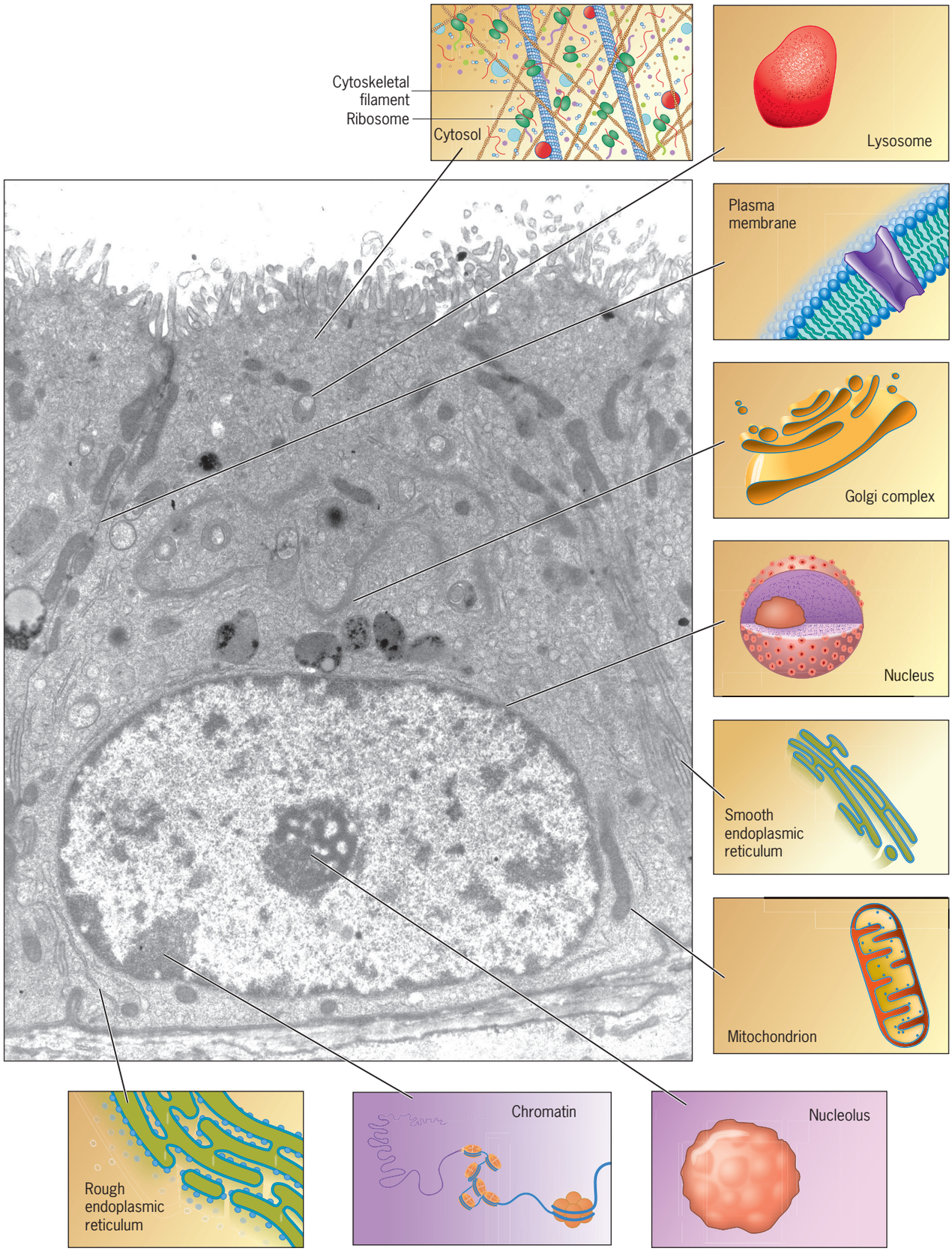
TABLE 1.1 A Comparison of Prokaryotic and Eukaryotic Cells

Features held in common by the two types of cells:

- Plasma membrane of similar construction
- Genetic information encoded in DNA using identical genetic code
- Similar mechanisms for transcription and translation of genetic information, including similar ribosomes
- Shared metabolic pathways (e.g., glycolysis and TCA cycle)
- Similar apparatus for conservation of chemical energy as ATP (located in the plasma membrane of prokaryotes and the mitochondrial membrane of eukaryotes)
- Similar mechanism of photosynthesis (between cyanobacteria and green plants)
- Similar mechanism for synthesizing and inserting membrane proteins
- Proteasomes (protein-digesting structures) of similar construction (between archaebacteria and eukaryotes)
- Cytoskeletal filaments built of proteins similar to actin and tubulin

Features of eukaryotic cells not found in prokaryotes:

- Division of cells into nucleus and cytoplasm, separated by a nuclear envelope containing complex pore structures
- Complex chromosomes composed of DNA and associated proteins that are capable of compacting into mitotic structures
- Complex membranous cytoplasmic organelles (includes endoplasmic reticulum, Golgi complex, lysosomes, endosomes, peroxisomes, and glyoxisomes)
- Specialized cytoplasmic organelles for aerobic respiration (mitochondria) and photosynthesis (chloroplasts)
- Complex cytoskeletal system (including actin filaments, intermediate filaments, and microtubules) and associated motor proteins
- Complex flagella and cilia
- Ability to ingest particulate material by enclosure within plasma membrane vesicles (phagocytosis)
- Cellulose-containing cell walls (in plants)
- Cell division using a microtubule-containing mitotic spindle that separates chromosomes
- Presence of two copies of genes per cell (diploidy), one from each parent
- Presence of three different RNA-synthesizing enzymes (RNA polymerases)
- Sexual reproduction requiring meiosis and fertilization



Source: David M. Phillips/Science Source.

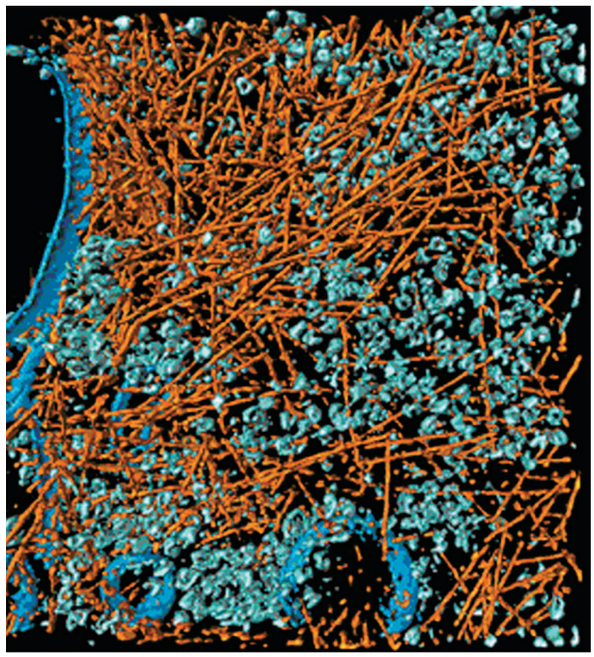
FIGURE 1.10 **The structure of a eukaryotic cell.** This epithelial cell lines the male reproductive tract in the rat. A number of different organelles are indicated and depicted in schematic diagrams surrounding the micrograph.

contain an array of membrane-bound organelles. Eukaryotic organelles include mitochondria, where chemical energy is made available to fuel cellular activities; an endoplasmic reticulum, where many of a cell's proteins and lipids are manufactured; Golgi complexes, where materials are sorted, modified, and transported to specific cellular destinations; and a variety of simple membrane-bound vesicles of different dimensions. Plant cells contain additional membranous organelles, including chloroplasts, which are the sites of photosynthesis, and often a single large vacuole that can occupy most of the volume of the cell. Taken as a group, the membranes of the eukaryotic cell serve to divide the cytoplasm into compartments, within which specialized activities can take place. In contrast, the cytoplasm of prokaryotic cells is essentially devoid of membranous structures. The complex photosynthetic membranes of the cyanobacteria are a major exception to this generalization (see Figure 1.15).

The cytoplasmic membranes of eukaryotic cells form a system of interconnecting channels and vesicles that function in the transport of substances from one part of a cell to another, as well as between the inside of the cell and its environment. Because of their small size, directed intracytoplasmic communication is less important in prokaryotic cells, where the necessary movement of materials can be accomplished by simple diffusion.

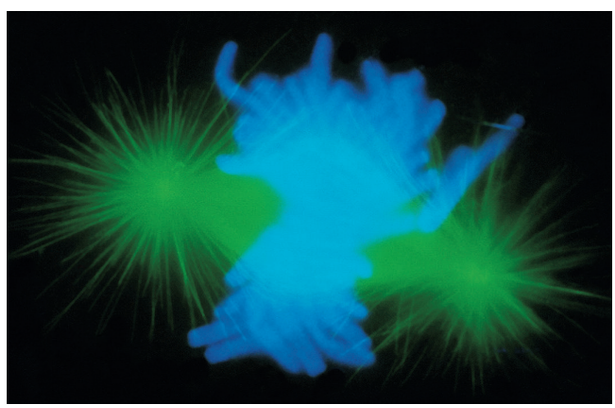
Eukaryotic cells also contain numerous structures lacking a surrounding membrane. Included in this group are the elongated tubules and filaments of the cytoskeleton, which participate in cell contractility, movement, and support. It was thought for many years that prokaryotic cells lacked any trace of a cytoskeleton, but primitive cytoskeletal filaments have been found in bacteria (see Section 9.13). It is still fair to say that the prokaryotic cytoskeleton is much simpler, both structurally and functionally, than that of eukaryotes. Both eukaryotic and prokaryotic cells possess ribosomes, which are nonmembranous particles that function as "workbenches" on which the proteins of the cell are manufactured. Even though ribosomes of prokaryotic and eukaryotic cells have considerably different dimensions (those of prokaryotes are smaller and contain fewer components), these structures participate in the assembly of proteins by a similar mechanism in both types of cells. **Figure 1.11** is a colorized electron micrograph of a portion of the cytoplasm near the thin edge of a single-celled eukaryotic organism. This is a region of the cell where membrane-bound organelles tend to be absent. The micrograph shows individual filaments of the cytoskeleton (orange) and other large macromolecular complexes of the cytoplasm (turquoise). Most of these complexes are ribosomes. It is evident from this type of image that the cytoplasm of a eukaryotic cell is extremely crowded, leaving very little space for the soluble phase of the cytoplasm, called the **cytosol**.

Other major differences between eukaryotic and prokaryotic cells can be noted. Eukaryotic cells divide by a complex process of mitosis in which duplicated chromosomes condense into compact structures that are segregated by an elaborate microtubule-containing apparatus (**Figure 1.12**).



Source: From Ohad Medalia et al., *Science* 298:1211, 2002, Figure 3*a*. ©2002, reprinted with permission from AAAS. Photo provided courtesy of Wolfgang Baumeister.

FIGURE 1.11 **The cytoplasm of a eukaryotic cell is a crowded compartment.** This colorized electron micrographic image shows a small region near the edge of a single-celled eukaryotic organism that had been quickly frozen prior to microscopic examination. The three-dimensional appearance is made possible by capturing two-dimensional digital images of the specimen at different angles and merging the individual frames using a computer. Cytoskeletal filaments are shown in orange, macromolecular complexes (primarily ribosomes) are turquoise, and portions of cell membranes are blue.

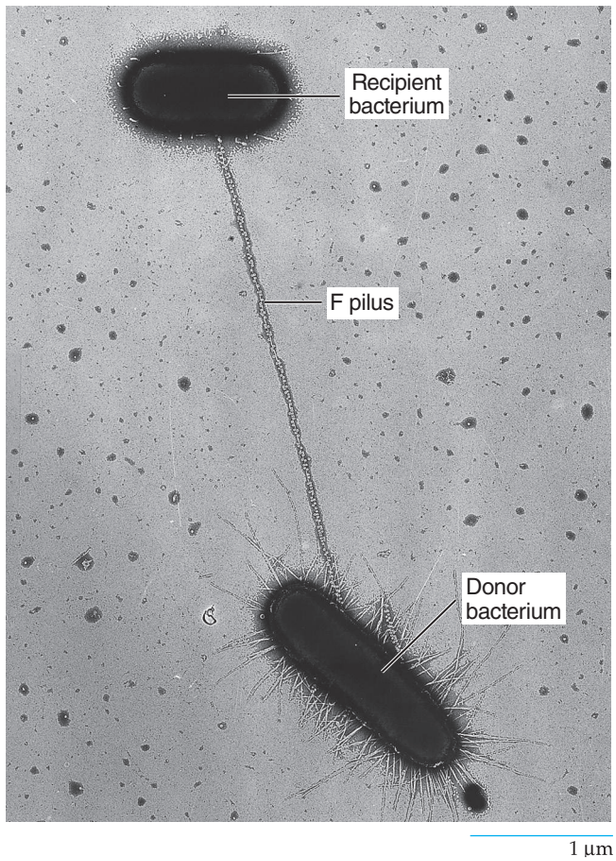


Source: Photograph by Dr. Conly L. Rieder, Wadsworth Center, Albany, New York 12201–0509.

FIGURE 1.12 **Cell division in eukaryotes** requires the assembly of an elaborate chromosome-separating apparatus called the mitotic spindle, which is constructed primarily of microtubules. The microtubules in this micrograph appear green because they are bound by an antibody that is linked to a green fluorescent dye. The chromosomes, which were about to be separated into two daughter cells when this cell was fixed, are stained blue.

This apparatus, called a *mitotic spindle*, allows each daughter cell to receive an equivalent array of genetic material. In prokaryotes, there is no mitotic spindle to separate the genome copies after replication. It was once thought that the two copies are separated by attaching the DNA to the cell surface, allowing the growth of the cell membrane to pull them apart. However, live cell imaging showed that the DNA separates faster than the cell grows, and the precise mechanism by which prokaryotes segregate their genomes remains an open question. Some current models are based on regulated compaction or folding of the DNA so that the two copies would fold into two separate masses.

For the most part, prokaryotes are nonsexual organisms. They contain only one copy of their single chromosome and have no processes comparable to meiosis, gamete formation, or true fertilization. Even though true sexual reproduction is lacking among prokaryotes, some are capable of *conjugation*, in which a piece of DNA is passed from one cell to another (**Figure 1.13**). However, the recipient almost never receives a whole chromosome from the donor, and the condition in which the recipient cell contains both its own and its partner's DNA is fleeting. The cell soon reverts back to possession of a single chromosome. Although prokaryotes may not be as efficient as eukaryotes in exchanging DNA with other members of their own species, they are more adept than eukaryotes at picking up and incorporating foreign DNA from their environment, which has had considerable impact on microbial evolution (Experimental Pathways feature in Section 1.4).

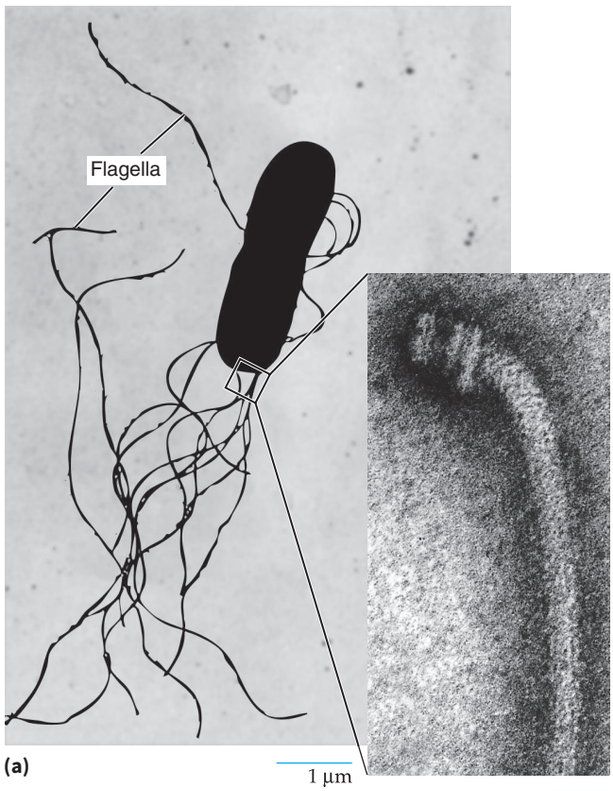


Source: Courtesy of Charles C. Brinton, Jr., and Judith Carnahan.

FIGURE 1.13 Bacterial conjugation. Electron micrograph showing a conjugating pair of bacteria joined by a structure of the donor cell, termed the F pilus, through which DNA is thought to be passed.

Eukaryotic cells possess a variety of complex locomotor mechanisms, whereas those of prokaryotes are relatively simple. The movement of a prokaryotic cell may be accomplished by a thin protein filament, called a *flagellum*, which protrudes from the cell and rotates (**Figure 1.14a**). The rotations of the flagellum, which can exceed 1000 times per second, exert pressure against the surrounding fluid, propelling the cell through the medium. Certain eukaryotic cells, including many protists and sperm cells, also possess flagella, but the eukaryotic versions (Figure 1.14*b*) are much more complex than the simple protein filaments of bacteria, and they generate movement by a different mechanism.

Many of the most important differences between the prokaryotic and eukaryotic levels of cellular organization have been mentioned in the preceding paragraphs. We will elaborate on many of these points in later chapters. Before you dismiss prokaryotes as inferior, keep in mind that these organisms have remained on Earth for more than three billion years, and at this very moment, trillions of them are clinging to the outer surface of your body and feasting on the nutrients within your digestive tract. We think of these organisms as individual, solitary creatures, but recent insights have shown that they live in complex, multispecies communities called *biofilms*. The layer of plaque that grows on our teeth is an example of a biofilm. Different cells in a biofilm may carry out different specialized activities, not unlike the cells in a plant or an animal. Consider also that, metabolically, prokaryotes are very sophisticated, highly evolved organisms. For example, a bacterium, such as *Escherichia coli*, a common inhabitant of both the human digestive tract and the laboratory culture dish, has the ability to live and prosper in a medium containing one or two low-molecular-weight organic compounds and a few inorganic ions. Other bacteria are able to live on a diet consisting solely of inorganic substances. One species of bacteria has been found in wells more than a thousand meters below the Earth's surface living on basalt rock and molecular hydrogen (H_2) produced by inorganic reactions. In contrast, even the most metabolically talented cells in your body require a variety of organic compounds, including a number of vitamins and other essential substances they cannot make on their own. In fact, many of these essential dietary ingredients are produced by the bacteria that normally live in the large intestine.



Source: (*a*) From Bernard R. Gerber, Lewis M. Routledge, and Shiro Takashima, *J. Mol. Biol.*, 71: 317–323, ©1972, with permission from Elsevier; (*b*) Juergen Berger/Science Source Inset: Don W. Fawcett/Science Source.

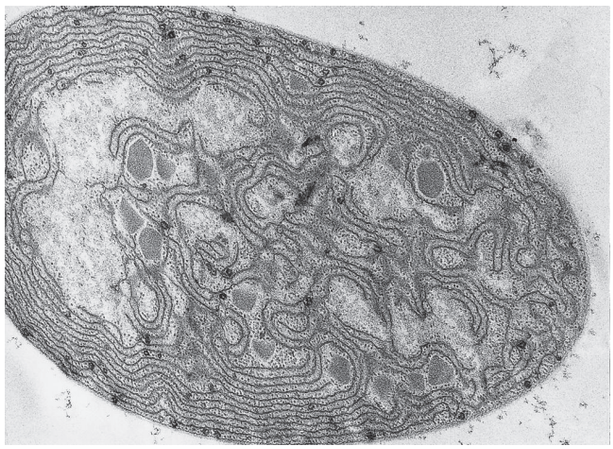
FIGURE 1.14 **The difference between prokaryotic and eukaryotic flagella.** **(a)** The bacterium *Salmonella* with its numerous flagella. Inset shows a high-magnification view of a portion of a single bacterial flagellum, which consists largely of a single protein called flagellin. **(b)** Each of these human sperm cells is powered by the undulatory movements of a single flagellum. The inset shows a cross section of the central core of a mammalian sperm flagellum. The flagella of eukaryotic cells are so similar that this cross section could just as well have been taken of a flagellum from a protist or green alga.

Types of Prokaryotic Cells

The distinction between prokaryotic and eukaryotic cells is based on structural complexity (as detailed in Table 1.1) and not on phylogenetic relationship. Prokaryotes are divided into two major taxonomic groups, or domains: the Archaea (or archaebacteria) and the Bacteria (or eubacteria). Members of the Archaea are more closely related to eukaryotes than they are to the other group of prokaryotes (the Bacteria). The experiments that led to the discovery that life is represented by three distinct branches are discussed in the Experimental Pathways feature in Section 1.4.

Domain Archaea and Domain Bacteria The domain Archaea includes several groups of organisms; their evolutionary ties are revealed by similarities in the nucleotide sequences of their nucleic acids. The best known Archaea are species that live in extremely inhospitable environments; they are often referred to as *extremophiles*. Included among the Archaea are the methanogens [prokaryotes capable of converting CO_2 and H_2 gases into methane (CH_4) gas]; the halophiles (prokaryotes that live in extremely salty environments, such as the Dead Sea or certain deep sea brine pools that possess a salinity equivalent to 5M $MgCl_2$); acidophiles (acid-loving prokaryotes that thrive at a pH as low as 0, such as that found in the drainage fluids of abandoned mine shafts); and thermophiles (prokaryotes that live at very high temperatures). Included in this last-named group are hyperthermophiles, which live in the hydrothermal vents of the ocean floor. The latest record holder among this group has been named “strain 121” because it is able to grow and divide in superheated water at a temperature of 121°C, which just happens to be the temperature used to sterilize surgical instruments in an autoclave. Recent analyses of soil and ocean microbes indicate that many members of the Archaea are also at home in habitats of normal temperature, pH, and salinity.

All other prokaryotes are classified in the domain Bacteria. This domain includes the smallest known cells, the mycoplasma (0.2 μm diameter), which are the only known prokaryotes to lack a cell wall and to contain a genome with fewer than 500 genes. Bacteria are present in every conceivable habitat on Earth, from the permanent ice shelf of the Antarctic to the driest African deserts, to the internal confines of plants and animals. Bacteria have even been found living in rock layers situated several kilometers beneath the Earth’s surface. Some of these bacterial communities are thought to have been cut off from life on the surface for more than one hundred million years. The most complex prokaryotes are the cyanobacteria. Cyanobacteria contain elaborate arrays of cytoplasmic membranes, which serve as sites of photosynthesis (**Figure 1.15a**). The membranes of cyanobacteria are very similar to the photosynthetic membranes present within the chloroplasts of plant cells. As in eukaryotic plants, photosynthesis in cyanobacteria is accomplished by splitting water molecules, which releases molecular oxygen.



(a)

(b)

Source: (*a*) Courtesy of Norma J. Lang. (*b*) Courtesy Zoological Society of San Diego.

FIGURE 1.15 **Cyanobacteria.** **(a)** Electron micrograph of a cyanobacterium showing the cytoplasmic membranes that carry out photosynthesis. These concentric membranes are very similar to the thylakoid membranes present within the chloroplasts of plant cells, a reminder that chloroplasts evolved from a symbiotic cyanobacterium. **(b)** Cyanobacteria living inside the hairs of these polar bears are responsible for the unusual greenish color of their coats.

Many cyanobacteria are capable not only of photosynthesis but also of **nitrogen fixation**, the conversion of nitrogen (N_2) gas into reduced forms of nitrogen (such as ammonia, NH_3), which can be used by cells in the synthesis of nitrogen-containing organic compounds, including amino acids and nucleotides. Those species capable of both photosynthesis and nitrogen fixation can survive on the barest of resources—light, N_2, CO_2, and H_2O. It is not surprising, therefore, that cyanobacteria are usually the first organisms to colonize the bare rocks rendered lifeless by a scorching volcanic eruption. Another unusual habitat occupied by cyanobacteria is illustrated in Figure 1.15*b*.

Prokaryotic Diversity For the most part, microbiologists are familiar only with those microorganisms that they are able to grow in a culture medium. When a patient suffering from a respiratory or urinary tract infection sees a physician, one of the first steps often taken is to culture the pathogen. Once it has been cultured, the organism can be identified and the proper treatment prescribed. It has proven relatively easy to culture *most* disease-causing prokaryotes, but the same is not true for those living free in nature. The problem is compounded by the fact that prokaryotes are barely visible in a light microscope and their morphology is often not very distinctive. To date, roughly 6000 species of prokaryotes have been identified by traditional techniques, which is less than one-tenth of 1 percent of the millions of prokaryotic species thought to exist on Earth! Our appreciation for the diversity of prokaryotic communities has increased dramatically in recent years with the use of molecular techniques that do not require the isolation of a particular organism.

Suppose that you wanted to learn about the diversity of prokaryotes living in the upper layers of the Pacific Ocean off the coast of California. Rather than trying to culture such organisms, which would prove largely futile, a researcher could concentrate the cells from a sample of ocean water, extract the DNA, and analyze certain DNA sequences present in the preparation. All organisms share certain genes, such as those that code for the RNAs present in ribosomes or the enzymes of certain metabolic pathways. Even though all organisms may share such genes, the sequences of the nucleotides that make up the genes vary considerably from one species to another. This is the basis of biological evolution. By using techniques that reveal the variety of DNA sequences of a particular gene in a particular habitat, you learn directly about the diversity of species living in that habitat. Recent sequencing techniques have become so rapid and cost-efficient that virtually all of the genes present in the microbes of a given habitat can be sequenced, generating a collective genome, or *metagenome*. This approach can provide information about the types of proteins these organisms manufacture and thus about many of the metabolic activities in which they engage.

These same molecular strategies are being used to explore the remarkable diversity among the trillions of "unseen passengers" that live on or within our own bodies, in habitats such as the intestinal tract, mouth, vagina, and skin. This collection of microbes, which is known as the human *microbiome*, is the subject of several international research efforts aimed at identifying and characterizing these organisms in people of different ages, diets, geographies, and states of health. It has already been demonstrated, for example, that obese and lean humans have markedly different populations of bacteria in their digestive tracts. As obese individuals lose weight, their bacterial profile shifts toward that of the leaner individuals. One study of fecal samples taken from 124 people of varying weight revealed the presence within the collective population of more than 1000 different species of bacteria. Taken together,

TABLE 1.2 **Number and Biomass of Prokaryotes in the World**

Environment	No. of prokaryotic cells, × 10^{28}	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

*1 petagram (Pg) = 10^{15}g.
Source: From W. B. Whitman et al., Proc. Nat'l. Acad. Sci. U.S.A. 95: 6578, 1998. ©1998 National Academy of Sciences, U.S.A. Reproduced with permission of National Academy of Sciences.

these microbes contained more than 3 million distinct genes—approximately 150 times as many as the number present in the human genome. Among the functions of proteins encoded by these microbial genomes are the synthesis of vitamins, the breakdown of complex plant sugars, and the prevention of growth of pathogenic organisms.

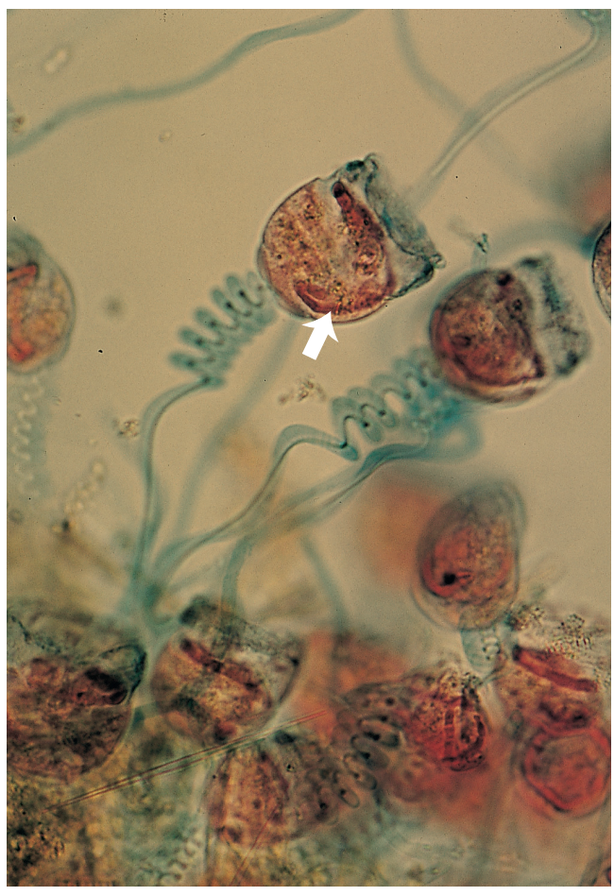
By using sequence-based molecular techniques, biologists have found that most habitats on Earth are teeming with previously unrecognized prokaryotic life. One estimate of the sheer numbers of prokaryotes in the major habitats of Earth is given in **Table 1.2**. It is noteworthy that more than 90 percent of these organisms are now thought to live in the subsurface sediments well beneath the oceans and upper soil layers. Nutrients can be so scarce in some of these deep sediments that microbes living there are thought to divide only once every several hundred years! Table 1.2 also provides an estimate of the amount of carbon (C) that is sequestered in the world's prokaryotic cells. To put this number into more familiar terms, it is roughly comparable to the total amount of carbon present in all of the world's plant life.

Types of Eukaryotic Cells

In many regards, the most complex eukaryotic cells are not found inside of plants or animals, but rather among the single-celled (*unicellular*) protists, such as those pictured in **Figure 1.16**. All of the machinery required for the complex activities in which this organism engages—sensing the environment, trapping food, expelling excess fluid, evading predators—is housed within the confines of a single cell.

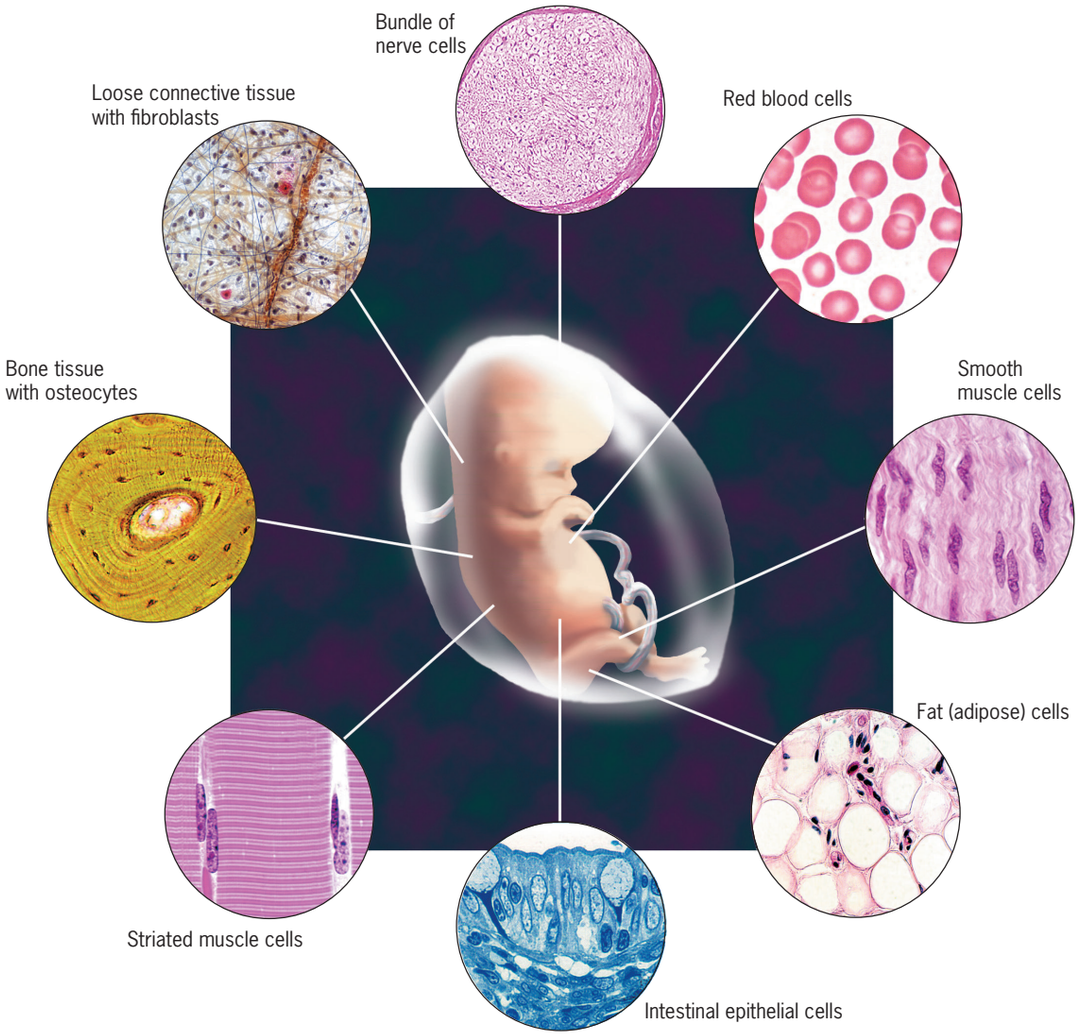
Cell Differentiation Complex unicellular organisms represent one evolutionary pathway. An alternate pathway has led to the evolution of multicellular organisms in which different activities are conducted by different types of specialized cells. Specialized cells are formed by a process called **differentiation**. A fertilized human egg, for example, will progress through a course of embryonic development that leads to the formation of approximately 250 distinct types of differentiated cells. Some cells become part of a particular digestive gland, others part of a large skeletal muscle, others part of a bone, and so forth (**Figure 1.17**). The pathway of differentiation followed by each embryonic cell depends primarily on the signals it receives from the surrounding environment; these signals in turn depend on the position of that cell within the embryo. As discussed in the Human Perspective feature, researchers are learning how to control the process of differentiation in the culture dish and are applying this knowledge to the treatment of complex human diseases.

As a result of differentiation, different types of cells acquire a distinctive appearance and contain unique materials. Skeletal muscle cells contain a network of precisely aligned filaments composed of unique contractile proteins; cartilage



Source: Carolina Biological/Medical Images

FIGURE 1.16 ***Vorticella*, a complex ciliated protist.** A number of these unicellular organisms are seen here; most have withdrawn their "heads" due to shortening of the blue-stained contractile ribbon in the stalk. Each cell has a single large nucleus, called a macronucleus (arrow), which contains many copies of the genes.



Source: Courtesy of Michael Ross, University of Florida.

FIGURE 1.17 Pathways of cell differentiation. A few of the types of differentiated cells present in a human fetus.

The Human Perspective

The Prospect of Cell Replacement Therapy

Many human diseases result from the deaths of specific types of cells. Type 1 diabetes, for example, results from the destruction of beta cells in the pancreas; Parkinson's disease occurs with the loss of dopamine-producing neurons in the brain; and heart failure can be traced to the death of cadiac muscle cells (cardiomyocytes) in the heart. Imagine the possibilities if we could isolate cells from a patient, convert them into the cells that are needed, and then infuse them back into the patient to restore the body's lost function. Recent studies have given researchers hope that one day this type of therapy will be commonplace. To better understand the concept of cell replacement therapy, consider a procedure used widely in current practice, known as *bone marrow transplantation*, in which cells are extracted from the pelvic bones of a donor and infused into the body of a recipient.

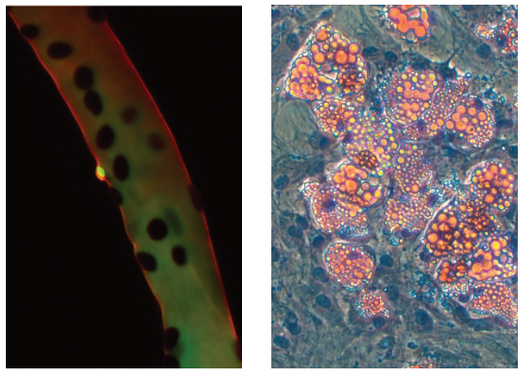
Bone marrow transplantation is used most often to treat lymphomas and leukemias, cancers that affect the nature and number of white blood cells. To carry out the procedure, the patient is exposed to a high level of radiation and/or toxic chemicals; this kills the cancer cells, but also kills all of the cells involved in the formation of red and white blood cells. The treatment has this effect because blood-forming cells are particularly sensitive to radiation and toxic chemicals. Once a person's blood-forming cells have been destroyed, they are replaced by bone marrow cells transplanted from a healthy donor. Bone marrow can regenerate the blood tissue of the transplant recipient because it contains a small percentage of cells that can proliferate and restock the patient's blood-forming bone marrow tissue.[1] These

blood-forming cells in the bone marrow, termed **hematopoietic stem cells** (or **HSCs**), were discovered in the early 1960s by Ernest McCulloch and James Till at the University of Toronto. HSCs are responsible for replacing the millions of red and white blood cells that age and die every minute in our bodies (see Figure 17.6). Amazingly, a *single* HSC is capable of reconstituting the entire hematopoietic (blood-forming) system of an irradiated mouse. An increasing number of parents are saving the blood from the umbilical cord of their newborn baby as a type of "stem-cell insurance policy" in case that child should ever develop a disease that might be treated by administration of HSCs. Now that you understand one type of cell replacement therapy, we can consider several other types with much wider therapeutic potential. We divide these potential therapies into four types.

Adult Stem Cells

Hematopoietic stem cells in the bone marrow are an example of an *adult* stem cell. **Stem cells** are defined as undifferentiated cells that are (1) capable of self-renewal, that is, production of more cells like themselves, and (2) *multipotent*, capable of differentiating into two or more mature cell types. HSCs of the bone marrow are only one type of adult stem cell. Most, if not all, of the organs in a human adult contain stem cells that are capable of replacing the particular cells of the tissue in which they are found. Even the adult brain, which is not known for its ability to regenerate, contains stem cells that can generate new neurons and glial cells (the supportive cells of the brain). **Figure 1a** shows an isolated stem cell present in adult skeletal muscle; these *satellite cells* are thought to divide and differentiate as needed for the repair of injured muscle tissue. Figure 1*b* shows a culture of adipose (fat) cells that have differentiated in vitro from adult stem cells present within fat tissue.

The adult human heart contains stem cells that are capable of differentiating into the cells that form both the muscle tissue of the heart (the cardiomyocytes of the myocardium) and the heart's blood vessels. It had been hoped that these cardiac stem cells might have the potential to regenerate healthy heart tissue in a patient who had experienced a serious heart attack. This hope has apparently been realized, based on the appearance of two landmark reports in late 2011. In clinical trials of patients who had suffered significant heart-tissue damage following heart attacks, stem cells were harvested during heart surgeries, expanded in number through in vitro culture, and then infused back into the heart. Over the next few months, a majority of treated patients experienced significant replacement (e.g., 50 percent) of the damaged heart muscle by healthy tissue derived from the infused stem cells. This regeneration of heart tissue was accompanied by a clear improvement in quality of life compared to patients in the placebo group, who did not receive stem cells. Adult stem cells are an ideal system for cell replacement therapies because they represent an *autologous* treatment; that is, the cells are taken from the same patient in which they are used. Consequently, these stem cells do not face the prospect of immune rejection. These dramatic results with cardiac stem cells rekindled interest in adult stem cells, which had waned after a number of failed attempts to direct stem cells isolated from bone marrow to regenerate diseased tissues. The great majority of adult stem cell therapies under development use a type of adult stem cell known as a *mesenchymal stem cell* (MSC). These can be obtained from bone marrow but they are different from the HSCs discussed previously; they do not produce blood cells but rather a variety of other cell types found in various tissues and organs. MSCs can also be obtained from fat tissue collected during liposuction procedures. Currently there are well over 100 controlled clinical trials underway for treating a wide range of diseases with MSC-derived cells, including heart disease, diabetes, and immune diseases such as Lupus and Crohn's disease. An MSC-based therapy called Prochymal became the first FDA-approved stem cell therapy. It is used to treat Crohn's disease and immune reactions in patients who receive bone marrow transplants.



(a) (b)

Source: (*a*) From Charlotte A. Collins et al., *Cell* 122:291, 2005; with permission of Elsevier; (*b*) Reprinted by permission from Springer Nature: Nathan Blow. *Nature* 451, pages 856–858, 2008.

FIGURE 1 An adult muscle stem cell. **(a)** A portion of a muscle fiber, with its many nuclei stained blue. A single stem cell (yellow) is seen to be lodged between the outer surface of the muscle fiber and an extracellular layer (or basement membrane), which is stained red. The undifferentiated stem cell exhibits this yellow color because it expresses a protein that is not present in the differentiated muscle fiber. **(b)** Adult stem cells undergoing differentiation into adipose (fat) cells in culture. Stem cells capable of this process are present in adult fat tissue and also bone marrow.

Embryonic Stem Cells

Much of the excitement that has been generated in the field over the past decade or two has come from studies on **embryonic stem (ES) cells**, a type of stem cell isolated from very young mammalian embryos (**Figure 2a**). These are the cells in the early embryo that give rise to all of the various structures of the mammalian fetus. Unlike adult stem cells, ES cells are *pluripotent*; that is, they are capable of differentiating into every type of cell in the body. In most cases, human ES cells have been isolated from embryos provided by in vitro fertilization clinics. Worldwide, dozens of genetically distinct human ES cell lines, each derived from a single embryo, are available for experimental investigation.

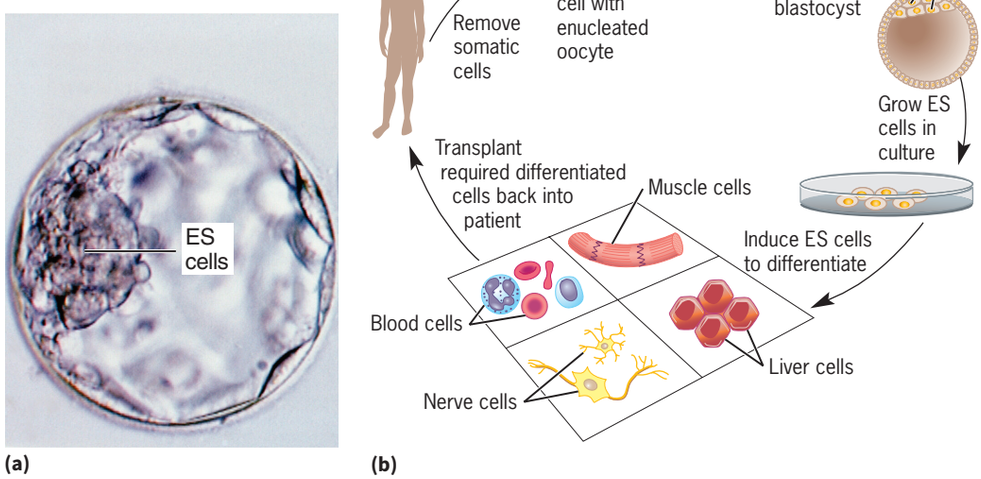
The long-range goal of clinical researchers is to learn how to coax ES cells to differentiate in culture into each of the many cell types that might be used for cell replacement therapy. Considerable progress has been made in this pursuit, and numerous studies have shown that transplants of differentiated, ES-derived cells can improve the condition of animals with diseased or damaged organs. The first trial in humans was begun in 2009 on patients who had experienced debilitating spinal cord injuries. The trial utilized cells, called oligodendrocytes, that produce the myelin sheaths which become wrapped around nerve cells (see Figure 4.5). The oligodendrocytes transplanted into these patients were differentiated from human ES cells cultured in a medium containing insulin, thyroid hormone, and a combination of certain growth factors. This particular

culture protocol had been found to direct the differentiation of ES cells into oligodendrocytes rather than any other cell type. Unfortunately, no significant improvement was reported in the treated patients, and the company conducting the trial decided to cease further involvement in the effort.

Embryonic stem cell therapy is currently under intense study as a treatment for retinal degeneration diseases such as macular degeneration. At the time of this writing, there are several government-approved clinical trials using ES cells induced to differentiate into retinal pigmented epithelial cells, a key cell type within the retina, in an attempt to cure different forms of retinal degeneration.

The primary risk with the therapeutic use of ES cells is the unnoticed presence of undifferentiated ES cells among the differentiated cell population. Undifferentiated ES cells are capable of forming a type of benign tumor, called a teratoma, which may contain a bizarre mass of various differentiated tissues, including hair and teeth. The formation of a teratoma within the central nervous system could have severe consequences. The culture of ES cells currently involves the use of nonhuman biological materials, which also poses potential risks.

The ES cells used in these early trials were derived from cell lines that had been isolated from human embryos unrelated to the patients being treated. Such cells face the prospect of immunologic rejection by the transplant recipient. It may be possible, however, to "customize" ES cells so that they possess the same genetic makeup as the individual being treated. This may be accomplished one day by a roundabout procedure called *somatic cell nuclear transfer* (*SCNT*), shown in Figure 2*b*, that begins with an unfertilized egg—a cell obtained from the ovaries of an unrelated woman donor. In this approach, the nucleus of the unfertilized egg would be replaced by the nucleus of a cell from the patient to be treated, to give the egg the same chromosome composition as that of the patient. The egg would then be allowed to develop to an early embryonic stage, and the ES cells would be removed, cultured, and induced to differentiate into the type of cells needed by the patient. Because this procedure involves the formation of a human embryo that is used only as a source of ES cells, major ethical questions must be settled before it could be practiced routinely. In addition, the process is very expensive and technically demanding, making it highly improbable that it could ever be practiced as part of any routine medical treatment. If ES cell–based therapy is ever practiced, it would more likely depend on the use of a bank of hundreds or thousands of different ES cells. Such a bank could contain cells that are close enough as a tissue match to be suitable for use in the majority of patients.



Source: Phanie/SuperStock.

FIGURE 2 Embryonic stem cells; their isolation and potential use. **(a)** Micrograph of a mammalian blastocyst, an early stage during embryonic development, showing the inner cell mass, which is composed of pluripotent ES cells. Once isolated, such cells are readily grown in culture. **(b)** A potential procedure for obtaining differentiated cells for use in cell replacement therapy. A small piece of tissue is taken from the patient, and one of the somatic cells is fused with a donor oocyte (egg) from which the nucleus was previously removed. The resulting oocyte, with the patient's cell nucleus, is allowed to develop into an early embryo, and the ES cells are harvested and grown in culture. A population of ES cells are induced to differentiate into the required cells, which are subsequently transplanted into the patient to restore organ function. (At the present time, it has not been possible to obtain blastocyst stage embryos, that is, ones with ES cells, from any primate species by the procedure shown here, although it has been accomplished using an oocyte from which the nucleus is *not* first removed. The ES cells generated in such experiments are triploid; that is, they have three copies of each chromosome—one from the oocyte and two from the donor nucleus—rather than two, as would normally be the case. Regardless, these triploid ES cells are pluripotent and capable of transplantation.)

Induced Pluripotent Stem Cells

It had long been thought that the process of cell differentiation in mammals was irreversible; once a cell had become a fibroblast, or white blood cell, or cartilage cell, it could never again revert to any other cell type. This belief was shattered in 2006 when Shinya Yamanaka and co-workers of Kyoto University announced a stunning discovery; his lab had succeeded in reprogramming a fully differentiated mouse cell—in this case, a type of connective tissue fibroblast—into a pluripotent stem cell. They accomplished the feat by introducing into the mouse fibroblast the genes that encoded four key proteins that are characteristic of ES cells. These genes (*Oct4, Sox2, Klf4,* and *Myc*, known collectively as OSKM) are thought to play a key role in maintaining the cells in an undifferentiated state and allowing them to continue to self-renew. The genes were introduced into cultured fibroblasts using gene-carrying viruses, and those rare cells that became reprogrammed were selected from the others in the culture by specialized techniques. They called this new type of cells *induced pluripotent stem cells* (*iPS cells*) and demonstrated that they were indeed pluripotent by injecting them into a mouse blastocyst and finding that they participated in the differentiation of all the cells of the body, including eggs and sperm. Within the next year or so, the same reprogramming feat had been accomplished in several labs with human cells. So researchers now have available to them an unlimited supply of pluripotent cells that can be directed to differentiate into various types of body cells using experimental protocols similar to those already developed for ES cells.

Indeed, iPS cells have already been used to correct certain disease conditions in experimental animals, including sickle cell anemia in mice as depicted in **Figure 3**. Based on the promising results of animal experiments, attempts to use iPS cells in patients are beginning. Small-scale clinical trials of iPS cell–based therapies have been carried out, mostly to determine whether there are harmful side effects. Based on promising results from these studies, larger-scale clinical trials are now being launched in several countries, including the United States. Similar to ongoing embryonic stem cell trials mentioned earlier, these trials are testing the use of iPS cell–derived retinal pigmented epithelial cells to treat macular degeneration.

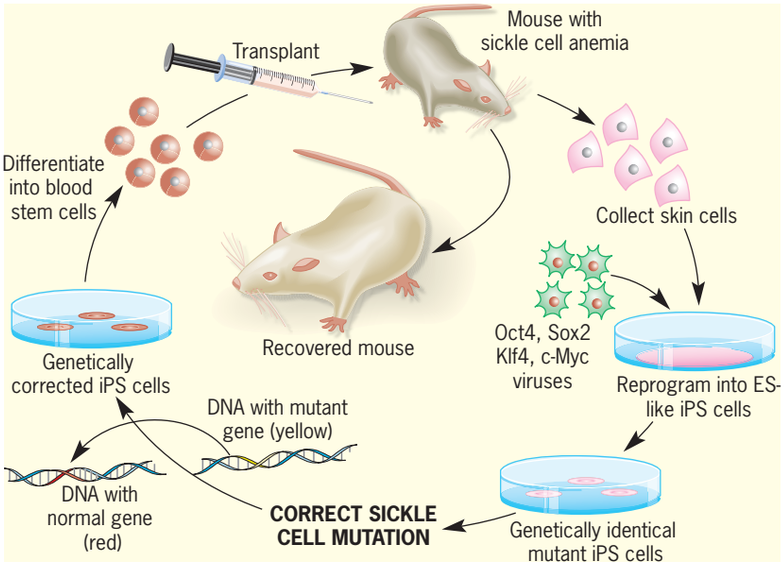
The utility of iPS cells may extend far beyond cell replacement therapy. iPS cells have also been prepared from adult cells taken from patients with a multitude of genetic disorders. Researchers are then able to follow the differentiation of these iPS cells in culture into the specialized cell types that are affected by the particular disease. It is hoped that such studies will reveal the mechanisms of disease formation as it unfolds in a culture dish just as it would normally occur in an unobservable way deep within the body. These "diseased iPS cells" have been referred to as "patients in a Petri dish." The clinical relevance of these cells can be illustrated by an example. iPS cells derived from patients with a heart disorder called long QT syndrome differentiate into cardiac muscle cells that exhibit irregular contractions (*beats*) in culture. This disease-specific phenotype seen in culture can be corrected by several medicines normally prescribed to treat this disorder. Moreover, when cardiomyocytes that had differentiated from the diseased iPS cells were exposed to the drug cisapride, the irregularity of their contractions increased. Cisapride, a drug that was used to treat heartburn, was pulled from the market in the United States after it was shown to cause heart arrhythmias in certain patients. Results of this type suggest that differentiated cells derived from diseased iPS cells will serve as valuable targets for screening potential drugs for their effectiveness in halting disease progression.

Unlike ES cells, the generation of iPS cells does not require the use of an embryo. This feature removes all of the ethical reservations that accompany work with ES cells and also makes it much easier to generate these cells in the lab. However, as research on iPS cells has increased, the therapeutic potential of these cells has become less clear. For the first several years of study, it was thought that iPS cells and ES cells were essentially indistinguishable. However, recent studies have shown that iPS cells lack the "high quality" characteristic of ES cells and that not all iPS cells are the same. For example, iPS cells exhibit certain genomic abnormalities that are not present in ES cells, including the presence of mutations and extra copies of random segments of the genome. In addition, the DNA-containing chromatin of iPS cells retains certain traces of the original cells from which they were derived, which means that they are not completely reprogrammed into ES-like, pluripotent cells. This residual memory of their origin makes it is easier to direct iPS cells toward differentiation back into the cells from which they were derived than into other types of cells. It may be that these apparent deficiencies in iPS cells will not be a serious impediment in their use to treat diseases that affect adult tissues, but it has raised important questions. There are other issues with iPS cells as well. It will be important to develop efficient cell reprogramming techniques that do not use genome-integrating viruses because such virus-infected cells carry the potential of developing into cancers. Progress has been made in this regard, but the efficiency of iPS cell formation typically drops when other procedures are used to introduce genes.

Like ES cells, undifferentiated iPS cells also give rise to teratomas, so it is essential that only fully differentiated cells are transplanted into human subjects. Also like ES cells, the iPS cells in current use have the same tissue antigens as the donors who originally provided them, so they would stimulate an immune attack if they were transplanted into other human recipients. Compared with the difficulties inherent in the formation of ES cells, however, clinicians will find it much easier to generate personalized, tissue-compatible iPS cells, because they can be derived from a simple skin biopsy. Still, as with ES cells, it does take considerable time, expense, and technical expertise to generate a population of iPS cells from a specific donor. Consequently, if iPS cells are ever developed for widespread therapeutic use, they would likely come from a large cell bank that could provide cells that are close tissue matches to most potential recipients. One day it may also be possible to remove all of the genes from iPS cells that normally prevent them from being transplanted into random recipients.

Direct Cell Reprogramming

In 2008, the field of cellular reprogramming took another unexpected turn with the announcement that one type of differentiated cell had been converted directly into another type of differentiated cell, a case of *transdifferentiation*. In this report, the acinar cells of the pancreas, which produce enzymes responsible for digestion of food in the intestine, were transformed into pancreatic beta cells, which synthesize and secrete the hormone insulin. The reprogramming process occurred directly, in a matter of a few days, without the cells passing through an intermediate stem cell state—and it occurred while the cells remained in their normal residence within the pancreas of a live mouse. This feat was accomplished by injection of viruses that carried three genes known to be important in differentiation of beta cells in the embryo. In this case, the recipients of the injection were diabetic mice, and the transdifferentiation of a significant number of acinar cells into beta cells allowed



Source: Adapted from an illustration by Rudolf Jaenisch, *Cell* 132:5, 2008, from Elsevier.

FIGURE 3 Steps taken to generate induced pluripotent stem (iPS) cells for use in correcting the inherited disease sickle cell anemia in mice. Skin cells are collected from the diseased animal, reprogrammed in culture by introducing the four required genes that are ferried into the cells by viruses, and allowed to develop into undifferentiated pluripotent iPS cells. The iPS cells are then treated so as to replace the defective (globin) gene with a normal copy, and the corrected iPS cells are caused to differentiate into normal blood stem cells in culture. These blood stem cells are then injected back into the diseased mouse, where they proliferate and differentiate into normal blood cells, thereby curing the disorder.

the animals to regulate their blood sugar levels with much lower doses of insulin. It is also noteworthy that the adenoviruses used to deliver the genes in this experiment do not become a permanent part of the recipient cell, which removes some of the concerns about the use of viruses as gene carriers in humans. Since this initial report, a number of laboratories have developed in vitro techniques to directly convert one type of differentiated cell (typically a fibroblast) into another type of cell, such as a neuron, cardiomyocyte, or blood-cell precursor, in culture, without passing through a pluripotent intermediate. In all of these cases, transdifferentiation occurs when the original cells are forced to express certain genes that play a role in the normal embryonic differentiation of the other cell type. It is too early to know whether direct cell reprogramming has therapeutic potential, but it certainly raises the prospect that diseased cells needing to be replaced might be formed directly from other types of cells within the same organ.

Reference

1. Bone marrow transplantation can be compared to a simple blood transfusion in which the recipient receives differentiated blood cells (especially red blood cells and platelets) present in the circulation.

cells become surrounded by a characteristic matrix containing polysaccharides and the protein collagen, which together provide mechanical support; red blood cells become disk-shaped sacks filled with a single protein, hemoglobin, which transports oxygen; and so forth. Despite their many differences, the various cells of a multicellular plant or animal are composed of similar organelles. Mitochondria, for example, are found in essentially all types of cells. In one type, however, they may have a rounded shape, whereas in another they may be highly elongated and thread-like. In each case, the number, appearance, and location of the various organelles can be correlated with the activities of the particular cell type. An analogy might be made to a variety of orchestral pieces: all are composed of the same notes, but varying arrangements give each its unique character and beauty.

Model Organisms

Living organisms are highly diverse, and the results obtained from a particular experimental analysis may depend on the particular organism being studied. As a result, cell and molecular biologists have focused considerable research activities on a small number of *representative* or **model organisms**. It is hoped that a comprehensive body of knowledge built on these studies will provide a framework to understand those basic processes that are shared by most organisms, especially humans. Many other organisms are widely used in the study of cell and molecular biology. Nevertheless, six model organisms—one prokaryote and five eukaryotes—have captured much of the attention: a bacterium, *E. coli*; a budding yeast, *Saccharomyces cerevisiae*; a flowering plant, *Arabidopsis thaliana*; a nematode, *Caenorhabditis elegans*; a fruit fly, *Drosophila melanogaster*; and

a mouse, *Mus musculus*. Each of the organisms pictured in **Figure 1.18** has specific advantages that make it particularly useful as a research subject for answering certain types of questions. A few of their advantages as research systems are described in the accompanying legend. We concentrate in this text on results obtained from studies on mammalian systems—mostly on the mouse and on cultured mammalian cells—because these findings are most applicable to humans. Because a large portion of what we know about mammalian cells was first discovered by experiments in other model organisms that are easier to study, on many occasions we describe research carried out on the cells of other species. You may be surprised to discover how similar you are to these much smaller and simpler organisms at the cell and molecular level.

The Sizes of Cells and Their Components

Figure 1.19 shows the relative size of a number of structures of interest in cell biology. Two units of linear measure are most commonly used to describe structures within a cell: the **micrometer** (μm) and the **nanometer** (nm). One μm is equal to 10^{-6} meters, and one nm is equal to 10^{-9} meters. The **angstrom** (Å), which is equal to one-tenth of an nm, is commonly employed by molecular biologists for atomic dimensions. One angstrom is roughly equivalent to the diameter of a hydrogen atom. Large biological molecules (i.e., macromolecules) are described in either angstroms or nanometers. Myoglobin, a typical globular protein, is approximately 4.5 nm × 3.5 nm × 2.5 nm; highly elongated proteins (such as collagen or myosin) are over 100 nm in length; and DNA is approximately 2.0 nm in width. Complexes of macromolecules, such as ribosomes, microtubules, and microfilaments, are between 5 and 25 nm in diameter. Despite their tiny dimensions, these macromolecular complexes constitute remarkably sophisticated "nanomachines" capable of performing a diverse array of mechanical, chemical, and electrical activities.

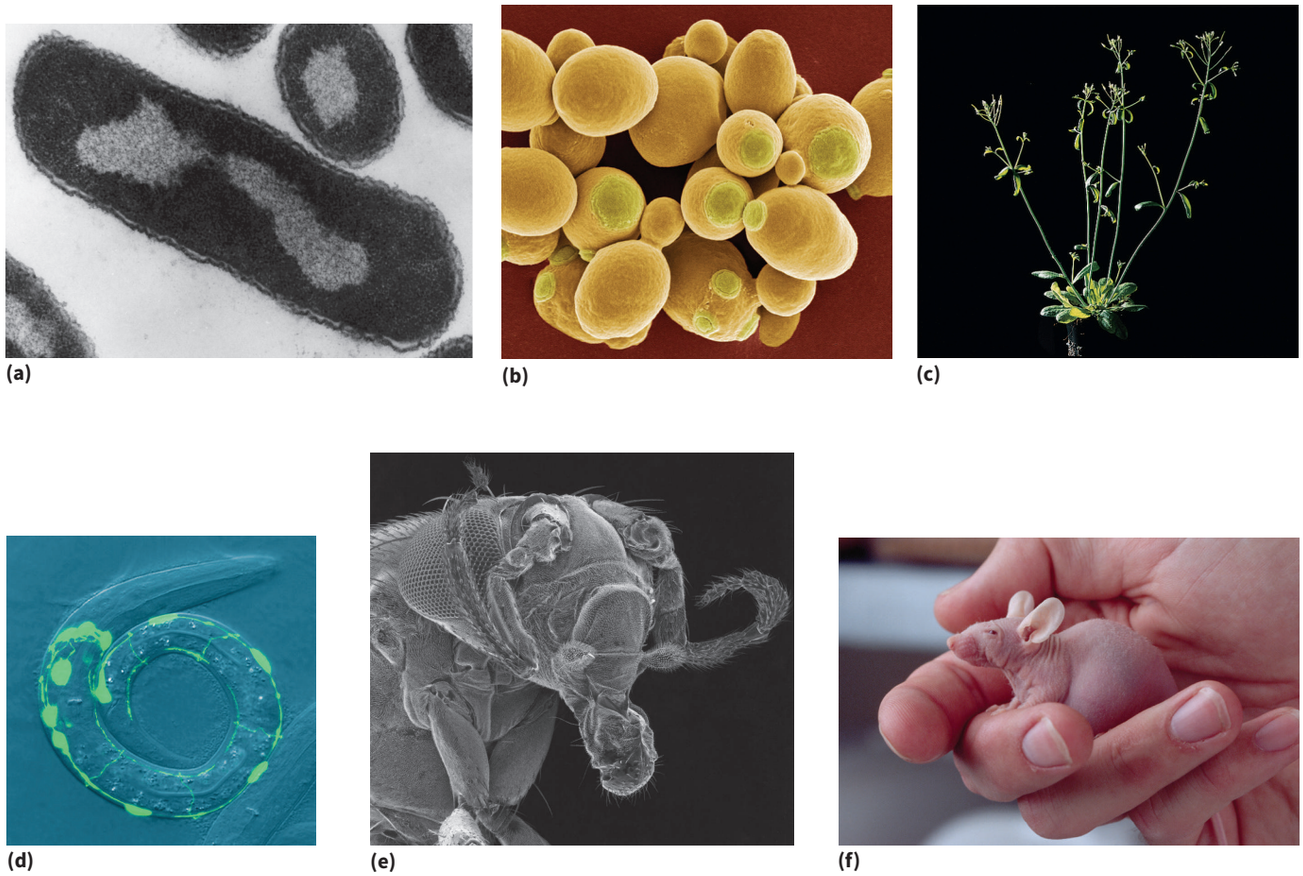
Cells and their organelles are more easily defined in micrometers. Nuclei, for example, are approximately 5–10 μm in diameter, and mitochondria are approximately 2 μm in length. Prokaryotic cells typically range in length from about 1 to 5 μm, eukaryotic cells from about 10 to 30 μm. There are a number of reasons most cells are so small. Consider the following.

- Most eukaryotic cells possess a single nucleus that contains only two copies of most genes. Because genes serve as templates for the production of information-carrying messenger RNAs, a cell can only produce a limited number of these messenger RNAs in a given amount of time. The greater a cell's cytoplasmic volume, the longer it will take to synthesize the number of messages required by that cell.
- As a cell increases in size, the surface area/volume ratio decreases.[3] The ability of a cell to exchange substances with its environment is proportional to its surface area. If a cell were to grow beyond a certain size, its surface would not be sufficient to take up the substances (e.g., oxygen, nutrients) needed to support its metabolic activities. Cells specialized for absorption of solutes, such as those of the intestinal epithelium, typically possess microvilli, which greatly increase the surface area available for exchange (see Figure 1.3). The interior of a large plant cell is typically filled by a large, fluid-filled vacuole rather than metabolically active cytoplasm (see Figure 8.37*b*).
- A cell depends to a large degree on the random movement of molecules (*diffusion*). Oxygen, for example, must diffuse from the cell's surface through the cytoplasm to the interior of its mitochondria. The time required for diffusion is proportional to the square of the distance to be traversed. For example, O_2 requires only 100 microseconds to diffuse a distance of 1 μm, but requires 10^6 times as long to diffuse a distance of 1 mm. As a cell becomes larger and the distance from the surface to the interior becomes greater, the time required for diffusion to move substances in and out of a metabolically active cell becomes prohibitively long.

Despite these constraints, some eukaryotic cells can be extremely large. The free-living single-celled organism *Stentor coeruleus*, which lives in freshwater ponds, grows to be more than a millimeter long, and the giant single-celled green alga *Acetabularia* is more than 10 cm long. The gargantuan single-celled green alga *Caulerpa* can grow to a length of several meters and contains millions of nuclei in a common cytoplasm. Examples of large cell size are not restricted to such strange organisms, however. Indeed, we have some examples in our own bodies. Neurons send out extremely long processes; motor neurons in the human spinal cord, for example, send out axons that can be as long as one meter.

The fact that cells are collections of nanomachines has inspired a research field known as *synthetic biology*, with the ultimate goal of creating some minimal type of living cell in the laboratory out of the same types of component parts found in real cells. Synthetic biology uses the molecules, molecular complexes, and organelles of a cell as buiding blocks, as suggested by the cartoon in **Figure 1.20**. One motivation of these researchers is simply to accomplish the feat and, in the process, demonstrate that life at the cellular level emerges spontaneously when the proper constituents are brought together from chemically synthesized materials. At this point in time,

[3]You can verify this statement by calculating the surface area and volume of a cube with sides 1 cm in length versus a cube with sides 10 cm in length. The surface area/volume ratio of the smaller cube is considerably greater than that of the larger cube.



Source: (*a*) Biophoto Associates/Science Source; (*b*) STEVE GSCHMEISSNER/Science Photo Library/Getty Images; (*c*) ISM/Jean-Claude RÉVY/Medical Images; (*d*) Courtesy of Erik Jorgensen, Department of Biology, University of Utah. From Trends Genetics, Vol. 14, cover #12. ©1998, with permission from Elsevier; (*e*) David Scharf/Science Source; (*f*) Ted Spiegel/Getty Images.

FIGURE 1.18 **Six model organisms.** **(a)** *Escherichia coli* is a rod-shaped bacterium that lives in the digestive tract of humans and other mammals. Much of what we discuss about the basic molecular biology of the cell, including the mechanisms of replication, transcription, and translation, was originally worked out on this one prokaryotic organism. The relatively simple organization of a prokaryotic cell is illustrated in this electron micrograph. **(b)** *Saccharomyces cerevisiae*, more commonly known as baker's yeast or brewer's yeast, is the least complex of the eukaryotes commonly studied, yet it contains a surprising number of proteins that are homologous to proteins in human cells. Such proteins typically have a conserved function in the two organisms. The species has a small genome encoding about 6200 proteins; it can be grown in a haploid state (one copy of each gene per cell rather than two as in most eukaryotic cells); and it can be grown under either *aerobic* (O_2-containing) or *anaerobic* (O_2-lacking) conditions. It is ideal for the identification of genes through the use of mutants. **(c)** *Arabidopsis thaliana*, a weed (called the thale cress) related to mustard and cabbage, has an unusually small genome (120 million base pairs) for a flowering plant, a rapid generation time, and large seed production, and grows to a height of only a few inches. **(d)** *Caenorhabditis elegans*, a microscopic-sized nematode, consists of a defined number of cells (roughly 1000), each of which develops according to a precise pattern of cell divisions. The animal is easily cultured, can be kept alive in a frozen state, has a transparent body wall, a short generation time, and facility for genetic analysis. This micrograph shows the larval nervous system, which has been labeled with the green fluorescent protein (GFP). **(e)** *Drosophila melanogaster*, the fruit fly, is a small but complex eukaryote that is readily cultured in the lab, where it grows from an egg to an adult in a matter of days. *Drosophila* has been a favored animal for the study of genetics, the molecular biology of development, and the neurological basis of simple behavior. Certain larval cells have giant chromosomes, with individual genes that can be identified for studies of evolution and gene expression. In the mutant fly shown here, a leg has developed where an antenna would be located in a normal (wild type) fly. **(f)** *Mus musculus*, the common house mouse, is easily kept and bred in the laboratory. Thousands of different genetic strains have been developed, many of which are stored simply as frozen embryos due to lack of space to house the adult animals. The "nude mouse" pictured here develops without a thymus gland and, therefore, is able to accept human tissue grafts.

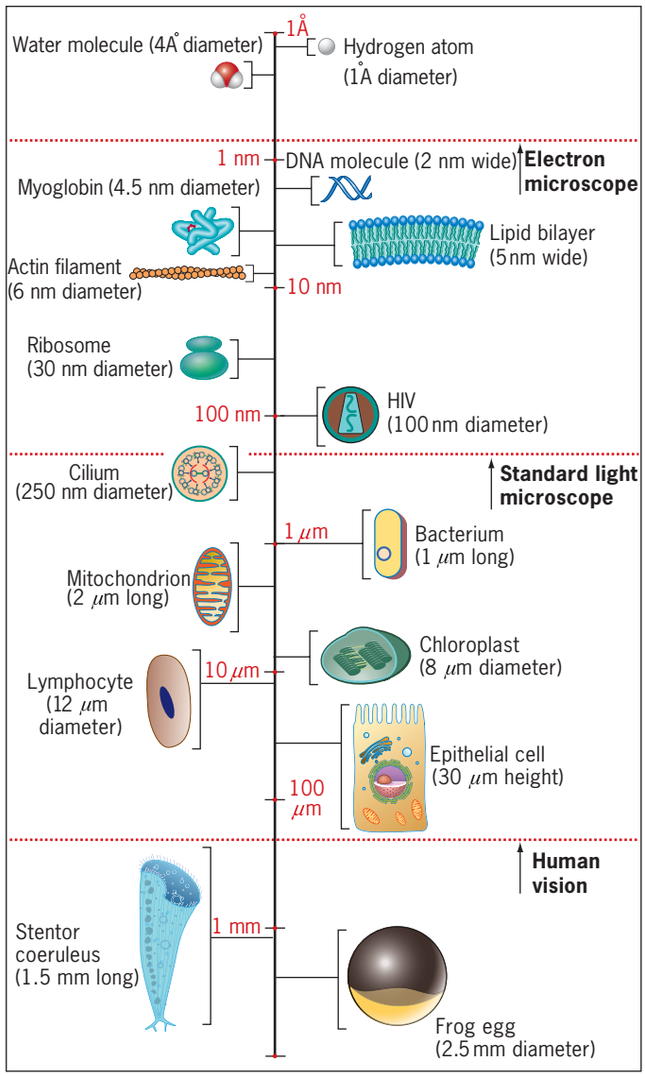


FIGURE 1.19 Relative sizes of cells and cell components. These structures differ in size by more than seven orders of magnitude.

biologists have only begun the first steps in this direction. Such work holds the potential to illuminate the possible origins of life and to launch an entirely new approach to biotechnology. However, creating life may raise interesting moral and even religious questions. A more modest goal of synthetic biology is to develop novel life forms, using existing organisms as a starting point, that have a unique value in medicine and industry, or in cleaning up the environment.

If, as most biologists would argue, the properties and activities of a cell spring from the genetic blueprint of that cell, then it should be possible to create a new type of cell by introducing a new genetic blueprint into the cytoplasm of an existing cell. This feat was accomplished by J. Craig Venter and colleagues in 2007, when they replaced the genome of one bacterium with a genome isolated from a closely related species, effectively transforming one species into the other. By 2010, after overcoming a number of stubborn technical roadblocks, the team was able to accomplish a similar feat using a copy of a bacterial genome that had been assembled (inside a yeast cell) from fragments of DNA that had been chemically synthesized in the laboratory. The synthetic copy of the donor genome, which totaled approximately 1.1 million base pairs of DNA, contained a number of modifications introduced by the researchers. The modified copy of the genome (from *Mycoplasma mycoides*) was transplanted into a cell of a closely related bacterial species (*M. capricolum*), where it replaced the host's original genome. Following genome transplantation, the recipient cell rapidly took on the characteristics of the species from which the donor DNA had been derived. In effect, these researchers have produced cells containing a "genetic skeleton" to which they can add combinations of new genes taken from other organisms.

Researchers around the world are attempting to genetically engineer organisms to possess metabolic pathways capable of producing pharmaceuticals, hydrocarbon-based fuel molecules, and other useful chemicals from cheap, simple precursors. Several companies are growing genetically engineered cyanobacteria capable of producing diesel fuel from sunlight, water, and CO_2. Researchers at one company have genetically engineered the common lab bacterium *E. coli* to ferment the complex polysaccharides present in seaweed into the biofuel ethanol. This feat required the introduction into *E. coli* of a combination of genes derived from three other bacterial species. Work has also begun on "rewriting" the yeast genome, signifying that eukaryotic cells have also become part of the effort to design genetically engineered biological manufacturing plants.

In principle, the work described in the Human Perspective feature, in which one type of cell is directed into the formation of an entirely different type of cell, is also a form of synthetic biology. As a result of these many efforts, biologists are no longer restricted to studying cells that are available in nature, but are turning their attention to cells that can become available through experimental manipulation.

Review

1. Compare prokaryotic and eukaryotic cells on the basis of their structural, functional, and metabolic differences.
2. Which group of prokaryotes is best known for containing many extremophiles?
3. What is the importance of cell differentiation?
4. Why are cells almost always microscopic?
5. If a mitochondrion were 2 µm in length, how many angstroms would it be? How many nanometers? How many millimeters?

Source: Courtesy of Jakob C. Schweizer.

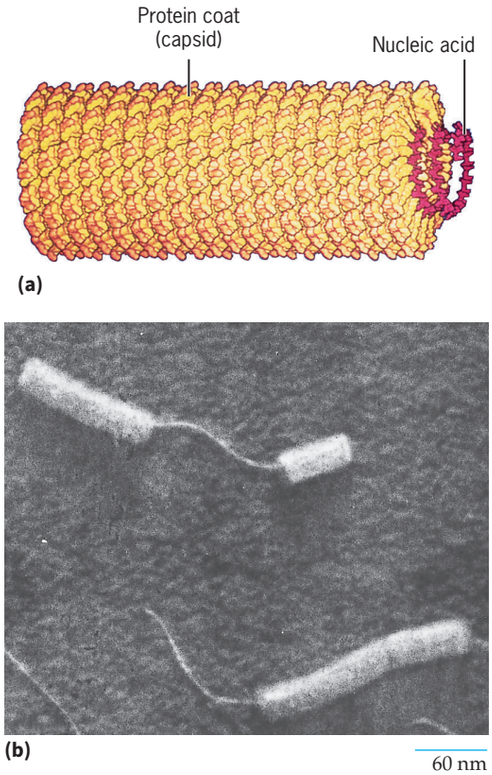
FIGURE 1.20 **The synthetic biologist's toolkit of the future?** Such a toolkit would presumably contain nucleic acids, proteins, lipids, and many other types of biomolecules.

1.4 Viruses and Viroids

By the end of the nineteenth century, the work of Louis Pasteur and others had convinced the scientific world that infectious diseases of plants and animals were due to bacteria. But studies of tobacco mosaic disease in tobacco plants and hoof-and-mouth disease in cattle pointed to the existence of another type of infectious agent. It was found, for example, that sap from a diseased tobacco plant could transmit mosaic disease to a healthy plant, even when the sap showed no evidence of bacteria in the light microscope. To gain further insight into the size and nature of the infectious agent, Dmitri Ivanovsky, a Russian biologist, forced the sap from a diseased plant through filters with pores so small that they retarded the passage of the smallest known bacterium. The filtrate was still infective, causing Ivanovsky to conclude in 1892 that certain diseases were caused by pathogens that were even smaller, and presumably simpler, than the smallest known bacteria. These pathogens became known as **viruses**.

In 1935, Wendell Stanley of the Rockefeller Institute reported that the virus responsible for tobacco mosaic disease could be crystallized and that the crystals were infective. Substances that form crystals have a highly ordered, well-defined structure and are vastly less complex than the simplest cells. Stanley mistakenly concluded that tobacco mosaic virus (TMV) was a protein. In fact, TMV is a rod-shaped particle consisting of a single molecule of RNA surrounded by a helical shell composed of protein subunits (**Figure 1.21**).

Viruses are responsible for dozens of human diseases, including acquired immunodeficiency syndrome (AIDS), polio, influenza, ebola, measles, and a few types of cancer. Viruses occur in a wide variety of very different shapes, sizes, and constructions, but all of them share certain common properties. All viruses are obligatory intracellular parasites; that is, they cannot reproduce unless present within a host cell. Depending on the specific virus, the host may be a plant, animal, or bacterial cell. Outside a living cell, the virus exists as a particle, or **virion**, which is little more than a macromolecular package. The virion contains a small amount of genetic material that, depending on the virus, can be single-stranded or double-stranded, RNA or DNA. Remarkably, some viruses have as few as three or four different genes, but others may have as many as several hundred. The genetic material of the virion is surrounded by a protein capsule, or *capsid*. Virions are macromolecular aggregates, inanimate particles that by themselves are unable to reproduce, metabolize, or carry on any of



Source: (*a*) Courtesy of Gerald Stubbs, Keuchi Namba, and Donald Caspar; (*b*) Courtesy of M.K. Corbett.

FIGURE 1.21 Tobacco mosaic virus (TMV). **(a)** Model of a portion of a TMV particle. The protein subunits, which are identical along the entire rod-shaped particle, enclose a single helical RNA molecule (red). **(b)** Electron micrograph of TMV particles after phenol has removed the protein subunits from the middle part of the upper particle and the ends of the lower particle. Intact rods are approximately 300 nm long and 18 nm in diameter.

the other activities associated with life. For this reason, viruses are not considered to be organisms and are not described as being alive.

Viral capsids are generally made up of a specific number of subunits. There are numerous advantages to construction by subunit, one of the most apparent being an economy of genetic information. If a viral coat is made of many copies of a single protein, as is that of TMV, or a few proteins, as are the coats of many other viruses, the virus needs only one or a few genes to code for its protein container. Many viruses have a capsid with subunits organized into a polyhedron, that is, a structure having planar faces. A particularly common polyhedral shape of viruses is the 20-sided *icosahedron*. For example, adenovirus, which causes respiratory infections in mammals, has an icosahedral capsid (**Figure 1.22a**). In many animal viruses, including the *human immunodeficiency virus* (*HIV*) responsible for AIDS, the protein capsid is surrounded by a lipid-containing outer envelope derived from the modified plasma membrane of the host cell as the virus buds from the host-cell surface (Figure 1.22*b*). Bacterial viruses, or *bacteriophages*, are among the most complex viruses (Figure 1.22*c*). They are also the most abundant biological entities on Earth. The T bacteriophages (which were used in key experiments that revealed the structure and properties of the genetic material) consist of a polyhedral head containing DNA, a cylindrical stalk through which the DNA is injected into the bacterial cell, and tail fibers, which together cause the particle to resemble a landing module for the moon (Figure 1.22*c*).

Each virus has on its surface a protein that is able to bind to a particular surface component of its host cell. For example, the protein that projects from the surface of the HIV particle (labeled gp120 in Figure 1.22*b*, which stands for glycoprotein of molecular mass 120,000 daltons[4]) interacts with a specific protein (called CD4) on the surface of certain white blood cells, facilitating entry of the virus into its host cell. The interaction between viral and host proteins determines the specificity of the virus, that is, the types of host cells that the virus can enter and infect. Some viruses have a wide *host range*, being able to infect cells from a variety of different organs or host species. The virus that causes rabies, for example, is able to infect many different types of mammalian hosts, including dogs, bats, and humans. Most viruses, however, have a relatively narrow host range. This is true, for example, of human cold and influenza viruses, which are generally able to infect only the respiratory epithelial cells of human hosts.

A change in host-cell specificity can have striking consequences. This point is dramatically illustrated by the 1918 influenza pandemic, which killed more than 30 million people worldwide. The virus was especially lethal in young adults, who do not normally fall victim to influenza. In fact, the 675,000 deaths from this virus in the United States temporarily lowered average life expectancy by several years. In one of the most acclaimed—and controversial—feats of the past few years, researchers have been able to determine the genomic sequence of the virus responsible for this pandemic and to reconstitute the virus in its full virulent state. This was accomplished by isolating the viral genes (which are part of a genome consisting of eight separate RNA molecules encoding 11 different proteins) from the preserved tissues of victims who had died from the infection 90 years earlier. The best preserved samples were obtained from a Native American woman who had been buried in the Alaskan permafrost. The sequence of the *1918 virus* suggested that the pathogen had jumped from birds to humans. Although the virus had accumulated a considerable number of mutations, which adapted it to a mammalian host, it had never exchanged genetic material with a human influenza virus as had been postulated.

Analysis of the sequence of the 1918 virus has provided some clues to explain why it was so deadly and how it spread so efficiently from one human to another. Using the genomic sequence, researchers reconstituted the virus into

[4]One dalton is equivalent to one unit of atomic mass, the mass of a single hydrogen (^{1}H) atom.

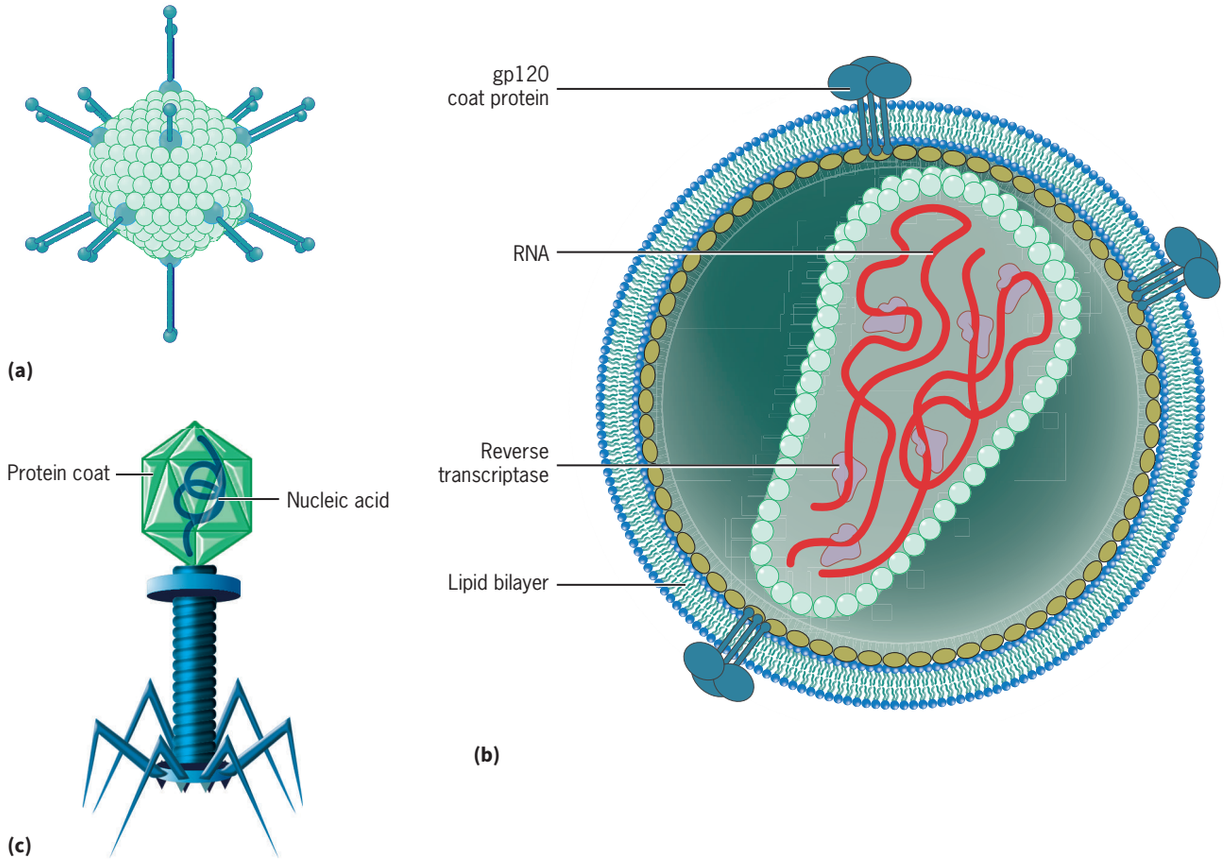
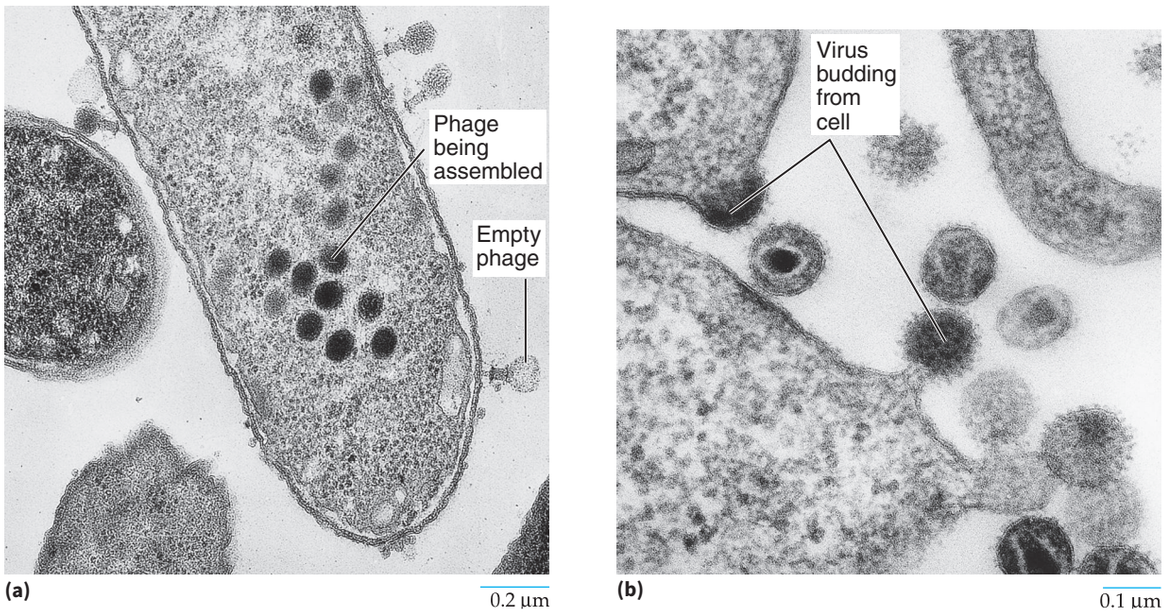


FIGURE 1.22 Virus diversity. The structures of **(a)** an adenovirus, **(b)** a human immunodeficiency virus (HIV), and **(c)** a T-even bacteriophage. *Note:* These viruses are not drawn to the same scale.

infectious particles, which were found to be exceptionally virulent in laboratory tests. Whereas laboratory mice normally survive infection by modern human influenza viruses, the reconstituted 1918 strain killed 100 percent of infected mice and produced enormous numbers of viral particles in the animals' lungs. Because of the potential risk to public health, publication of the full sequence of the 1918 virus and its reconstitution went forward only after approval by governmental safety panels and the demonstration that existing influenza vaccines and drugs protect mice from the reconstituted virus.

There are two basic types of viral infection. (1) In most cases, the virus arrests the normal synthetic activities of the host and redirects the cell to use its available materials to manufacture viral nucleic acids and proteins, which assemble into new virions. Viruses, in other words, do not grow like cells; they are assembled from components directly into the mature-sized virions. Ultimately, the infected cell ruptures (*lyses*) and releases a new generation of viral particles capable of infecting neighboring cells. An example of this type of *lytic* infection is shown in **Figure 1.23a**. (2) In other cases, the infecting virus does not lead to the death of the host cell, but instead inserts (*integrates*) its DNA into the DNA of the host cell's chromosomes. The integrated viral DNA is called a **provirus**. An integrated provirus can have different effects depending on the type of virus and host cell. For example,

- Bacterial cells containing a provirus behave normally until exposed to a stimulus, such as ultraviolet radiation, that activates the dormant viral DNA, leading to the lysis of the cell and release of viral progeny.
- Some animal cells containing a provirus produce new viral progeny that bud at the cell surface without lysing the infected cell. Human immunodeficiency virus (HIV) acts in this way; an infected cell may remain alive for a period, acting as a factory for the production of new virions (Figure 1.23*b*).
- Some animal cells containing a provirus lose control over their own growth and division and become malignant. This phenomenon is readily studied in the laboratory by infecting cultured cells with the appropriate tumor virus.



Source: (*a*) Courtesy of Jonathan King and Erika Hartwig; (*b*) Courtesy of Hans Gelderblom.

FIGURE 1.23 A virus infection. **(a)** Micrograph showing a late stage in the infection of a bacterial cell by a bacteriophage. Virus particles are being assembled within the cell, and empty phage coats are still present on the cell surface. **(b)** Micrograph showing HIV particles budding from an infected human lymphocyte.

Viruses are not without their virtues. Because the activities of viral genes mimic those of host genes, investigators have used viruses for decades as a research tool to study the mechanism of DNA replication and gene expression in their much more complex hosts. In addition, viruses are now being used as a means to introduce foreign genes into human cells, a technique that will likely serve as the basis for the treatment of human diseases by gene therapy. Last, insect- and bacteria-killing viruses may play an increasing role in the war against insect pests and bacterial pathogens. Bacteriophages have been used for decades to treat bacterial infections in eastern Europe and Russia, while physicians in the West have relied on antibiotics. Given the rise in antibiotic-resistant bacteria, bacteriophages may be making a comeback on the heels of promising studies on infected mice. Several biotechnology companies are now producing bacteriophages intended to combat bacterial infections and to protect certain foods from bacterial contamination.

It came as a surprise in 1971 to discover that viruses are not the simplest types of infectious agents. In that year, T. O. Diener of the U.S. Department of Agriculture reported that potato spindle-tuber disease, which causes potatoes to become gnarled and cracked, is caused by an infectious agent consisting of a small circular RNA molecule that totally lacks a protein coat. Diener named the pathogen a **viroid**. The RNAs of viroids range in size from about 240 to 600 nucleotides, one-tenth the size of the smaller viruses. No evidence has been found that the naked viroid RNA encodes any proteins. Rather, any biochemical activities in which viroids engage take place using host-cell proteins. For example, duplication of the viroid RNA within an infected cell utilizes the host's RNA polymerase II, an enzyme that normally transcribes the host's DNA into messenger RNAs. Viroids are thought to cause disease by interfering with the cell's normal path of gene expression. The effect on crops can be serious: A viroid disease called cadang-cadang has devastated the coconut palm groves of the Philippines, and another viroid has wreaked havoc on the chrysanthemum industry in the United States. The discovery of a different type of infectious agent even simpler than a viroid is described in the Human Perspective feature on Protein Misfolding in Section 2.7.

Review

1. **What properties distinguish a virus from a bacterium?**
2. **What types of infections are viruses able to cause?**
3. **Compare and contrast: nucleoid and nucleus; the flagellum of a bacterium and that of a sperm; an archaebacterium and a cyanobacterium; nitrogen fixation and photosynthesis; bacteriophages and tobacco mosaic virus; a provirus and a virion.**

Experimental Pathways

The Origin of Eukaryotic Cells

You have seen in this chapter that cells can be divided into two groups: prokaryotic cells and eukaryotic cells. Almost from the time this division of cellular life was proposed, biologists have been fascinated by the question: What is the origin of the eukaryotic cell? It is generally agreed that prokaryotic cells (1) arose before eukaryotic cells and (2) gave rise to eukaryotic cells. The first point can be verified directly from the fossil record, which shows that prokaryotic cells were present in rocks approximately 2.7 billion years old (Section 1.3), which is roughly one billion years before any evidence is seen of eukaryotes. The second point follows from the fact that the two types of cells must be related to one another because they share many complex traits (e.g., very similar genetic codes, enzymes, metabolic pathways, and plasma membranes) that could not have evolved independently in different organisms.

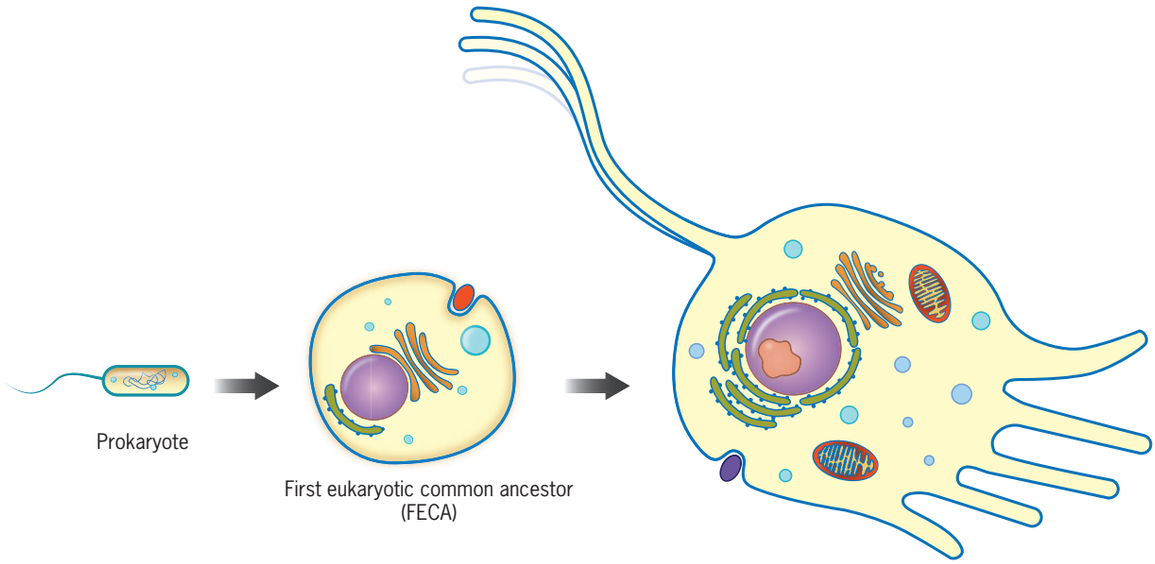
Until about 1970, it was generally believed that eukaryotic cells evolved from prokaryotic cells by a process of gradual evolution in which the organelles of the eukaryotic cell became progressively more complex. Acceptance of this concept changed dramatically about that time, largely through the work of Lynn Margulis, then at Boston University. Margulis resurrected an idea that had been proposed earlier, and dismissed, that certain organelles of a eukaryotic cell—most notably the mitochondria and chloroplasts—had evolved from smaller prokaryotic cells that had taken up residence in the cytoplasm of a larger host cell.[1] This hypothesis is referred to as the **endosymbiont theory** because it describes how a single "composite" cell of greater complexity could evolve from two or more separate, simpler cells living in a symbiotic relationship with one another.

Our earliest prokaryotic ancestors were presumed to have been anaerobic heterotrophic cells: *anaerobic* meaning they derived their energy from food matter without employing molecular oxygen (O_2), and *heterotrophic* meaning they were unable to synthesize organic compounds from inorganic precursors (such as CO_2 and water), but instead had to obtain preformed organic compounds from their environment. These prokaryotic ancestors are thought to have then acquired the ability to form internal membrane compartments, allowing formation of a nucleus by containing the DNA within an internal membrane. This development of internal membranes produced the first organism that would be considered eukaryote-like in terms of having a nucleus or other internal compartments (**Figure 1**). Because this is the first organism that subsequently gave rise to all eukaryotes, it is known as the *first eukaryotic common ancestor (FECA)*. Although the presence of internal membranes was once thought to be an exclusively eukaryotic trait, it is now known that some bacteria can in fact form extensive complex internal membrane systems. The most dramatic example known to date is the bacterium *Gemmata obscuriglobus*, which forms a variety of complex internal membranes (**Figure 2**). However, careful three-dimensional reconstructions of *G. obscuriglobus* structure show that these membranes do not form closed compartments like eukaryotic organelles.[2] It thus appears that the key step in producing the FECA was not formation of internal membranes per se, but the further development of these membranes into closed internal compartments, particularly a compartment surrounding the DNA to produce a nucleus.

According to the endosymbiont theory, the next step in the evolution of modern eukaryotes was when a descendant of the FECA cell ingested a small, aerobic prokaryote which somehow resisted digestion within the cytoplasm, taking up residence as a permanent endosymbiont. As the host cell reproduced, so did the endosymbiont, so that a colony of these composite cells was soon produced. Over many generations, endosymbionts lost many of the traits no longer required for survival, and the once-independent oxygen-respiring microbes evolved into precursors of modern-day mitochondria. A cell with ancestors that had formed through the sequence of symbiotic events just described could have given rise to a line of cells which evolved other basic characteristics of eukaryotic cells, including additional internal organelles (endoplasmic reticulum, Golgi complex, lysosomes), a complex cytoskeleton including cilia, intron splicing, and both mitotic and meiotic cell division. These characteristics, which are shared among all existing eukaryotic lineages, are proposed to have arisen by a gradual process of evolution, rather than in a single step as might occur through acquisition of an endosymbiont. All eukaryotes alive today descended from the cell that acquired these traits, and it is therefore known as the *last eukaryotic common ancestor* (LECA). Current research on evolutionary cell biology is focused on reconstructing the molecular, structural, and functional features of the FECA and LECA by comparing features of existing eukaryotic and prokaryotic lineages. The oldest fossils thought to be the remains of eukaryotes date back about 1.8 billion years.

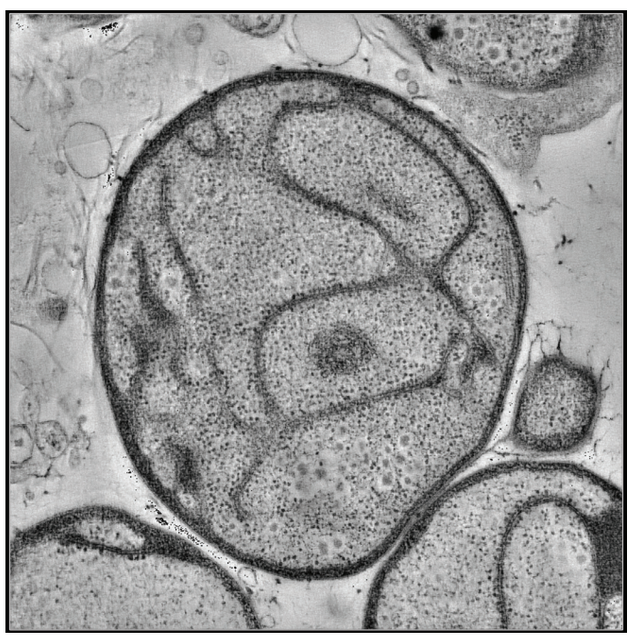
Margulis proposed that the acquisition of another endosymbiont, specifically a cyanobacterium, converted an early heterotrophic eukaryote into an ancestor of photosynthetic eukaryotes: the green algae and plants.[3] The acquisition of chloroplasts (roughly one billion years ago) must have been one of the last steps in the sequence of endosymbioses because these organelles are only present in plants and algae. In contrast, all known groups of eukaryotes either (1) possess mitochondria or (2) show definitive evidence they have evolved from organisms that possessed these organelles.[a] The concept that mitochondria and chloroplasts arose

[a]There are a number of anaerobic unicellular eukaryotes (e.g., the intestinal parasite *Giardia*) in which mitochondria have lost their respiratory functions but still contain organelles that are clearly related to mitochondria in terms of protein composition. However, in recent years a group of unicellular eukaryotes known as Preaxostyla have been found to include species that completely lack any organelle related to mitochondria. The metabolic functions normally performed by mitochondria are replaced with genes obtained from bacteria. Based on sequence comparisons of nonmitochondrial genes, we know that the Preaxostyla lacking mitochondria evolved from ancestors that had mitochondria, a phenomenon known as *secondary loss*. Thus, all evidence remains consistent with the idea that the ancestor of all existing eukaryotes contained a mitochondrion.



Source: Adapted from F.D. Mast et al., *Trends Cell Biol*. 24:435–442, 2014.

FIGURE 1 **A model depicting stages in the evolution of eukaryotes.** Starting from a prokaryotic ancestor, internal compartments began to develop, leading to an organism with internal membrane compartments such as a nucleus. Such an organism is known as the first eukaryotic common ancestor (FECA). The molecular machinery for making internal membranes then allowed the FECA to engulf and maintain endosymbiotic organisms, allowing acquisition of mitochondria. Additional evolutionary innovations gave rise to cellular features common to all eukaryotic lineages, including cilia, intron splicing, and meiosis. The organism that had all of these traits, and therefore gave rise to all existing eukaryotic lineages, is known as the last eukaryotic common ancestor (LECA). After the LECA arose, further evolutionary steps, such as endosymbiosis of photosynthetic bacteria to produce chloroplasts, gave rise to different classes of eukaryotic cells.



Source: Rachel Santarella-Mellwig, Sabine Pruggnaller, Norbert Roos, Iain W. Mattaj, and Damien P. Devos.

FIGURE 2 **Prokaryotes with complex internal membrane systems.** Electron micrograph of *Gemmata obscuriglobus*, a bacterium with a complex set of internal membranes. Although these membranes do not form closed organelles as they would in eukaryotes, they show that a potential for membrane organization exists even in prokaryotes.

via evolution from symbiotic organisms is now supported by an overwhelming body of evidence, some of which will be described in numerous chapters of this text.

The division of all living organisms into two categories, prokaryotes and eukaryotes, reflects a basic dichotomy in the structures of cells, but it is not necessarily an accurate *phylogenetic* distinction, that is, one that reflects the evolutionary relationships among living organisms. How do we determine evolutionary relationships among organisms that have been separated in time for billions of years, such as prokaryotes and eukaryotes? Modern taxonomic schemes that attempt to classify organisms are based on comparisons of the DNA sequences of living organisms.[4] Differences between organisms in the sequence of nucleotides that make up a nucleic acid are the result of mutations in DNA that have been transmitted to offspring. Mutations can accumulate in a given gene at a relatively constant rate over long periods of time. Consequently, comparisons of nucleotide sequences can be used to determine how closely organisms are related to one another. For example, two organisms that are closely related, that is, have diverged only recently from a common ancestor, should have fewer sequence differences in a particular gene than two organisms that are distantly related, that is, do not have a recent common ancestor. Using this type of sequence information as an "evolutionary clock," researchers can construct phylogenetic trees showing proposed pathways by which different groups of living organisms may have diverged from one another during the course of evolution.

Beginning in the mid-1970s, Carl Woese and his colleagues at the University of Illinois began a series of studies comparing the nucleotide sequence in different organisms of the RNA molecule that resides in the small subunit of the ribosome. This RNA—which is called the 16S rRNA in prokaryotes or the 18S rRNA in eukaryotes—was chosen because it is present in large quantities in all cells, is easy to purify, and tends to change only slowly over long periods of evolutionary time, which means that it could be used to study relationships of very distantly related organisms. In one of their first studies, Woese and his colleagues analyzed the rRNA present in the ribosomes of chloroplasts from the photosynthetic protist *Euglena*.[5] They found that the sequence of this chloroplast rRNA molecule was much more similar to that of the 16S rRNA found in ribosomes of cyanobacteria than it was to its 18S counterpart in the ribosomes from eukaryotic cytoplasm. This finding provided strong evidence for the symbiotic origin of chloroplasts from cyanobacteria.

In 1977, Woese and George Fox published a landmark paper in the study of molecular evolution.[6] Comparing the nucleotide sequences of small-subunit rRNAs that had been purified from 13 different prokaryotic and eukaryotic species, they found that the sequences clustered into three distinct groups, such that the rRNAs within each group are much more similar to one another than they are to rRNAs of the other two groups. The first of the groups contained only eukaryotes; the second group contained the "typical" bacteria (gram-positive, gram-negative, and cyanobacteria); and the third group contained several species of methanogenic (methane-producing) "bacteria." Woese and Fox concluded, to their surprise, that the methanogenic organisms "appear to be no more related to typical bacteria than they are to eukaryotic cytoplasms." These results suggested that the members of these three groups represent three distinct evolutionary lines that branched apart from one another at a very early stage in the evolution of cellular organisms. Consequently, they assigned these organisms to three different kingdoms, which they named the Urkaryotes, Eubacteria, and Archaebacteria, a terminology that divided the prokaryotes into two fundamentally distinct groups. It was further proposed that a member of the Urkaryote group combined with a member of Eubacteria to produce the first eukaryote.

Subsequent research provided support for the concept that prokaryotes could be divided into two distantly related lineages, and it expanded the ranks of the archaebacteria to include at least two other groups, the thermophiles, which live in hot springs and ocean vents, and the halophiles, which live in very salty lakes and seas. In 1989, two published reports rooted the tree of life and suggested that the archaebacteria were actually more closely related to eukaryotes than they were to eubacteria.[7,8] Both groups of researchers compared the amino acid sequences of several proteins that were present in a wide variety of different prokaryotes, eukaryotes, mitochondria, and chloroplasts. A phylogenetic tree constructed from sequences of ribosomal RNAs, which comes to the same conclusion, is shown in **Figure 3a**.[9] In the latter paper, Woese and colleagues proposed a revised taxonomic scheme, which has been widely accepted. In this scheme, the archaebacteria, eubacteria, and eukaryotes are assigned to separate domains, which are named Archaea, Bacteria, and Eucarya, respectively.[b] Similar DNA sequence analysis studies have shown that eukaryotes then split into six distinct lineages (Figure 3*b*); animals, including humans, fall into a group known as *opisthokonts*. According to the model in Figure 3*a*, the first major split in the tree of life produced two separate lineages, one leading to the Bacteria and the other leading to both the Archaea and the Eucarya. If this view is correct, it was an archaebacterium, not a Urkaryote, that took in a eubacterium as a symbiont and gave rise to the lineage that led to the first eukaryotic cells. Although the host prokaryote was presumably an archaebacterium, the symbionts that evolved into mitochondria and chloroplasts were almost certainly eubacteria, as indicated by their close relationship with modern members of this group.

Until 1995, phylogenetic trees of the type shown in Figure 3*a* were based primarily on the analysis of the gene encoding the 16S–18S rRNA. By then, phylogenetic comparisons of a number of other genes were suggesting that the scheme depicted in Figure 3*a* might be oversimplified. Questions about the origin of prokaryotic and eukaryotic cells came into sharp focus between 1995 and 1997 with the publication of the entire sequences of a number of prokaryotic genomes, both archaebacterial and eubacterial, and the genome of a eukaryote, the yeast *Saccharomyces cerevisiae*. Researchers could now compare the sequences of hundreds of

[b]Many biologists dislike the terms *archaebacteria* and *eubacteria*. Although these terms have gradually faded from the literature, being replaced simply by *archaea* and *bacteria*, many researchers in this field continue to use the former terms in published articles. Given that this is an introductory chapter in an introductory text, we have continued to refer to these organisms as archaebacteria and eubacteria to avoid possible confusion over the meaning of the term *bacterial*.