GLOBAL EDITION

BROCK BIOLOGY OF MICROORGANISMS

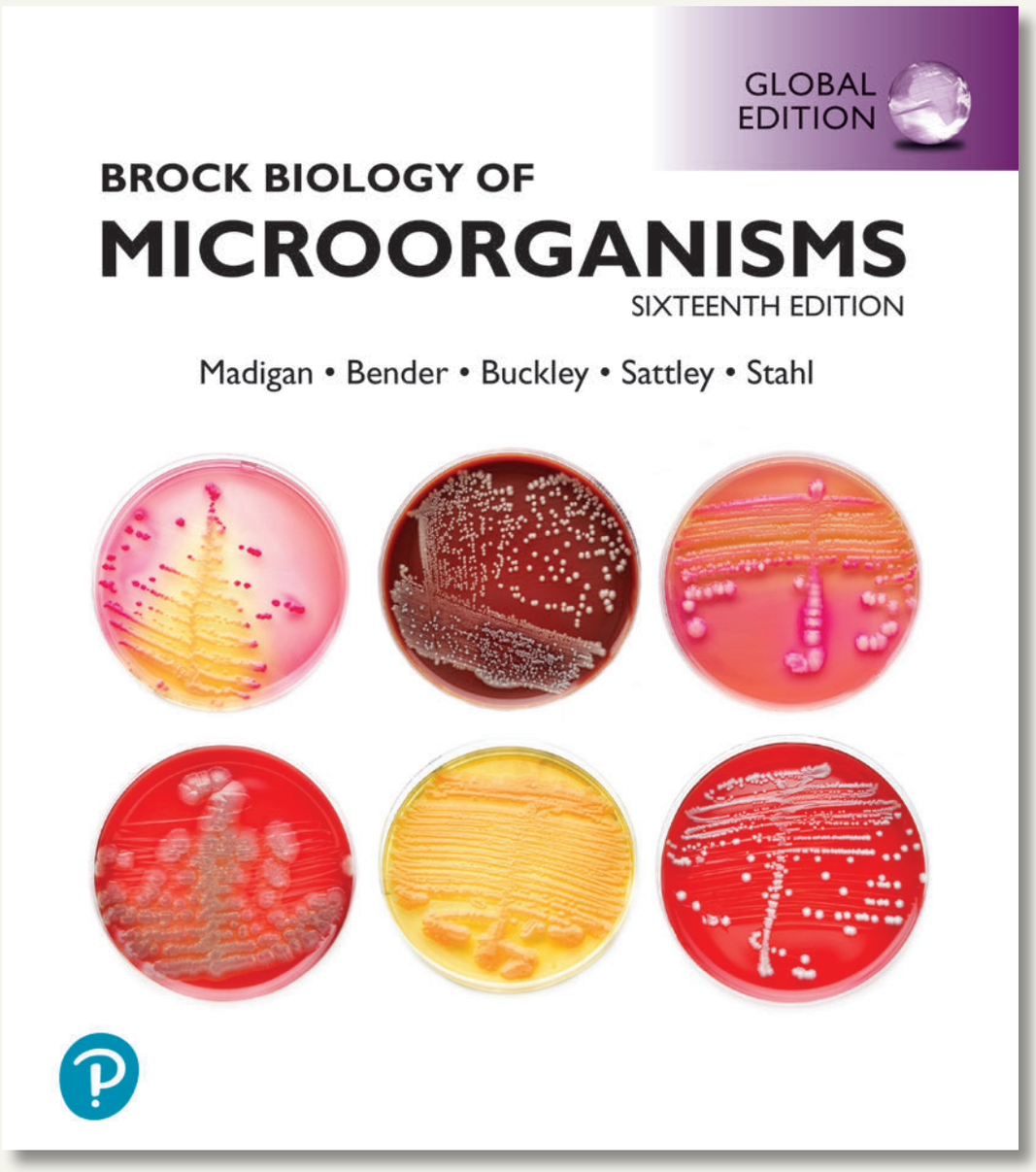
SIXTEENTH EDITION

Madigan • Bender • Buckley • Sattley • Stahl

Brief Contents

Authoritative. Accurate. Accessible.

Brock Biology of Microorganisms **is the leading microbiology text for majors, setting the standard for impeccable scholarship, accuracy, a visually stunning art program, and the use of cutting-edge research to illustrate basic concepts.**



Pearson

Making Connections Across

UPDATED! Each chapter is carefully cross-referenced to connect students with related material found earlier (◀) or later (▶) in the book.

I • Bacterial Cell Division

Prokaryotic cell division is preceded by chromosome replication and the synthesis of new cell wall material in a way that defines cell shape. Cell division is orchestrated in a carefully controlled fashion by protein complexes whose activities can be visualized by powerful light microscopic techniques.

Most cells divide by binary fission (◀ Section 4.6 and Figure 4.8), and this process occurs in a defined series of steps such that each daughter cell obtains a copy of the genome. During the division cycle, the cell must also produce new peptidoglycan and cytoskeleton elements to prevent bursting from osmotic forces. This cytoskeleton gives the cell its distinct morphology (◀ Figure 1.8). To successfully orchestrate all of these events, various regulatory cascades are put into play. In this first part of the chapter we focus on the molecular mechanisms employed by two well-studied gram-negative bacteria, *Escherichia coli* and *Caulobacter crescentus*, and introduce advanced microscopic techniques that have revealed the major molecular events that underlie cell division and cell morphology.

NEW! Key Concept statements at the start of each key topic of a chapter give students a big-picture view of the content to come before they dive in and immerse themselves in the details.

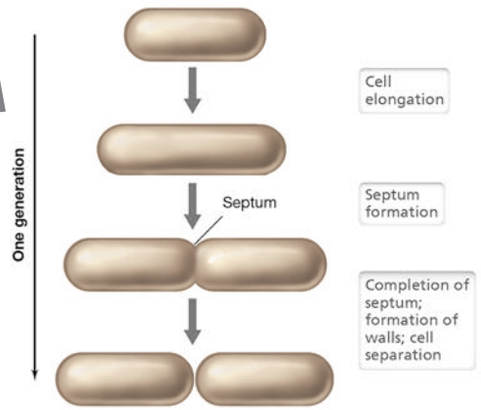


Figure 4.8 Binary fission in a rod-shaped bacterium. Cell numbers (and all components of the cells) double every generation.

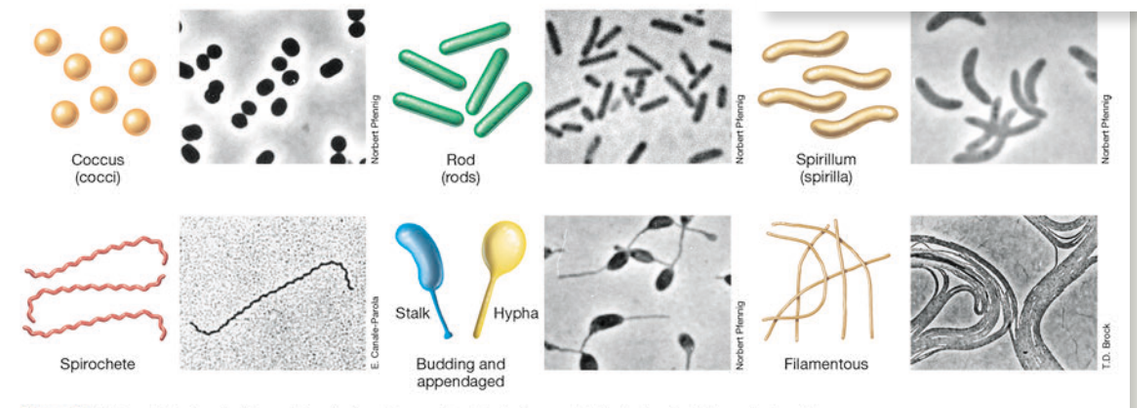
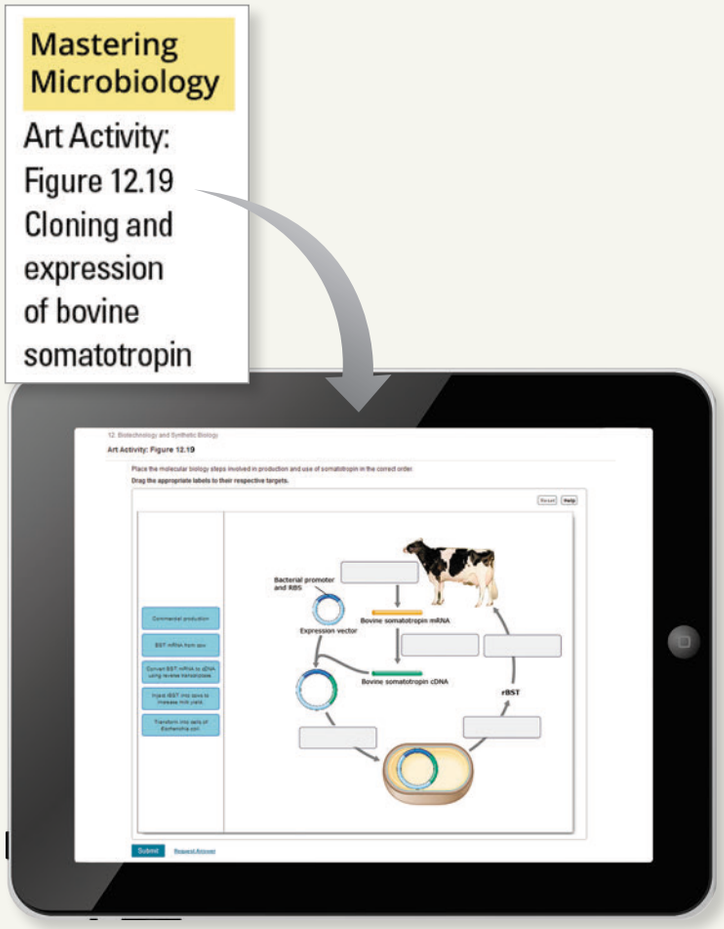


Figure 1.8 Cell morphologies. Beside each drawing is a phase-contrast photomicrograph of cells showing that morphology. Coccus (cell diameter in photomicrograph, 1.5 μm); rod (1 μm); spirillum (1 μm); spirochete (0.25 μm); budding (1.2 μm); filamentous (0.8 μm). All photomicrographs are of species of *Bacteria*. Not all of these morphologies are known among the *Archaea*, but cocci, rods, and spirilla are common.

Concepts in Microbiology

NEW! Marginal annotations highlight some of the best material available for instructors to assign in Mastering Microbiology, guiding students along their journey with insightful materials that support and strengthen the learning experience.



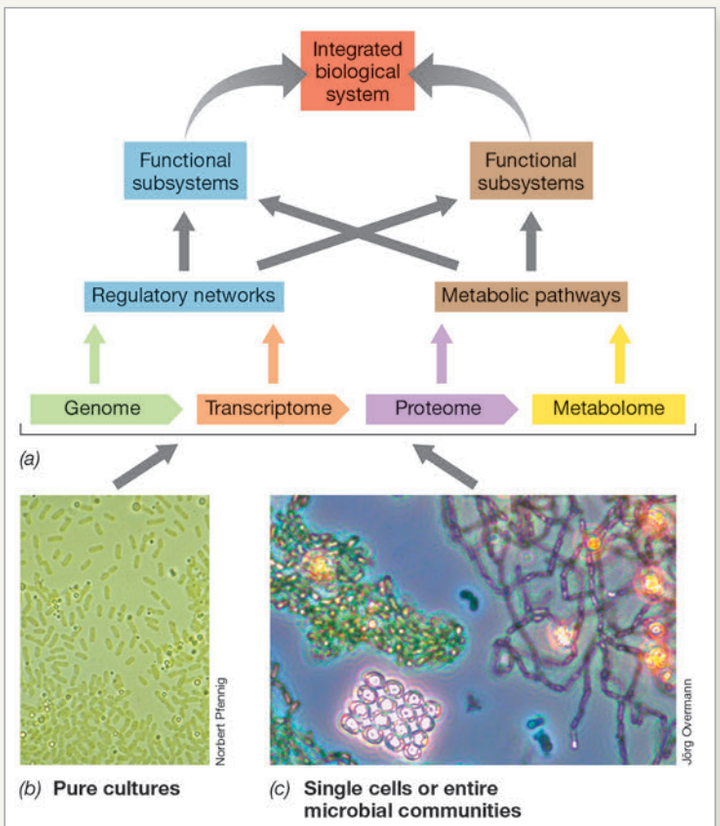


Figure 10.29 The components of systems biology. *(a)* The results of various "omics" analyses are combined and successively integrated into higher-level views of the entire biology of a pure culture, such as *(b)* that of the green sulfur bacterium *Chlorobium*; or of a mixed microbial community, such as *(c)* that of phototrophic sulfur bacteria obtained from a lake; or of a single cell isolated from a microbial community (see Figure 10.30).

Genomics, and the various "omics" it has spawned, is woven into every chapter of the text, providing students with concrete examples of how powerful tools have allowed microbiologists to probe deeper and farther into the microbial world than ever before.

Cutting-Edge Content

MICROBIOLOGYNOW

When Antibiotics Fail, Bacteriophage Therapy to the Rescue

Acquiring an antibiotic-resistant infection or "superbug" is one of medicine's biggest nightmares. What can medical practitioners do to treat the patient? Besides drugs, viruses known as bacteriophages have been recruited to specifically target and kill bacteria.

Despite microbiologists' tinkering with using bacteriophages as antimicrobials for decades, their actual application in medicine has been minimal. However, the emergence of antibiotic resistance has led to renewed focus on using these tiny microbes as therapeutic agents. The photo above shows Ella Balasa (right side of photo), a microbiologist who has cystic fibrosis. Cystic fibrosis is a genetic disease that results in a buildup of thick mucus in the lungs. This mucus allows bacteria to flourish in the lungs, which results in infections and subsequent lung damage that can be fatal. Ella had been treated numerous times with strong antibiotics specific for a respiratory infection caused by the bacterial pathogen *Pseudomonas aeruginosa*, but the microbial cells had become unresponsive to the drugs. At the time of this photo, the recurrent infection had decreased her lung function to the point where she required constant supplemental oxygen.

As an alternative treatment route, Dr. Benjamin Chan (on the left) took mucus from Ella's lungs infected with *P. aeruginosa* and isolated a bacteriophage that specifically killed the pathogen (see zones of clearing on Petri plate). This bacteriophage was propagated and then poured into a device so that Ella could inhale the therapy. The result of her treatment? Amazingly, the bacteriophage therapy along with a mixture of antibiotics resulted in the infection clearing a few weeks later!

While bacteriophage therapy is l
specificity and the ability of the path
become resistant to viral infection, t
success stories illustrate the future
therapy when all other options fail.

Source: Kortright, K.E., B.K. Chan, J.
Phage therapy: A renewed approach
bacteria. *Cell Host Microbe* 25(2): 21

NEW! Thirty-four Microbiology Now chapter opening vignettes were composed for this edition, each designed to introduce a chapter's theme through a recent discovery in the field of microbiology. These exciting accounts will draw students into the chapter and show how the chapter content connects with real-world problems.

NEW! Several new Explore the Microbial World features provide fascinating stories that highlight how important chapter concepts have evolved from research in the microbial world.

Explore the Microbial World

Pattern Recognition Receptors of Hydrothermal Vent Tube Worms Facilitate Endosymbiosis

Invertebrates and plants lack adaptive immunity but have a well-developed innate immune response to a wide variety of pathogens. As discussed in Section 26.6, virtually all multicellular organisms respond to pathogen invasion by recognizing signature molecules found on pathogen surfaces. These molecules contain conserved, repetitive structures called pathogen-associated molecular patterns (PAMPs) that include molecules such as the lipopolysaccharide (LPS) and flagellin of gram-negative bacteria, the peptidoglycan of gram-positive bacteria, and the double-stranded RNA of certain viruses. The term *microbe-associated molecular pattern*, or MAMP, is also commonly used to describe signature molecules found on microorganisms. However, "MAMP" is a broader term than "PAMP" because it includes components found on microorganisms that are not pathogenic.

Unlike PAMPs, which are exploited specifically for innate defense against pathogens, MAMPs found on nonpathogenic bacteria can serve an entirely different purpose—that of facilitating, through host pattern recognition receptors (PRRs), a mutualistic partnership rather than a confrontation between a host and the bacteria that colonize it. As we learned in Chapter 23, a wide variety of plants and animals maintain symbiotic relationships with microorganisms. There we discussed the association of tube worms that develop near hydrothermal vents in the deep sea with autotrophic, sulfur-oxidizing bacteria (SOB) that inhabit their *trophosome*, a spongy internal organ that comprises most of the volume of the 1- to 2-m-long worms (Figure 1). These SOB form an endosymbiosis with the worms in which the bacteria provide all organic carbon requirements for their animal host in exchange for a steady supply of essential metabolites, in particular H_2S, O_2, and CO_2. H_2S is the energy source for the SOB; they oxidize it to S^0 and then SO_4^{2-} and respire the electrons to generate a proton motive force that drives ATP synthesis. Oxygen (O_2) is required as a terminal acceptor of electrons that have traversed the electron transport chain. CO_2 is the carbon source and is incorporated into bacterial cell material by way of the Calvin cycle, the major means of autotrophy in chemolithotrophic bacteria.

This fascinating association raises the question of how it is established. Specifically, how does the tube worm populate its trophosome with SOB to the exclusion of other, potentially pathogenic, bacteria? The answer appears to be closely linked to MAMPs associated with the endosymbiotic SOB. Although host PRRs are typically used to recognize and eliminate pathogens, the study of tube worms and other animals that harbor endosymbiotic microbes shows a broader functionality for PRRs in that they can also interact beneficially with MAMPs to selectively populate a host with nonpathogenic symbionts.

The tube worm trophosome contains a large number of specialized host immune cells called *bacteriocytes*, and it is within these cells that the bacterial symbionts take up residence. The tube-worm bacteriocytes express high levels of PRRs that recognize MAMPs, such as specific cell surface lipoproteins associated with SOB. This positive interaction locates the bacteria to the trophosome and, with a steady supply of simple nutrients from the hydrothermal vent system delivered by blood circulating in the worm, stimulates colonization and growth of the symbionts in their animal host.

As this example illustrates, in addition to providing a rapid response to pathogen challenge, innate immune mechanisms—specifically, the interaction of PRRs with MAMPs—may also serve the primary role in governing host–symbiont interactions and the establishment of endosymbiotic relationships. Given the critical role innate immune mechanisms play in maintaining the animal–bacterial symbiosis within the tube-worm trophosome, it is likely that similar tightly choreographed molecular mechanisms constitute a host–microbe "dialogue" that helps to maintain balanced communities of beneficial microbiota in virtually all animals, including humans.

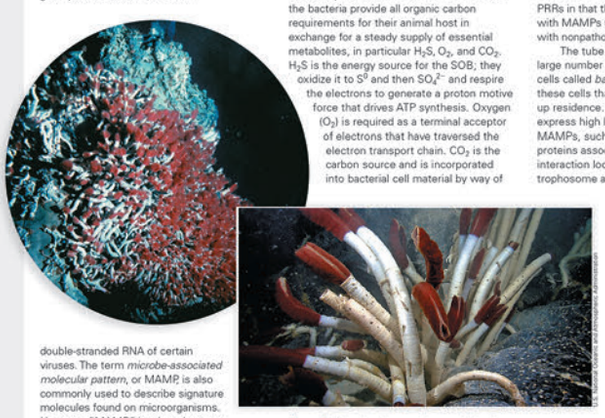


Figure 1 Hydrothermal vent tube worms harboring endosymbiotic sulfur-oxidizing bacteria. Top: A "black smoker" hydrothermal vent community containing several tube worms that obtain organic carbon from sulfur-oxidizing chemolithotrophic bacteria (SOB) living within them. Bottom: A close-up view of tube worms; each worm is 1–2 m long. The red area on the top of each worm, called the plume, is where O_2 and H_2S are taken in to be fed to the worm's SOB endosymbionts residing in the trophosome.

NEW! A section on immunotherapy highlights exciting advancements in the use of genetic engineering and molecular immunology to treat cancer.

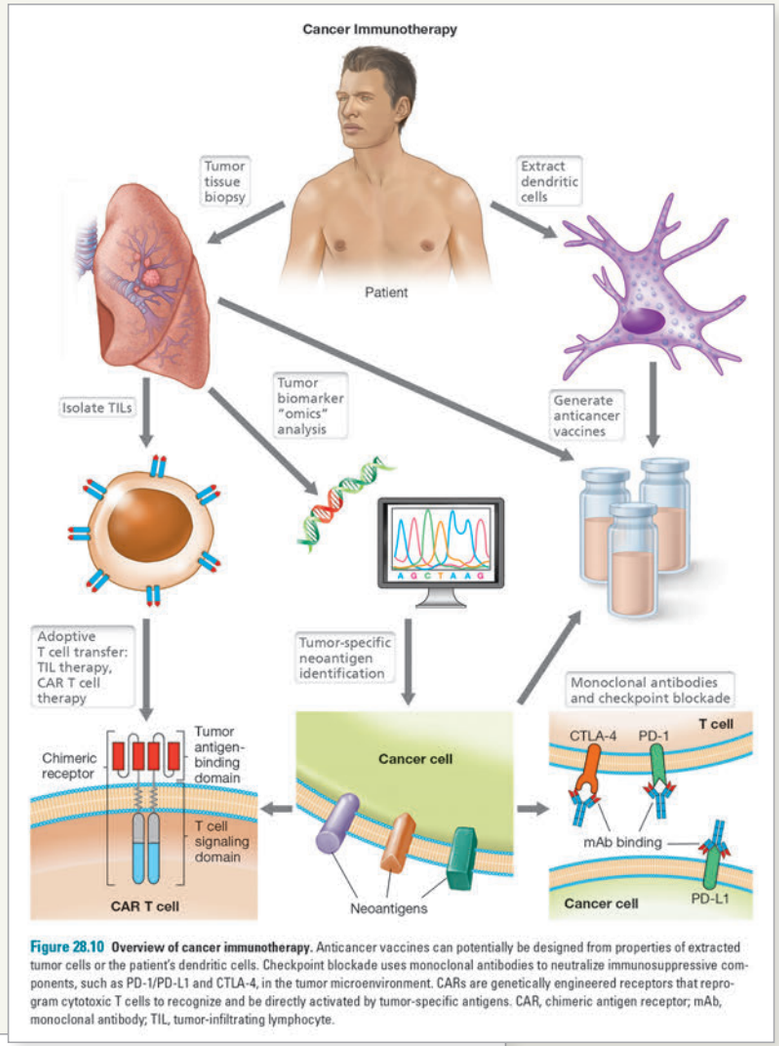


Figure 28.10 Overview of cancer immunotherapy. Anticancer vaccines can potentially be designed from properties of extracted tumor cells or the patient's dendritic cells. Checkpoint blockade uses monoclonal antibodies to neutralize immunosuppressive components, such as PD-1/PD-L1 and CTLA-4, in the tumor microenvironment. CARs are genetically engineered receptors that reprogram cytotoxic T cells to recognize and be directly activated by tumor-specific antigens. CAR, chimeric antigen receptor; mAb, monoclonal antibody; TIL, tumor-infiltrating lymphocyte.

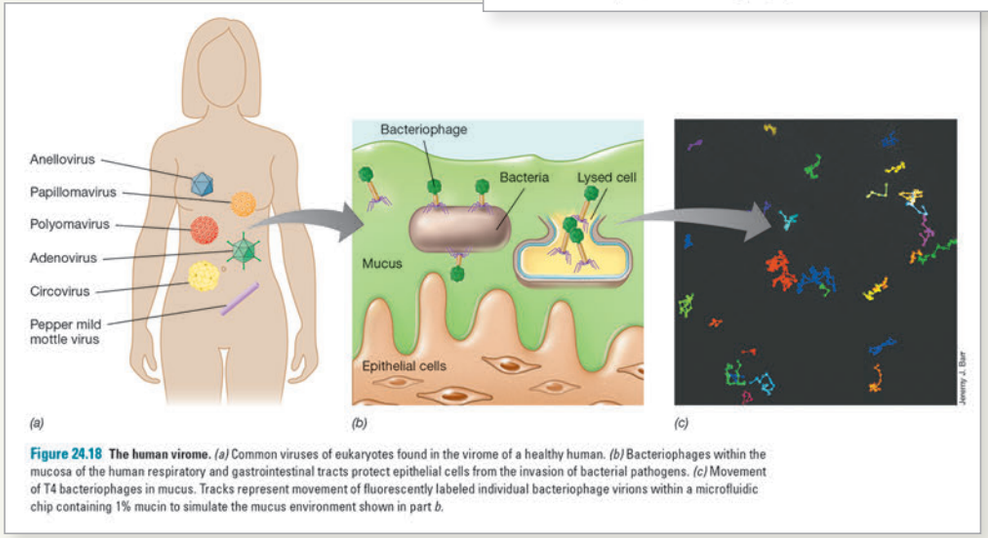
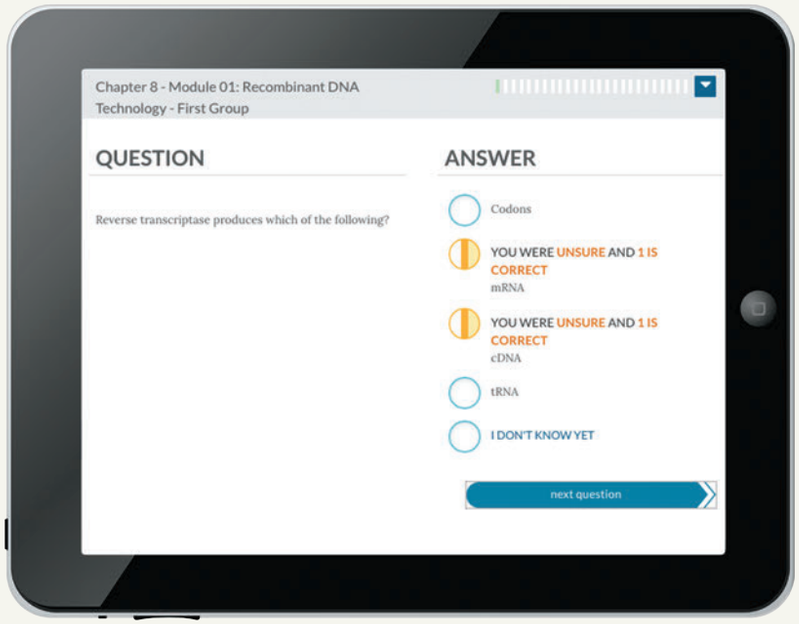


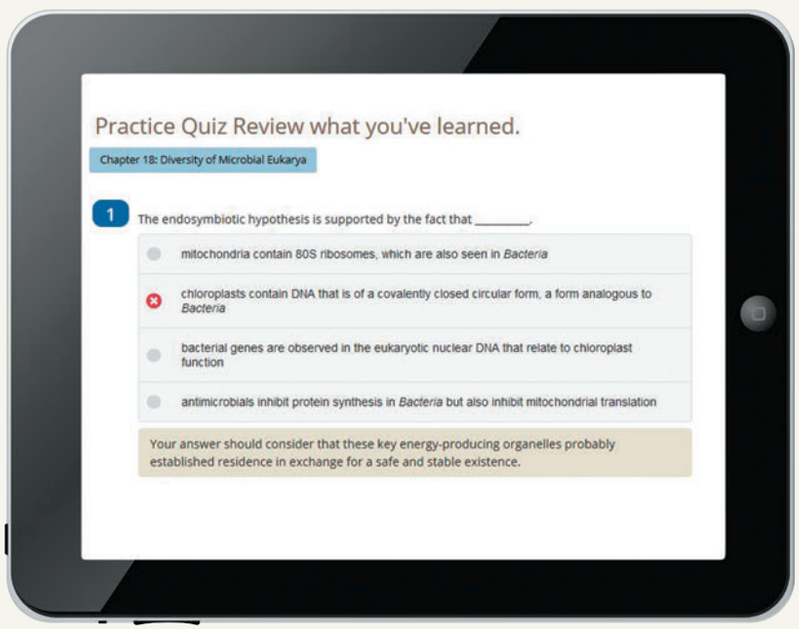
Figure 24.18 The human virome. *(a)* Common viruses of eukaryotes found in the virome of a healthy human. *(b)* Bacteriophages within the mucosa of the human respiratory and gastrointestinal tracts protect epithelial cells from the invasion of bacterial pathogens. *(c)* Movement of T4 bacteriophages in mucus. Tracks represent movement of fluorescently labeled individual bacteriophage virions within a microfluidic chip containing 1% mucin to simulate the mucus environment shown in part *b*.

NEW! The chapter on the human microbiome now includes a new section on the human virome, describing how metagenomics is aiding the discovery and isolation of many new viruses. Extensive coverage is provided of the impact of early life events on the development of the newborn gut microbiome and of recent successes in probiotic therapy for preventing newborn intestinal diseases.

Empower Each Learner

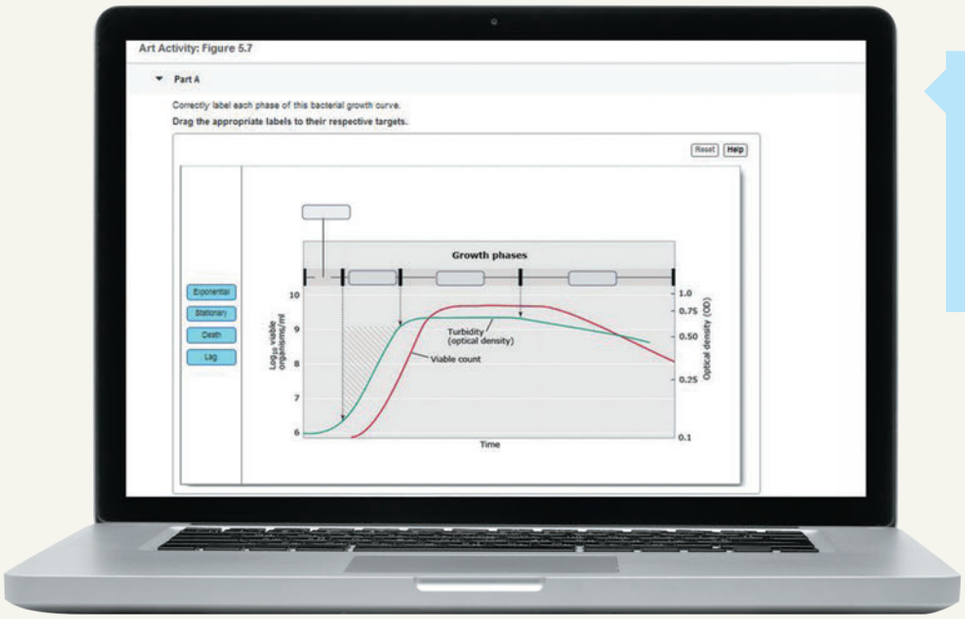


NEW! Dynamic Study Modules help students study course topics by adapting to their performance in real time. Students build the confidence they need to deepen their understanding, participate meaningfully, and perform better—in and out of class. Available on smartphones, tablets, and computers.



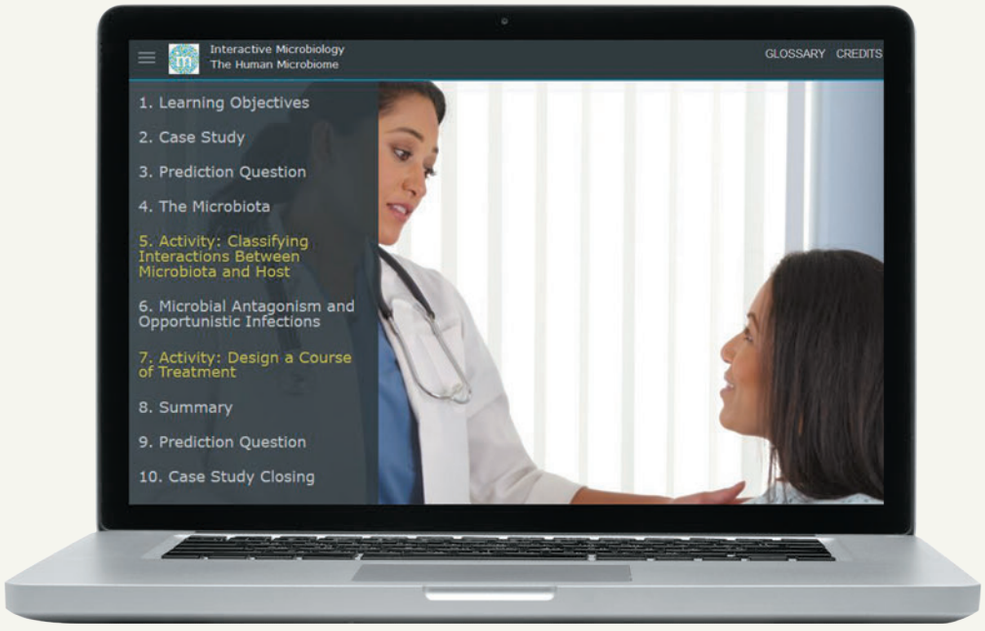
Mastering is so much more than homework. The **Pearson Study Area** allows students to access multiple study tools in one place. They don't need to search Google to find study tools that align with their course materials. Students have access to practice quizzes, videos and animations, vocabulary tools, and more.

with Mastering Microbiology



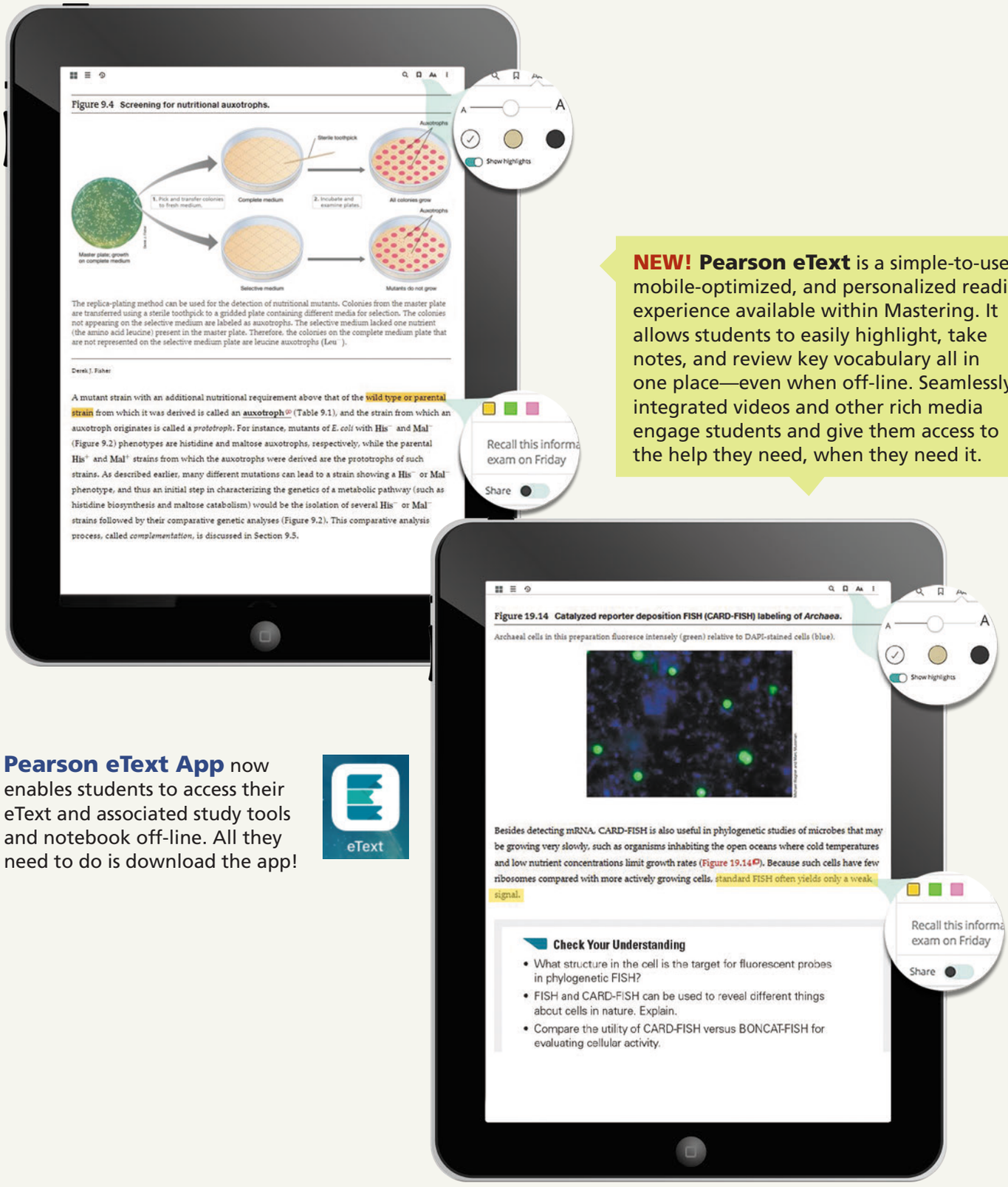
Get students engaged with content by assigning a variety of questions in Mastering Microbiology. These include:

- Reading Questions
- Art-Based Activities
- Coaching Activities and more



Interactive Microbiology is a dynamic suite of interactive tutorials and animations that teach key microbiology concepts including Operons, Biofilms and Quorum Sensing, Complement, Human Microbiota, and Antibiotic Resistance. Interactive Microbiology actively engages students with each topic, enabling them to learn from manipulating variables, predicting outcomes, and answering formative and summative assessment questions. Each tutorial presents the concept within a real healthcare scenario in order to emphasize problem solving and interest students from the beginning.

Pearson eText: A Whole New Reading Experience



NEW! Pearson eText is a simple-to-use, mobile-optimized, and personalized reading experience available within Mastering. It allows students to easily highlight, take notes, and review key vocabulary all in one place—even when off-line. Seamlessly integrated videos and other rich media engage students and give them access to the help they need, when they need it.

Pearson eText App now enables students to access their eText and associated study tools and notebook off-line. All they need to do is download the app!

BROCK BIOLOGY OF MICROORGANISMS

SIXTEENTH EDITION
GLOBAL EDITION

MICHAEL T. MADIGAN
Southern Illinois University Carbondale

KELLY S. BENDER
Southern Illinois University Carbondale

DANIEL H. BUCKLEY
Cornell University

W. MATTHEW SATTLEY
Indiana Wesleyan University

DAVID A. STAHL
University of Washington Seattle

Pearson

Please contact https://support.pearson.com/getsupport/s/ with any queries on this content.

Cover photo subject and credits: Mixed of bacteria colonies in various petri dish. Courtesy of Jarun Ontakrai.

Attributions of third-party content appear on page 1075, which constitutes an extension of this copyright page.

PEARSON, ALWAYS LEARNING, and MASTERING are exclusive trademarks owned by Pearson Education, Inc. or its affiliates in the U.S. and/or other countries.

Pearson Education Limited
KAO Two
KAO Park
Hockham Way
Harlow
CM17 9SR
United Kingdom
and Associated Companies throughout the world

Visit us on the World Wide Web at: www.pearsonglobaleditions.com
© Pearson Education Limited 2022

The rights of Michael T. Madigan, Kelly S. Bender, Daniel H. Buckley, W. Matthew Sattley, and David A. Stahl to be identified as the authors of this work have been asserted by them in accordance with the Copyright, Designs and Patents Act 1988.

Authorized adaptation from the United States edition, entitled *Brock Biology of Microorganisms*, 16th Edition, ISBN 978-0-13-487440-1 by Michael T. Madigan, Kelly S. Bender, Daniel H. Buckley, W. Matthew Sattley, and David A. Stahl published by Pearson Education © 2021.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without either the prior written permission of the publisher or a license permitting restricted copying in the United Kingdom issued by the Copyright Licensing Agency Ltd, Saffron House, 6–10 Kirby Street, London EC1N 8TS.

All trademarks used herein are the property of their respective owners. The use of any trademark in this text does not vest in the author or publisher any trademark ownership rights in such trademarks, nor does the use of such trademarks imply any affiliation with or endorsement of this book by such owners. For information regarding permissions, request forms, and the appropriate contacts within the Pearson Education Global Rights and Permissions department, please visit www.pearsoned.com/permissions.

This eBook may be available as a standalone product or integrated with other Pearson digital products like MyLab and Mastering. This eBook may or may not include all assets that were part of the print version. The publisher reserves the right to remove any material in this eBook at any time.

ISBN 10: 1-292-40479-5
ISBN 13: 978-1-292-40479-0
eBook ISBN 13: 978-1-292-40506-3

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

1 21

eBook formatted by B2R Technologies Pvt. Ltd.

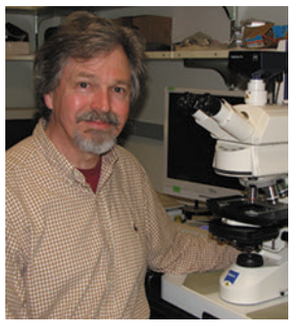
About the Authors

Michael T. Madigan received his B.S. in Biology and Education from Wisconsin State University–Stevens Point (1971) and his M.S. (1974) and Ph.D. (1976) in Bacteriology from the University of Wisconsin–Madison in the laboratory of Thomas Brock. Following a postdoc at Indiana University with Howard Gest, Mike moved to Southern Illinois University Carbondale, where he taught courses in introductory microbiology and bacterial diversity as a professor of microbiology for 33 years. In 1988 Mike was selected as the Outstanding Teacher in the College of Science and in 1993, the Outstanding Researcher. In 2001 he received the SIUC Outstanding Scholar Award and Distinguished Professor title. In 2003 Mike received the Carski Award for Distinguished Undergraduate Teaching from the American Society for Microbiology (ASM), and he is an elected Fellow of the American Academy of Microbiology (ASM) and the American Association for the Advancement of Science (AAAS). He has also been recognized by the American Red Cross as a major volunteer blood donor for the 24 gallons of blood he has donated since 1967. Mike's research is focused on phototrophic bacteria that inhabit extreme environments, and for the past 20 years his emphasis has been Antarctic microbiology. Mike has co-edited a major treatise on phototrophic bacteria and served for 10 years as chief editor of the journal *Archives of Microbiology.* He currently serves on the editorial board of the journals *Environmental Microbiology* and *Antonie van Leeuwenhoek*. Mike's other interests include forestry, swimming, reading, and caring for his dogs and horses. He lives on a small farm near a quiet lake with his wife, Nancy, three dogs (Kato, Nut, and Merlyn), and three horses (Eddie, Georgie, and Roscoe).

Kelly S. Bender received her B.S. in Biology from Southeast Missouri State University (1999) and her Ph.D. (2003) in Molecular Biology, Microbiology, and Biochemistry from Southern Illinois University Carbondale. Her dissertation research focused on the genetics of perchlorate-reducing bacteria. During her postdoctoral fellowship, Kelly worked on the genetic regulation of sulfate-reducing bacteria in the laboratory of Judy Wall at the University of Missouri–Columbia. She also completed a transatlantic biotechnology fellowship at Uppsala University in Sweden researching regulatory small RNAs in bacteria. In 2006, Kelly returned to her alma mater, Southern Illinois University Carbondale, as an Assistant Professor in the Department of Microbiology and in 2012 was tenured and promoted to Associate Professor. She has served as Chair of the SIUC Department of Microbiology since 2018. Her lab studies a range of topics including regulation in sulfate-reducing bacteria, the microbial community dynamics of sites impacted by acid mine drainage, and diversity of phototrophic heliobacteria. Kelly teaches courses in introductory microbiology and microbial diversity, has served on numerous federal grant review panels, and is an active member of the American Society for Microbiology (ASM). Her other interests include spending time with her daughter, Violet, and husband, Dick.

Daniel H. Buckley is a Professor at Cornell University in the School of Integrative Plant Science and the Department of Microbiology. He earned his B.S. in Microbiology (1994) at the University of Rochester and his Ph.D. in Microbiology (2000) at Michigan State University. His graduate research in the laboratory of Thomas M. Schmidt explored environmental factors that influence microbial diversity in soils. Dan then received a National Science Foundation Postdoctoral Fellowship to work with Pieter T. Visscher, University of Connecticut, investigating linkages between microbial diversity and biogeochemistry within microbial mats and stromatolites. Dan moved to Cornell in 2003 where he investigates the ecology and evolution of the diverse microorganisms that live in soils. He has taught both introductory and advanced courses in microbiology, microbial diversity, and microbial genomics. He received a National Science Foundation Faculty Early Career Development (CAREER) award in 2005 for excellence in integrating research and education, and served as Co-Director of the MBL Microbial Diversity summer course in Woods Hole, Massachusetts (2009–2013). He currently serves on the editorial boards of *Applied and Environmental Microbiology* and *Environmental Microbiology*. Dan lives in Ithaca, New York, with his wife, Merry, and sons, Finn and Colin.

W. Matthew Sattley received his B.A. in Biology in 1998 from Blackburn College (Illinois) and his Ph.D. (2006) in Molecular Biology, Microbiology, and Biochemistry from Southern Illinois University Carbondale. His graduate studies focused on the microbiology of sulfur cycling and other biogeochemical processes in permanently ice-covered lakes of Antarctica. In his postdoctoral research at Washington University in Saint Louis, he studied the physiology and genomics of anoxygenic phototrophic bacteria in Robert Blankenship's laboratory. Matt then accepted a faculty appointment to the Department of Biology at MidAmerica Nazarene University (Kansas), where he supervised undergraduate research and taught courses in microbiology, environmental science, and cell biology. In 2010, Matt transitioned to the Division of Natural Sciences at Indiana Wesleyan University (IWU), where he is a Professor of Biology and has served as the Director of the Hodson Research Institute, a faculty-led summer research program for undergraduate students in the Natural Sciences. Matt's research group investigates the ecology, diversity, and genomics of bacteria that inhabit extreme environments, and in 2017, he was the recipient of IWU's Outstanding Scholarship Award. Matt is a member of the American Society for Microbiology (including its Indiana Branch) and the Indiana Academy of Science. Matt lives in Marion, Indiana, with his wife, Ann, and sons, Josiah and Samuel. Outside of teaching and research, Matt enjoys playing drums, reading, motorcycling, and baseball.



David A. Stahl received his B.S. degree in Microbiology from the University of Washington, Seattle, and completed graduate studies in microbial phylogeny and evolution with Carl Woese in the Department of Microbiology at the University of Illinois at Urbana–Champaign. Subsequent work as a postdoctoral fellow with Norman Pace, then at the National Jewish Hospital in Colorado, involved early applications of 16S rRNA-based sequence analysis to the study of natural microbial communities. In 1984 Dave joined the faculty at the University of Illinois with appointments in Veterinary Medicine, Microbiology, and Civil Engineering. In 1994 he moved to the Department of Civil Engineering at Northwestern University, and in 2000 returned to the University of Washington as professor in the Departments of Civil and Environmental Engineering and Microbiology. Dave is known for his work in microbial evolution, ecology, and systematics, and received the 1999 Bergey Award and the 2006 ASM Procter & Gamble Award in Applied and Environmental Microbiology. Dave is an elected fellow of the American Academy of Microbiology and a member of the National Academy of Engineering. His main research interests surround the biogeochemistry of nitrogen and sulfur and the microbial communities that sustain the associated nutrient cycles. His laboratory was the first to culture ammonia-oxidizing *Archaea,* a group believed to be the key mediators of this process in the nitrogen cycle. Dave has taught several courses in environmental microbiology, was one of the founding editors of the journal *Environmental Microbiology*, and has served on many advisory committees. Outside the lab, Dave enjoys hiking, bicycling, spending time with family, reading a good science fiction book, and—with his wife, Lin—renovating an old farmhouse on Bainbridge Island.

Dedications

Michael T. Madigan

dedicates this book to the 10^{31} (more or less) microbial cells on and within Earth that maintain our planet in a habitable state. Keep up the good work, guys.

Kelly S. Bender

dedicates this book to the memory of her grandmother, Alberta, whose biggest regret in life was not being able to attend school past the fifth grade.

Daniel H. Buckley

dedicates this book to his father, Ron, who taught me ingenuity and persistence.

W. Matthew Sattley

dedicates this book to the memory of his father, Steven, and to his mother, Patrice, for demonstrating the benefits of working hard and seeking knowledge.

David A. Stahl

dedicates this book to his wife, Lin. My love, and one that helps me keep the important things in perspective.

This page is intentionally left blank

Preface

Welcome to the best learning resource in microbiology education today: the visually stunning 16th Edition of *Brock Biology of Microorganisms* (*BBOM*). The 16th Edition is the most student-friendly and accessible edition yet and presents the most exciting and recent picture of the science of microbiology available today. For three generations, both students and instructors alike have praised the accuracy, authority, consistency, and teachability of *BBOM* for exploring the principles of microbiology in a readable, connected, and visually appealing way.

Both students and instructors will benefit from at least four important strengths of the 16th Edition: (1) our approach of using cutting-edge research to solidify basic concepts; (2) the seamless integration of molecular and ecological microbiology with coverage of evolution, diversity, the immune system, and infectious diseases; (3) the spectacular art program complemented with striking and compelling photos; and (4) the wide assortment of teaching and learning tools that accompany the book itself. With an extremely strong author team that employs experts in each major theme, *BBOM* 16th Edition leads the way in presenting the essential principles of microbiology that students need to master today.

What's New in the 16th Edition?

The 16th Edition guides students through the six major themes of microbiology as outlined by the American Society for Microbiology Conference on Undergraduate Education (ASMCUE): Evolution, Cell Structure and Function, Metabolic Pathways, Information Flow and Genetics, Microbial Systems, and the Impact of Microorganisms. With new and revised artwork complemented by over 60 new photos, *BBOM* 16th Edition (16e) presents microbiology as the visual science it is. Thirty-four new MicrobiologyNow chapter-opening vignettes were composed for this edition, each designed to introduce a chapter's theme through a recent discovery in the field of microbiology. These exciting accounts will naturally draw students into the chapter and show how the chapter's content connects with real-world problems. Several new Explore the Microbial World features were also developed for this edition, each designed to give students a feel for exciting special topics in microbiology and to fuel their scientific curiosity.

Genomics, and all of the various "omics" it has spawned, support content in every chapter of *BBOM* 16e, reflecting the reality of how omics has transformed all of biology, especially microbiology. The result is a robust and modern treatment of microbiology that guides students through the maze of omics with concrete examples of how these powerful tools have allowed microbiologists to probe deeper and farther into the microbial world than ever before.

To reinforce the learning experience, the 16e debuts a new pedagogical aid called Key Concepts. These brief summaries of each chapter part are written in clear and straightforward language that give students a heads-up as to what is coming in the following sections. Complementing the Key Concepts, each numbered section is summarized in the chapter review and accompanied by a review question that links concept review with concept mastery.

BBOM 16e is supported by Mastering Microbiology, Pearson's online homework, tutorial, and assessment system that assists students in pacing their learning and keeps instructors current on class performance. Mastering Microbiology includes a new feature, Dynamic Study Modules, which adapt to the student's performance in real time to help each student's study of course topics. Students build the confidence they need to deepen their understanding, participate meaningfully, and perform better in and out of class. Other highlights include chapter-specific reading quizzes, MicroLab Tutorials, MicrobiologyNow coaching activities, Clinical Case and MicroCareer coaching activities, animation quizzes, MCAT Prep questions, and many additional study and assessment tools. Collectively, the content and presentation of *BBOM* 16e, coupled with the powerful learning tools of Mastering Microbiology, create an unparalleled educational experience in microbiology.

Revision Highlights

UNIT 1 The Foundations of Microbiology

Chapter 1

- The microbial world is introduced in an exciting and novel way by weaving together core concepts in microbiology with the historical events that led to their discovery. The foundations of microbiology are revealed through introductions to microscopy, laboratory cultivation, microbial evolution, and the molecular principles that unify all life.
- Some highlights: Vibrant new images help connect students with the diverse and numerous ways in which microbiology impacts our world. Coverage of cell size and morphology is introduced here rather than in Chapter 2 in order to draw students into the microscopic world early on and introduce them to actual microbes and their properties.

Chapter 2

- In the microbial world, cellular structures are tightly linked to cell functions, and Chapter 2 offers a complete guide to the features that define and differentiate microbial cells and their functions. Updated coverage of nutrient transport here rather than in the growth chapter places this critical cellular activity firmly within the context of the cell envelope.
- Some highlights: Electron cryotomography has provided new insight into cell biology and is incorporated in new views of peptidoglycan structure, S-layers, and diversity in cell envelope organization. Vivid new illustrations developed from cutting-edge

microscopic images of the flagellum, the archaellum, and the rotating proteins that confer gliding motility provide a fresh new look at how these structures move prokaryotic cells about their environments.

Chapter 3

- This chapter remains focused on the fundamentals of metabolism and has been revised to simplify metabolic concepts and emphasize the modularity of metabolism. The chapter starts with the essential principles and then provides examples of their application while guiding the student though the major metabolic processes that define microbial life.
- Some highlights: New art provides greater clarity and realism in understanding electron transport reactions, making this process easier to understand and easier to teach. Modularity of metabolism and the importance of the proton motive force receive greater emphasis by providing simple examples of chemolithotrophy and phototrophy to reinforce the student's understanding of energy conservation as a unifying concept in biology. Updates to fermentation clarify and distinguish this process from anaerobic respiration, and an overview of autotrophy and nitrogen fixation emphasize the connectivity between anabolic and catabolic processes in the cell.

Chapter 4

- This chapter on microbial growth and its control moves up one slot from the previous edition to better prepare students for dealing with concepts in molecular biology and genetics where microbial growth plays a central role.
- Some highlights: The essentials of microbial nutrition and laboratory culture are introduced here with a segue to counting methods and quantitative aspects of microbial growth. The dynamics of microbial growth are emphasized with exciting new coverage of the biofilm mode of growth and alternatives to binary fission. The latter includes organisms that display budding division such as *Caulobacter*—the prime model for developmental studies of bacteria—and bacteria that grow by hyphal extensions characteristic of filamentous bacteria such as *Streptomyces*, a major producer of antibiotics.

Chapter 5

- This introduction to virology moves up from its position in Unit 2 in the previous edition to round out the foundations of microbiology theme of Unit 1. This move gives earlier visibility to the importance of viruses as microbes, clearly explains how they differ from cells, and lays the necessary groundwork for dealing with the genetics, genomics, and molecular biology that follows in Unit 2.
- Some highlights: Emphasis remains on the basic principles of virology including how viruses and cells can be viewed as both similar and different and how methods for replicating viruses resemble those for growing cells. Bacteriophage T4 is used as a model lytic virus, and coverage of eukaryotic viruses is expanded beyond just animal viruses to include some major viruses of plants. This highly visual chapter is embellished with over a dozen new photos of exciting, newly discovered viruses along with supporting art that underscores the fundamentals of virology.

UNIT 2 Molecular Biology and Genetics

Chapter 6

- Moved forward two slots from its position in the previous edition to better fit as the kick-off to Unit 2, this chapter lays the necessary groundwork in molecular biology for tackling microbial genetics and genomics and the fast-moving fields of synthetic biology, molecular microbial ecology and diversity, the human microbiome, and diagnostic microbiology.
- Some highlights: Reorganized coverage of DNA supercoiling precedes new and more realistic depictions of the seminal processes of replication, transcription, and translation. New coverage of transcriptional processes in *Archaea* and their relationship to those in *Eukarya* and updated coverage of protein secretion round out this essential primer in microbe molecular biology that every student needs to master.

Chapter 7

- Because microbes must coordinate cellular processes to optimize their chances for survival and reproduction, Chapter 7 is central to Unit 2 in describing how prokaryotic cells control the seminal processes of replication, transcription, and translation. Microbial regulatory systems are highly diverse and sometimes tiered, but an appreciation for how control systems work is key to understanding metabolic diversity, pathogenesis, and synthetic biology.
- Some highlights: Reorganized and expanded coverage of gene expression in *Bacteria* and *Archaea* including activation and repression/derepression as well as chemotaxis and global controls. New coverage of two-component systems for regulating nitrogen assimilation and updated coverage of the phosphate regulon, heat shock response, and riboswitch activity exemplify the comprehensive nature of this chapter.

Chapter 8

- This chapter continues the molecular theme of Unit 2 by building on the major topics of Chapters 4, 6, and 7 in the context of the mechanisms that underlie microbial growth and differentiation. Knowledge of the molecular biology of microbial growth is central to mastering the biology of microbial populations and is keenly relevant to the topics of antibiotic efficacy, antibiotic resistance and persistence, and infectious disease microbiology in general.
- Some highlights: New high-resolution time-course images highlight the molecular processes of growth and cell shape determination. We expand coverage of biofilm formation and the signaling molecule cyclic-di-GMP in *Bacteria* and provide new coverage of biofilm formation in *Archaea*. The chapter also includes new coverage of endospore germination and phenotypic heterogeneity to encompass more topics within the evolving field of microbial growth from a molecular perspective.

Chapter 9

- This chapter rounds out Unit 2 by discussing the foundation for microbial diversity—how microbes undergo genetic change while still maintaining genomic integrity. This essential primer of microbial genetics also lays the groundwork for tackling the hot areas of

microbial omics and synthetic biology and provides the fundamental background necessary to comprehend the most recent concepts of microbial evolution that will unfold in later chapters.
- Some highlights: New and updated visual depictions of DNA exchange between microbes as well as updated coverage on natural competence and the role of pili in DNA uptake. Reorganized and new coverage of barriers to DNA transfer including CRISPR, the important bacterial and archaeal "immune system" whose applications are revolutionizing biology and clinical medicine.

UNIT 3: Genomics, Synthetic Biology, and Evolution

Chapter 10

- Because the genome is the blueprint for all biological traits, this chapter kicks off Unit 3 by discussing not only microbial genomics, but also methods to assay large pools of biological molecules. Various omics studies can be combined to provide a detailed picture of the vast range of capabilities possessed by a specific microbe or groups of microbes, which is essential to the topics of genetic engineering, synthetic biology, and microbial ecology.
- Some highlights: New and exciting coverage of functional genomics and high-throughput techniques to determine the role of individual genes. Reorganized and updated coverage of microbial genome content, proteomic applications, and systems biology highlight the ever-advancing field of omics.

Chapter 11

- This chapter continues the theme of Unit 3 by focusing on the unique genomes of viruses and the diverse mechanisms by which viral genomes are replicated. Knowledge of the molecular biology underlying viral replication is central not only to understanding how viruses infect their hosts and how they persist, but also for developing new clinical strategies for treating viral diseases of humans and other animals.
- Some highlights: New coverage of viral taxonomy precedes updated coverage of viruses that infect *Archaea*. Reorganized topics of bacteriophage genome replication and regulation of lysogeny in lambda directly link to foundational material in Chapter 5.

Chapter 12

- This high-energy chapter entitled "Biotechnology and Synthetic Biology" covers the essential tools of twenty-first-century biotechnology and describes how they have been applied to yield game-changing medical and other commercial products from the activities of genetically engineered microbes. Expanded coverage is provided of the rapidly advancing fields of synthetic biology and CRISPR genome editing—the latest revolutions to hit biology since discovery of the polymerase chain reaction (PCR). Text and art have been updated throughout.
- Some highlights: New coverage of how biobricks contribute to the construction of synthetic pathways and synthetic cells; the use of recombineering to revolutionize molecular cloning; genetically engineered delivery of human therapeutic agents; refactoring metabolic pathways; targeted microbial delivery of human drugs; and how gene drives could finally conquer malaria.

Chapter 13

- This chapter on microbial evolution was moved from the diversity unit into Unit 3 to emphasize its now closer ties to the unit theme of genomics. In addition to origin of life coverage, the chapter now focuses on how evolution affects the genome and ultimately the biology of the organism. The chapter ends with streamlined coverage of microbial systematics and the definition of a microbial species as a prelude to coverage of microbial diversity in Unit 4.
- Some highlights: New and expanded coverage of the evolution of both cells and viruses, including new art on cellular origins from hydrothermal systems and early bioenergetics; more extensive discussion of the mechanisms of microbial evolution from a genomic perspective, including genomic changes that occur during both vertical and horizontal gene transmission; broadened coverage of experimental evolution and genome dynamics.

UNIT 4 Microbial Diversity

Chapter 14

- Recent years have seen a flurry of fundamental new discoveries about how anaerobic organisms conserve energy. Chapter 14 has been updated to integrate information from new discoveries that lie at the heart of diverse metabolic pathways, including the discovery of electron bifurcation and energy-converting hydrogenases.
- Chapter 14 now includes a new introductory section that summarizes foundational principles of microbial physiology. This new section boils the diversity of the microbial world down into a few key principles that students can follow throughout the chapter. In addition, the chapter includes new art illustrating electron bifurcation, as well as electron flow in organisms such as sulfate reducers and methanogens. Old favorites throughout the chapter are also updated to account for recent discoveries in the field.

Chapter 15

- Chapter 15 has been reorganized and updated to emphasize relationships between metabolic and ecological diversity. New photos have been added to emphasize the morphological diversity of anoxygenic phototrophs and to demonstrate how microorganisms work together to modify their environments.

Chapter 16

- Chapter 16 has new coverage of difficult-to-cultivate bacteria, such as *Acidobacteria*, *Planctomycetes*, and *Fusobacteria*. The widespread application of metagenomic techniques have revealed that these *Bacteria* are of considerable importance in a range of habitats, including the human microbiome, but have only recently been obtained in laboratory culture.

Chapter 17

- Metagenomics has contributed greatly to our knowledge of archaeal diversity. Chapter 17 now exploits this and unveils the TACK, DPANN, and Asgard *Archaea*, some of which are the closest known relatives of the eukaryotes. We also update the diversity of mechanisms of methanogenesis in the archaeal domain.

Chapter 18

- Along with major updates on eukaryotic phylogeny, a new section is devoted to the haptophytes, including the globally and ecologically important coccolithophore *Emiliania huxleyi*. Coccolithophores play a major role in regulating global climate, illustrating the power that microbes exert over our biosphere.

UNIT 5 Microbial Ecology and Environmental Microbiology

Chapter 19

- The chapter begins a unit on ecology and environmental microbiology. The modern tools of the microbial ecologist are described with examples of how each has helped sculpt the science.
- Some highlights: A new method to visualize protein synthesis in single cells allows study of microbial activity in the environment. Metabolomics exploits new methods in mass spectrometry to unravel the complex metabolic interactions sustaining microbial communities. Nanosensor technologies are revealing how microbes alter the chemical landscape of three-dimensional surfaces. A new section explores multi-omics, which combines multiple state-of-the-art analytical tools to more fully characterize microbial communities.

Chapter 20

- The properties and microbial diversity of major microbial ecosystems including soils and aquatic systems are compared and contrasted in exciting ways.
- Some highlights: Expansive coverage of surface-attached microbial communities and how those communities are responding to plastic pollution of the environment. New understanding of the ecology of iron-oxidizing bacteria revealed by the isolation of new members of this biogeochemically significant group. The discovery in deep ocean sediments of novel *Archaea* that link this domain with *Eukarya*. Extensive coverage of marine viruses, their abundance and diversity, and how they alter the physiology of organisms they infect. Humans traveling to 10,000-meter depths in the oceans discover the most pressure-tolerant bacterium known.

Chapter 21

- Extensive coverage of the major nutrient cycles in nature and the microbes that catalyze them are presented in a fashion that allows the cycles to be taught as individual entities or as interrelated metabolic loops.
- Some highlights: Expanded coverage of the biogeochemistry of sulfur compounds highlights the importance of volatile microbial products such as dimethyl sulfide for cloud formation. Advances in the biochemistry of extracellular electron transfer add new understanding to how the ecology and diversity of microorganisms drive the biogeochemical cycling of iron and manganese. The mystery of how methane is generated (typically a strictly anoxic process) in highly oxygenated ocean surface waters is solved by discoveries in the phosphorus cycle described in a new Explore the Microbial World.

Chapter 22

- This chapter on the built environment shows how humans create new microbial habitats through construction of buildings, supporting infrastructure, and habitat modification, and which microbes take advantage of these habitats and why.
- Some highlights: The microbial metabolism of biologically produced and manufactured chlorinated organics has been expanded, as has the basis for the bioremediation of major chemical pollutants. How microbes are responding to the mountains of plastics contaminating the environment and the discovery of novel bacteria capable of degrading plastic bottles are described. New technology that improves the efficiency of wastewater treatment using granular sludge technology is presented, and the microbial response to the excessive use of common household cleansers is considered.

Chapter 23

- A chapter devoted to nonhuman microbial symbioses describes the major microbial partners that live in symbiotic associations with other microbes, with plants, and with animals other than humans.
- Some highlights: Newly revised section on symbioses between microorganisms addresses the ecological significance of phototroph switching in lichens and how certain bacterial species use electrically conductive structures to form intimate symbiotic associations. Several updates include how insect symbionts are used to combat transmission of major viral diseases of humans and how defensive chemicals produced by symbionts protect insects from predation. Detailed coverage is given to the elaborate "cross-talk" between microbe and animal needed to establish the squid light organ.

UNIT 6 Microbe–Human Interactions and the Immune System

Chapter 24

- A chapter on the human microbiome launches the unit on microbe–human interactions and the immune system by introducing and updating advances in our understanding of the microbes that inhabit the human body and their relationship to health and disease.
- Some highlights: The discovery of ultrasmall bacteria in the mouth parasitizing other bacteria brings a new twist to the microbial ecology of the oral cavity. A new section on the human virome describes how metagenomics is driving the discovery and isolation of interesting new viruses. Extensive coverage is devoted to the impact of early-life events on the development of the newborn gut microbiome and of recent successes in probiotic therapy for preventing newborn intestinal diseases.

Chapter 25

- Beginning with this chapter, the book shifts its focus to pathogenic microorganisms, the immune system, and disease. Part I of this chapter addresses microbial adherence, colonization and invasion, and pathogenicity, including important sections on virulence and virulence attenuation. Part II highlights key enzymes and toxins produced by microbes that contribute to pathogenesis.

- Some highlights: The updated text includes expanded coverage of bacterial adhesins supported by a new, two-part figure that highlights new discoveries in staphylococcal adherence. Revised coverage of virulence attenuation includes new artwork to show how this principle can be exploited for development of effective vaccines. An updated discussion of botulinum toxins reflects new findings and clearly presents both the neurotoxic mechanism and the surprising clinical utility of these extremely potent substances.

Chapter 26

- Chapter 26 opens with an overview of the immune system and the body's first-line barriers to infection. This is followed by a brief discussion of hematopoiesis before focusing on innate immune responses to pathogen invasion. The chapter provides a natural progression into adaptive immune responses covered in Chapter 27.
- Some highlights: In addition to a new chapter opener highlighting breakthroughs that link Alzheimer's disease to microbial infection, this chapter contains heavily edited text that includes a more comprehensive discussion of leukocyte diversity and an all-new description of the role of amyloid-β protein as an innate defense in the brain. Other highlights include expanded coverage of interferons and the role of natural killer cells as the primary effectors of antibody-dependent cell-mediated cytotoxicity. Finally, a fascinating new Explore the Microbial World highlights the role of pattern recognition receptors in establishing host–microbe mutualisms using hydrothermal vent tube worms as an example.

Chapter 27

- Chapter 27 begins with an essential discussion of the principles that define adaptive immunity: specificity, immune memory, lymphocyte selection, and immune tolerance. This is followed by sections that discuss the functional mechanisms of the key cells and proteins (immunoglobulins, major histocompatibility complexes, and T cell receptors) that drive adaptive immunity.
- Some highlights: The text has been heavily edited throughout, and this has produced a clearer and more informative presentation of B and T lymphocyte selection and tolerance, including a new discussion of T-dependent versus T-independent antigens. In addition, a new section dedicated to T cell activation and anergy clearly presents the important concept of the second signal required for T cell activation.

Chapter 28

- The newly reorganized Chapters 28 and 29 have emerged from materials presented in Chapter 28 of the 15th edition. Treating immune disorders and antimicrobial therapy (Chapter 28) separately from clinical diagnostic methods (Chapter 29) has produced a more teachable format, making these topics more accessible for students and easier for the instructor to plan course assignments.
- Some highlights: The text progresses smoothly from immune disorders and deficiencies to methods used to train and hone the immune response for disease prevention and treatment. New coverage of mRNA and plant-based vaccines shares the latest innovations in vaccinology. An all-new section on immunotherapy, supported by vibrant new artwork, highlights exciting advancements in the use of genetic engineering and molecular immunology to treat cancer.

UNIT 7 Infectious Diseases

Chapter 29

- To bring better focus to the material, this chapter is now solely dedicated to the clinical microbiology laboratory and includes information on lab safety, healthcare-associated infections, and a wide array of both culture-dependent and culture-independent techniques used to diagnose infectious diseases.
- Some highlights: The chapter launches with the description of an exciting new method of diagnosing tuberculosis—humanity's most notorious scourge. The text has been edited throughout for better organization and clarity, and art modifications help clarify complex diagnostic techniques. Updated terminology includes an introduction to point-of-care diagnostics.

Chapter 30

- This chapter introduces the topics and terminology of the science of epidemiology and public health. Historical and modern examples throughout emphasize key concepts such as emerging (and reemerging) diseases, epidemics and pandemics, and the public health threat associated with the development and use of weaponized microorganisms.
- Some highlights: incorporation of the most up-to-date statistics available on disease incidence and outbreaks throughout the text and in figures and tables, as well as an all-new section supported by photos on the emergence of the important healthcare-associated pathogen *Clostridioides* (*Clostridium*) *difficile*.

Chapter 31

- This is the first of four highly visual chapters that take an ecological approach to pathogenic microorganisms by considering infectious diseases based on their modes of transmission. Bacterial and viral diseases transmitted person to person by way of airborne particles, direct contact, or sexual contact are the focus here.
- Some highlights: Statistical data regarding key emerging and reemerging diseases, including measles, pertussis, influenza, hepatitis, HIV/AIDS, gonorrhea, and syphilis have been updated to reflect the most recent data available; an all-new discussion with supporting photo of the neglected tropical disease yaws helps impart knowledge and awareness of this lingering scourge.

Chapter 32

- In this chapter we examine pathogens transmitted to humans through either an animal vector or soil-contaminated wounds or objects. Many of these diseases have high morbidity and mortality rates, and in most cases, effective vaccines are not yet available.
- Some highlights: The text and figures include the most up-to-date statistics for diseases throughout the chapter, including rabies, hantavirus, spotted fever rickettsiosis, ehrlichiosis and anaplasmosis, Lyme disease, and the major tropical hemorrhagic fevers. In addition, the text now includes updated discussions of the emergence of key tickborne diseases in the United States and coverage of new strategies against dengue fever, including description of a new vaccine and the use of the bacterial endosymbiont *Wolbachia* to control the dengue virus–infected mosquito population.

Chapter 33

- Pathogens in contaminated water or food are easily transmitted to humans, with waterborne diseases being especially common in developing countries lacking adequate water treatment facilities. This chapter highlights the most prevalent water- and foodborne diseases and emphasizes the importance of clean water and proper food preparation and preservation in preventing these physically uncomfortable and occasionally fatal illnesses.
- Some highlights: Updated statistics have been incorporated for all major water- and foodborne diseases, including *Campylobacter* infections, which have now overtaken salmonellosis as the leading cause of bacterial food infection in the United States. New discussions cover recently elucidated norovirus pathology and new food safety developments, including the use of eBeam technology and bacteriophage sprays. A new overview figure of cholera infection integrates photos with artwork to emphasize key aspects of this devastating and all too common disease.

Chapter 34

- Eukaryotic pathogens present a special challenge to medicine because, on a cellular level, they are not that different from our own cells. Thus, it can be difficult to find selective targets for chemotherapeutic drugs. Yet the microbes highlighted in this highly visual chapter cause some of the most devastating and prevalent diseases today.
- Some highlights: New color photos adorn the chapter, including two stunning fluorescent micrographs of *Entamoeba histolytica*, the causative agent of amebic dysentery. Broader coverage of distinctive features of several diseases, including cyclosporiasis, toxoplasmosis, and malaria, has been seamlessly incorporated. All statistics have been updated with the most recent surveillance data to yield a global picture of fungal and parasitic diseases.

Acknowledgments

An excellent textbook is an educational resource that can only emerge from the combined contributions of a dedicated book team. In addition to the authors, the *Brock Biology of Microorganisms (BBOM)* team was composed of folks both inside and outside of Pearson. Content Manager Josh Frost paved the way for the 16th Edition of *BBOM* and provided the resources necessary for the authors to produce a spectacular revision in a timely fashion. Importantly, Josh also brought to *BBOM* his extensive experience as Content Manager of *Campbell Biology*, the leading textbook of biology worldwide. This greatly benefited *BBOM*, a book whose educational philosophy has traditionally paralleled that of strong majors-level biology books. The *BBOM* coauthor team greatly appreciated the guidance and input that Josh brought to our book.

BBOM 16e editorial and production were headed up by Michele Mangelli (Mangelli Productions), and both cover and interior designs were created by Gary Hespenheide (Hespenheide Design). Michele assembled and managed the production team and kept editorial and production on mission, on budget, and on time, and did so tirelessly in a helpful, author-friendly, and accommodating manner. The artistic magic of Gary is clearly visible in the outstanding internal design that smoothly ushers the reader through the book with highly effective organizational and navigational cues. Gary also designed the book's cover—a spectacular display of microbial diversity (photo courtesy of Professor Dr. Jörg Overmann, Braunschweig, Germany). The art team at Imagineering Art (Toronto, Canada) did an outstanding job in helping the authors link art with text and provided many helpful suggestions and options for art presentation, consistency, and style. Many thanks are extended to Michele, Gary, Jörg, and Imagineering for their outstanding efforts on *BBOM* 16e.

Many other people were part of the book production, editorial, or marketing team, including Karen Gulliver, Jean Lake, Kristin Piljay, Betsy Dietrich, Maureen Johnson, Susan Wang, Greg Anderson, and Elizabeth McPherson. Karen was our excellent and highly efficient production editor; she kept manuscript and pages moving smoothly through the wheels of production, graciously tolerated the authors' many requests, and accommodated our time constraints. Jean was our art coordinator, efficiently tracking and routing art and handling interactions between the art studio and the authors to ensure quality control and a timely schedule. Betsy worked with Jean and Karen to ensure an art program and text free of both bloopers and subtle errors. Kristin was our photo researcher who dug out some of the hard-to-find specialty photos that grace *BBOM* 16e. Susan, Greg, and Elizabeth composed an excellent accuracy review team and made numerous very helpful comments. The authors thank Karen, Jean, Kristin, Betsy, Susan, Greg, and Elizabeth for their combined contributions to the book you see in front of you today.

Special thanks go to Anita Wagner Hueftle, our spectacular copyeditor and a key part of the book team. Anita is not only a master wordsmith; her amazing gift of being able to keep track of *where* everything was said in this book and *how* everything was said in this book has helped us make the most readable, accurate, and consistent textbook of microbiology available today. Thank you kindly, Anita; you made outstanding contributions to this edition.

We thank Alysun Estes and Krista Clark for their much-appreciated marketing support.

We are also grateful to the top-notch educators who constructed the Mastering Microbiology program that accompanies this text; these include: Candice Damiani, Jennifer Hatchel, Bryan Jennings, Ann Paterson, Emily Booms, Ines Rauschenbach, and Monica Togna.

And last but not least, no textbook in microbiology could be published without reviewing of the manuscript and the gift of new photos from experts in the field. We are therefore extremely grateful for the assistance of the many individuals who kindly provided manuscript reviews, unpublished results, and new photos. Complete photo credits in this book are found either alongside a photo or in the photo credits listed in the back of the book. Reviewers and photo suppliers included:

Jônatas Abrahão, *Universidade Federal de Minas Gerais (Brazil)*
Sonja-Verena Albers, *University of Freiburg (Germany)*
Rebecca Albright, *California Academy of Sciences*
Douglas Bartlett, *Scripps Institution of Oceanography*
Bonnie Bassler, *Princeton University*
David Battigelli, *University of North Carolina Greensboro*
Tom Ian Battin, *Ecole Polytechnique Federale de Lausanne (Switzerland)*
J. Thomas Beatty, *University of British Columbia (Canada)*
Veysel Berk, *Wallit! Inc.*
Edward Bernard, *University of Maine*
Tanmay Bharat, *University of Oxford (England)*
Benjie Blair, *Jacksonville State University*
Ilka Bischofs, *Max Planck Institute for Terrestrial Biology (Germany)*
Robert Blankenship, *Washington University in St. Louis*
Antje Boetius, *Max Planck Institute for Marine Microbiology (Germany)*
Gary Borisy, *The Forsyth Institute*
Ariane Briegel, *Leiden University (Netherlands)*
Volker Brinkmann, *Max Planck Institute for Infection Biology, Berlin*
Pamela Brown, *University of Missouri*
Jennifer Brum, *Louisiana State University*
Marie Bulínová, *Charles University (Czech Republic)*
Gustavo Caetano-Anollés, *University of Illinois*
Christian Cambillau, *Centre National de la Recherche Scientifique Aix-Marseille University Marseille (France)*
Hans Carlson, *Lawrence Berkeley National Laboratory*
Dale Casamatta, *University of North Florida*
Clara S. Chan, *University of Delaware*
Matthew Wook Chang, *National University of Singapore*
Beat Christen, *ETH Zürich (Switzerland)*
Pascale Cossart, *Pasteur Institute (France)*
Matt Cruzen, *Biola University*
Michele Culumber, *Weber State University*

Ankur Dalia, *Indiana University*
Bertram Daum, *University of Exeter (England)*
Svetlana N. Dedysh, *Winogradsky Institute of Microbiology (Russia)*
David J. Des Marais, *NASA Ames*
Adam Deutschbauer, *Lawrence Berkeley National Laboratory*
Omar Din, *University of California, San Diego*
Alice Dohnalkova, *Pacific Northwest National Laboratory (Richland, WA)*
Steven Dominy, *Cortexyme, Inc.*
Paul Dunlap, *University of Michigan*
David Emerson, *Bigelow Laboratory for Ocean Sciences (Maine)*
Susanne Erdmann, *University of New South Wales (Australia)*
Belinda Ferrari, *University of New South Wales (Australia)*
Derek J. Fisher, *Southern Illinois University*
Jason Flowers, *Murraysmith (Seattle, WA*)
Bruce Fouke, *University of Illinois Urbana–Champaign*
Andreas Giesen, *Royal HaskoningDHV (The Netherlands)*
Mariana Gomes de Pinho, *Universidade Nova de Lisboa (Portugal)*
Hans-Peter Grossart, *Leibniz-Institute of Freshwater Ecology and Inland Fisheries (Germany)*
Ricardo Guerrero, *University of Barcelona (Spain)*
Jennifer Hatchel, *College of Coastal Georgia*
Roland Hatzenpichler, *Montana State University*
Jennifer Hess, *Aquinas College*
Donald Hilvert, *ETH Zürich (Switzerland)*
Jay Hodgson, *Georgia Southern University*
William Inskeep, *Montana State University*
Anthony James, *University of California, Irvine*
Joshua Jenkins, *University of Bristol Dental School (England)*
Grant Jensen, *California Institute of Technology*
Deborah O. Jung, *Southeast Missouri State University*
Kathryn Kauffman, *Massachusetts Institute of Technology*
Vjollca Konjufca, *Southern Illinois University*
Klaus Koren, *University of Copenhagen (Denmark)*
Michael Kovach, *Baldwin Wallace University*
Michael Kühl, *University of Copenhagen (Denmark)*
Philippe Laissue, *University of Essex (England)*
Christian Lesterlin, *Institut de Biologie et Chimie des Protéines (France)*
Ruth E. Ley, *Max-Planck Institute for Developmental Biology (Germany)*
Shee-Mei Lok, *Duke-NUS Medical School (Singapore)*
E. Erin Mack, *Dupont Corporate Remediation Group (Newark, DE)*
Jessica Mark Welch, *Marine Biological Laboratory, Woods Hole*
Francis Martin, *Lab of Excellence ARBRE, INRA-Nancy (France)*
Sean McAllister, *University of Delaware*
Margaret McFall-Ngai, *University of Hawaii at Manoa*
Jeffrey McLean, *University of Washington, Seattle*
Nancy Moran, *University of Texas*
Phillip Nadeau, *Massachusetts Institute of Technology*
National Aeronautics and Space Administration *(USA)*
Jeniel Nett, *University of Wisconsin*
Daniela Nicastro, *University of Texas Southwestern*
Trent Northen, *Lawrence Berkeley National Laboratory*
Gal Ofir, *Weizmann Institute of Science (Israel)*
Catherine Oikonomou, *California Institute of Technology*
Jörg Overmann, *Leibniz Institute DSMZ Braunschweig (Germany)*
Niki Parenteau, *NASA Ames*
Donghyun Park, *Yale University School of Medicine*
Nicolás Pinel, *Universidad de Antioquia (Colombia)*
Marie Pizzorno, *Bucknell University*
Joe Pogliano, *University of California, San Diego*
Martin Polz, *Massachusetts Institute of Technology*
Tessa Quax, *University of Freiburg (Germany)*
Katherine Ralston, *University of California*
Ines Rauschenbach, *Rutgers University*
Tara Renbarger, *Indiana Wesleyan University*
Niels Peter Revsbech, *Aarhus University (Denmark)*
Ned Ruby, *University of Hawaii at Manoa*
Bernhard Schink, *University of Konstanz (Germany)*
Susan Schlimpert, *John Innes Centre (England)*
Andrey Shkoporov, *University College Cork (Ireland)*
Simon Silver, *University of Illinois Chicago*
Janice Speshock, *Tarleton State University*
Emily Stowe, *Bucknell University*
Shunichi Takahashi, *National Institute for Basic Biology (Japan)*
Francisco Tenllado, *Centro de Investigaciones Biológicas (Spain)*
Andreas Teske, *University of North Carolina*
David Valentine, *University of California, Santa Barbara*
Mikel Valle, *Asociación Centro de Investigación Cooperativa en Biociencias (Spain)*
Nicholas Verola, *Verola Studio (Vero Beach, FL)*
Marilyn B. Vogel, *Auburn University*
Judy Wall, *University of Missouri*
Dave Ward, *Montana State University*
Bishop Wash, *The Richards Group (Dallas TX)*
Jillian L. Waters, *Max Planck Institute for Developmental Biology (Germany)*
Nicki Watson, *Massachusetts Institute of Technology*
Gunter Wegener, *Max Planck Institute of Marine Microbiology (Germany)*
Mari Winkler, *University of Washington*
Gerry Wright, *McMaster University*
Vamsi Yadavalli, *Virginia Commonwealth University*
Jeremy R. Young, *University College London (England)*
Carl Zeiss AG *(Jena, Germany)*
Jizhong (Joe) Zhou, *University of Oklahoma*
Steve Zinder, *Cornell University*

As hard as a publishing team may try, no textbook can ever be completely error-free. Although we are confident the reader will be hard pressed to find errors in *BBOM* 16e, any errors that do exist, either of commission or omission, are the responsibility of the authors. In past editions, users have been kind enough to contact us when they spot an error so we can fix it in a subsequent printing. Users should feel free to continue to contact the authors directly about any errors, concerns, questions, or suggestions they have about the book. We are always happy to hear from our readers; through the years, your comments have helped make the book stronger.

Michael T. Madigan (madigan@siu.edu)
Kelly S. Bender (bender@siu.edu)
Daniel H. Buckley (dbuckley@cornell.edu)
W. Matthew Sattley (matthew.sattley@indwes.edu)
David A. Stahl (dastahl@uw.edu)

Acknowledgments for the Global Edition

Pearson would like to acknowledge and thank the following for the Global Edition:

Contributors

Fiona Baird, *Griffith University (Australia)*
Aurélien Carlier, *Universiteit Gent (Belgium)*
Lara Grollo, *Australian Catholic University (Australia)*
HE Jianzhong, *National University of Singapore (Singapore)*
Wendy Ying Ying Liu, *Quest International University (Malaysia)*
Joy Pang, *Singapore Institute of Technology (Singapore)*
Jörg Simon, *Technical University of Darmstadt (Germany)*
Wei-Qin Zhuang, *University of Auckland (New Zealand)*

Reviewers

Oluwapelumi Adeyemi, *University of Leeds (England)*
Siti Khayriyyah Mohd Hanafiah, *Universiti Sains Malaysia (Malaysia)*
Quek Choon Lau, *Ngee Ann Polytechnic (Singapore)*
Maria del Carmen Montero-Calasanz, *Newcastle University (England)*

This page is intentionally left blank

Contents

UNIT 4 Microbial Diversity

14 Metabolic Diversity of Microorganisms 460

15 Ecological Diversity of *Bacteria* 514

16 Phylogenetic Diversity of *Bacteria* 555

ASM Recommended Curriculum Guidelines for Undergraduate Microbiology

The American Society for Microbiology (ASM) endorses a concept-based curriculum for undergraduate microbiology, emphasizing skills and concepts that have lasting importance beyond the classroom and laboratory. The ASM (in its *Curriculum Guidelines for Understanding Microbiology Education*) recommends deep understanding of 27 key concepts, 4 scientific thinking competencies, and 7 key skills. These guidelines follow scientific literacy reports and recommendations from the American Association for the Advancement of Science and the Howard Hughes Medical Institute by encouraging an active learning, student-based course. Consider these guiding statements as you progress through this book and master principles, problem solving, and laboratory skills in microbiology.

ASM Guideline Concepts and Statements

Evolution: Chapters 1, 9, 10–14, 20, 30

- Cells, organelles (e.g., mitochondria and chloroplasts), and all major metabolic pathways evolved from early prokaryotic cells.
- Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.
- Traditional concept of species is not readily applicable to microbes due to asexual reproduction and the frequent occurrence of horizontal gene transfer.
- Evolutionary relatedness of organisms is best reflected in phylogenetic trees.
- Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).

Cell Structure and Function: Chapters 1, 2, 5, 8, 11, 18

- Structure and function of microorganisms have been revealed by the use of microscopy (including bright-field, phase contrast, fluorescence, super-resolution, and electron).
- Bacteria have unique cell structures that can be targets for antibiotics, immunity, and phage infection.
- *Bacteria* and *Archaea* have specialized structures (e.g., flagella, endospores, and pili) that often confer critical capabilities.
- While microscopic eukaryotes (for example, fungi, protozoa, and algae) carry out some of the same processes as bacteria, many of the cellular properties are fundamentally different.
- Replication cycles of viruses (lytic and lysogenic) differ among viruses and are determined by their unique genomes.

Metabolic Pathways: Chapters 1, 3, 4, 7, 8, 12, 14

- *Bacteria and Archaea* exhibit extensive, and often unique, metabolic diversity (e.g., nitrogen fixation, methane production, anoxygenic photosynthesis).
- Interactions of microorganisms among themselves and with their environment are determined by their metabolic abilities (e.g., quorum sensing, oxygen consumption, nitrogen transformations).
- Survival and growth of any microorganism in a given environment depends on its metabolic characteristics.
- Growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means.

Information Flow and Genetics: Chapters 1, 5–13

- Genetic variations can impact microbial functions (e.g., in biofilm formation, pathogenicity, and drug resistance).
- Although the central dogma is universal in all cells, the processes of replication, transcription, and translation differ in *Bacteria*, *Archaea*, and eukaryotes.
- Regulation of gene expression is influenced by external and internal molecular cues and/or signals.
- Synthesis of viral genetic material and proteins is dependent on host cells.
- Cell genomes can be manipulated to alter cell function.

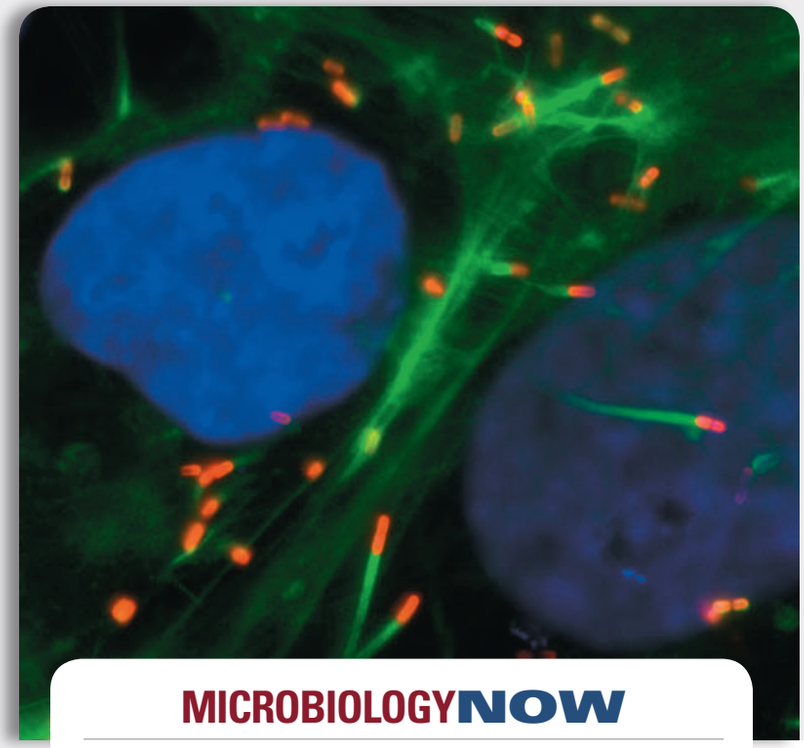
Microbial Systems: Chapters 1, 15–34

- Microorganisms are ubiquitous and live in diverse and dynamic ecosystems.
- Many bacteria in nature live in biofilm communities.
- Microorganisms and their environment interact with and modify each other.
- Microorganisms, cellular and viral, can interact with both human and nonhuman hosts in beneficial, neutral, or detrimental ways.

Impact of Microorganisms: Chapters 1, 6–8, 12, 19–34

- Microbes are essential for life as we know it and the processes that support life (e.g., in biogeochemical cycles and plant and/or animal microbiota).
- Microorganisms provide essential models that give us fundamental knowledge about life processes.
- Humans utilize and harness microorganisms and their products.
- Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.

The Microbial World 1



MICROBIOLOGY**NOW**

Microbiology in Motion

The microbial world is strange and fierce. It is teeming with life, ancient, diverse, and constantly changing. Microorganisms are Earth's life support system, and from our first breath they influence nearly every moment of our lives. Microbes are in our water and our food, and we carry them on us and in us. Indeed, microbes abound in any natural environment that will support life, including many environments too hostile for higher life forms.

While the microbial world is invisible, we can explore it through the science of microbiology. Microbiology evolves at a breathtaking pace. Even the microscope continues to evolve, providing an ever more detailed picture of the microbial world. The image above was made with a fluorescence microscope that uses lasers, guided by a computer, to map the three-dimensional structure of cells. The image shows neighboring human cells with their nuclei stained blue and actin filaments stained green. These cells are infected with the foodborne bacterial pathogen *Listeria monocytogenes*, stained red.

Listeria are soil organisms that sometimes find their way into our food. In soils they infect other microbes such as amoebae. Our cells are similar in many ways to those of microscopic organisms, and so *Listeria* finds itself well adapted to live within us. This bacterium has the unique ability to hijack cellular systems, causing actin to polymerize and propel the cell like a rocket within the host cytoplasm. The force of this propulsion causes *Listeria* to penetrate adjacent cells (image, lower left), spreading the infection. *Listeria* can also invade host vacuoles (not shown), where it hides and survives. This persistent state can prolong infection and promote resistance to antibiotic therapy. Research on *Listeria* has provided new insights on the biology of this pathogen and an ever-changing view of a microbial world in motion.

Source: Kortebi, M., et al. 2017. *Listeria monocytogenes* switches from dissemination to persistence by adopting a vacuolar lifestyle in epithelial cells. *PLoS Pathog. 13*: e1006734.

This chapter launches our journey into the microbial world. Here we will begin to discover what the science of microbiology is all about and what microorganisms are, what they do, and how they can be studied. We also place microbiology in historical context, as a process of scientific discovery driven by simple (yet powerful) experiments and insightful minds.

I • Exploring the Microbial World

The microbial world consists of microscopic organisms that have defined structures, unique evolutionary histories, and are of enormous importance to the biosphere.

1.1 Microorganisms, Tiny Titans of the Earth

Microorganisms (also called *microbes*) are life forms too small to be seen by the unaided human eye. These microscopic organisms are diverse in form and function, and they inhabit every environment on Earth that supports life. Many microbes are undifferentiated single-celled organisms, but some can form complex structures, and some are even multicellular. Microorganisms typically live in complex **microbial communities** (Figure 1.1), and their activities are regulated by interactions with each other, with their environments, and with other organisms. The science of microbiology is all about microorganisms, who they are, how they work, and what they do.

Microorganisms were teeming on the land and in the seas for billions of years before the appearance of plants and animals, and their diversity is staggering. Microorganisms represent a major fraction of Earth's biomass, and their activities are essential to sustaining life. Indeed, the very oxygen (O_2) we breathe is the result of microbial activities. Plants and animals are immersed in a world of microbes, and their evolution and survival are heavily influenced by microbial activities, by microbial symbioses, and by *pathogens*—those microbes that cause disease. Microorganisms are woven into the fabric of human life as well (Figure 1.2), from infectious diseases, to the food we eat, the water we drink, the fertility of our soils, the health of our animals, and even the fuel we put in automobiles. Microbiology is the study of the dominant form of life on Earth, and the effect that microbes have on our planet and all of the living things that call it home.

Microbiologists have many tools for studying microorganisms. Microbiology was born of the microscope, and microscopy is foundational to microbiology. Microbiologists have developed an array of methods for visualizing microorganisms, and these microscopic techniques are essential to microbiology. The cultivation of microorganisms is also foundational to microbiology. A microbial **culture** is a collection of cells that have been grown in or on a nutrient medium. A **medium** (plural, media) is a liquid or solid nutrient mixture that contains all of the nutrients required for a microorganism to grow. In microbiology, we use the word **growth** to refer to the increase in cell number as a result of cell division. A single microbial cell placed on a solid nutrient medium can grow and divide into millions or even billions of cells that form a visible **colony** (Figure 1.3). The formation of visible colonies makes it easier to see and grow microorganisms. Comprehension of the microbial basis of disease and microbial biochemical diversity has relied on the ability to grow microorganisms in the laboratory.

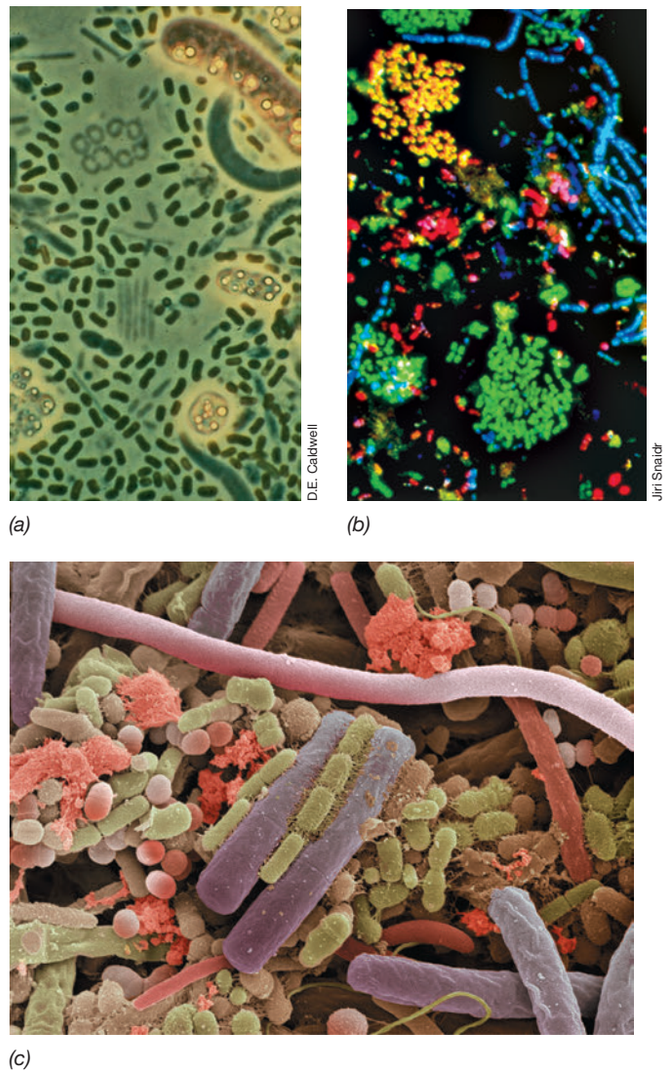


Figure 1.1 Microbial communities. *(a)* A bacterial community that developed in the depths of a small Michigan lake, including cells of various phototrophic bacteria. The bacteria were visualized using phase-contrast microscopy. *(b)* A bacterial community in a sewage sludge sample. The sample was stained with a series of dyes, each of which stained a specific bacterial group. From *Journal of Bacteriology 178*: 3496–3500, Fig. 2b. © 1996 American Society for Microbiology. *(c)* Colorized scanning electron micrograph of a microbial community scraped from a human tongue.

The ability to grow microorganisms rapidly under controlled conditions makes them highly useful for experiments that probe the fundamental processes of life. Most discoveries relating to the molecular and biochemical basis of life have been made using microorganisms. The study of molecules and their interactions is essential to defining the workings of microbial cells, and the tools of molecular biology and biochemistry are foundational to microbiology. Molecular biology has also provided a variety of tools to study microorganisms without need for their cultivation in the laboratory. These molecular tools have greatly expanded our knowledge of microbial ecology and diversity. Finally, the tools of genomics and molecular genetics are also cornerstones of modern

Figure 1.2 Microbial applications. Microorganisms have major impacts on the world in which we live. In the chapters that follow we will learn how microorganisms impact our health, the foods we eat, the water we drink, and even the air we breathe. We will learn how microbes can be used to produce valuable products and the many ways in which microorganisms touch our lives.

microbiology and allow microbiologists to study the genetic basis of life, how genes evolve, and how they regulate the activities of cells.

In the next section, we explore the basic elements of microbial cell structure and summarize the major physiological activities that take place in all cells, regardless of their structure.

Check Your Understanding

- In what ways are microorganisms important to humans?
- Why are microbial cells useful for understanding the basis of life?
- What is a microbial colony and how is one formed?

1.2 Structure and Activities of Microbial Cells

Microbial cells are living compartments that interact with their environment and with other cells in dynamic ways. We purposely exclude viruses in most of this discussion because although they resemble cells in many ways, viruses are not cells but instead a special category of microorganism. We consider the structure, diversity, and activities of viruses in Section 1.4 and in Chapters 5 and 11.

Elements of Microbial Structure

All cells have much in common and contain many of the same components (Figure 1.4). All cells have a permeability barrier called the **cytoplasmic membrane** that separates the inside of the cell, the **cytoplasm**, from the outside. The cytoplasm is an aqueous mixture of **macromolecules** (for example proteins, lipids, nucleic acids, and polysaccharides), small organic molecules (mostly the precursors of macromolecules), various inorganic ions, and ribosomes. All cells also contain **ribosomes**, which are the structures responsible for protein synthesis. Some cells have a **cell wall** that lends structural strength to a cell. The cell wall is a relatively permeable structure located outside the cytoplasmic membrane and is a much stronger layer than the membrane itself. Cell walls are typically found in plant cells and most microorganisms but are not found in animal cells.

There are two fundamental cell types that differ categorically in cellular organization: those having **prokaryotic** cell structure, and those having **eukaryotic** cell structure (Figure 1.4). Cells having eukaryotic cell structure are found in a group of organisms called the *Eukarya*. This group includes plants and animals as well as diverse microbial eukaryotes such as algae, protozoa, and fungi. Eukaryotic cells contain an assortment of membrane-enclosed cytoplasmic structures called **organelles** (Figure 1.4*b*). These include, most prominently, the DNA-containing nucleus but also mitochondria and chloroplasts, organelles that specialize in supplying the cell with energy, and various other organelles.

Mastering Microbiology
Art Activity: Figure 1.3 Common elements of prokaryotic/eukaryotic cells

Prokaryotic cell structure is found within two different groups of organisms we know as *Bacteria* and *Archaea*. Prokaryotic cells have few internal structures, they lack a nucleus, and they typically lack organelles (Figure 1.4*a*). *Bacteria* and *Archaea* appeared long before the evolution of eukaryotes (Section 1.5). While all *Archaea* and

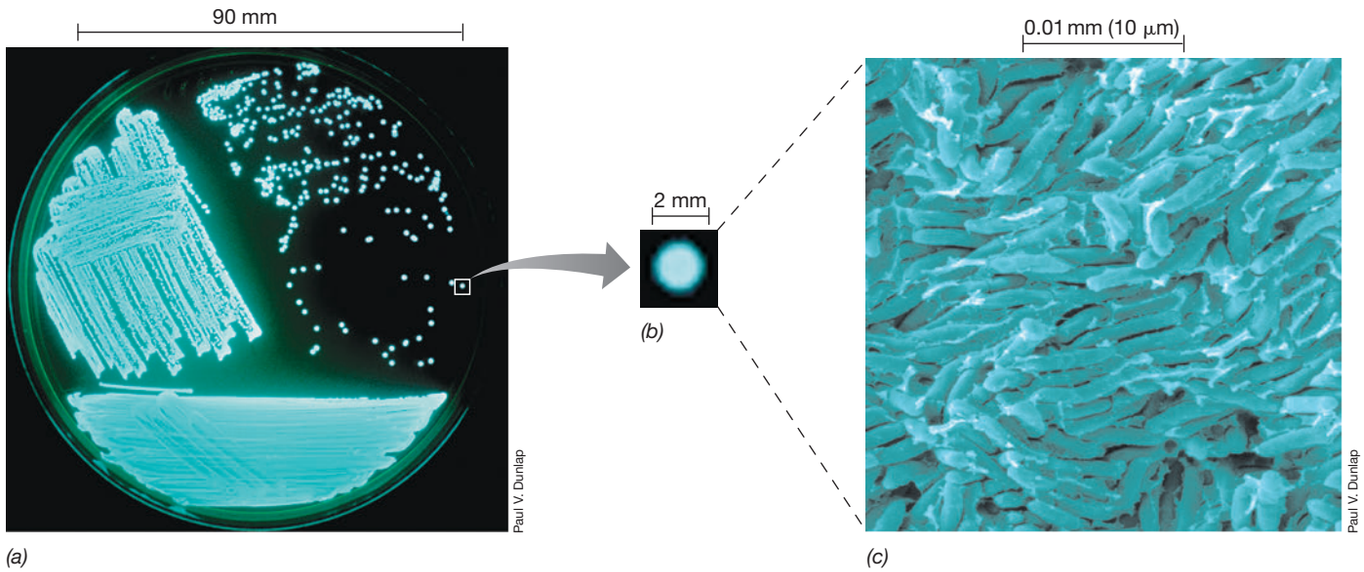


Figure 1.3 Microbial cells. *(a)* Bioluminescent (light-emitting) colonies of the bacterium *Photobacterium* grown in laboratory culture on a Petri plate. *(b)* A single colony can contain more than 10 million (10^7) individual cells. *(c)* Colorized scanning electron micrograph of cells of *Photobacterium*.

Bacteria have prokaryotic cell structure, these two groups diverged very early in the history of life and as a result many of their molecular and genetic characteristics differ at a fundamental level. Indeed, we will see later that in many ways *Archaea* and *Eukarya* are more similar to each other than either is to *Bacteria*.

Genes, Genomes, Nucleus, and Nucleoid

In addition to a cytoplasmic membrane and ribosomes, all cells also possess a DNA **genome**. The genome is the full set of genes in a cell. A gene is a segment of DNA that encodes a protein or an RNA molecule. The genome is the living blueprint of an organism; the characteristics, activities, and very survival of a cell are governed by its genome.

The genomes of prokaryotic cells and eukaryotic cells are organized into structures called **chromosomes**. In eukaryotic cells, DNA is present as several linear molecules (each one formed into its own chromosome) within the membrane-enclosed **nucleus**. By contrast, the genomes of *Bacteria* and *Archaea* are typically closed circular chromosomes (though some prokaryotic cells have linear chromosomes). The chromosome aggregates within the prokaryotic cell to form the **nucleoid**, a mass that is visible in the electron microscope (Figure 1.4*a*) but which is *not* enclosed by a membrane. Most prokaryotic cells have only a single chromosome, but many also contain one or more small circles of DNA distinct from that of the chromosome, called **plasmids** (Figure 1.4*a*). Plasmids typically contain genes that are not essential but often confer some special property on the cell (such as a unique metabolism, or antibiotic resistance). The genomes of *Bacteria* and *Archaea* are typically small and compact, and most contain between 500 and 10,000 genes encoded by 0.5 to 10 million base pairs of DNA. Eukaryotic cells typically have much larger and much less streamlined genomes than prokaryotic cells. A human cell, for example, contains approximately 3 billion base pairs, which encode about 20,000–25,000 genes.

Mastering Microbiology
Art Activity: Figure 1.4 Basic properties of microbial cells

Activities of Microbial Cells

To be competitive in nature, a microorganism must survive and reproduce. **Figure 1.5** considers structure and some of the activities that are performed by cells to drive survival and reproduction. All cells show some form of **metabolism** through which nutrients are acquired from the environment and transformed into new cellular materials and waste products. During these transformations, energy is used to support synthesis of new structures. Production of these new structures culminates in the division of the cell to form two cells. Microbial growth results from successive rounds of cell division.

Genes contain information that is used by the cell to perform the work of metabolism. Genes are decoded to form proteins that regulate cellular processes. **Enzymes**, those proteins that have catalytic activity, carry out reactions that supply energy and perform biosynthesis within the cell. Enzymes and other proteins are synthesized during *gene expression* in the sequential processes of transcription and translation. **Transcription** is the process by which the information encoded in DNA sequences is copied into an RNA molecule, and **translation** is the process whereby the information in an RNA molecule is used by a ribosome to synthesize a protein (Chapter 6). Gene expression and enzyme activity in a microbial cell are coordinated and highly regulated to ensure that the cell remains optimally tuned to its surroundings. Ultimately, microbial growth requires replication of the genome through the process of **DNA replication**, followed by cell division. All cells carry out the processes of transcription, translation, and DNA replication.

Microorganisms have the ability to sense and respond to changes in their local environment. Many microbial cells are capable of **motility**, typically by self-propulsion (Figure 1.5). Motility allows cells to relocate in response to environmental conditions. Some microbial cells undergo **differentiation**, which may result

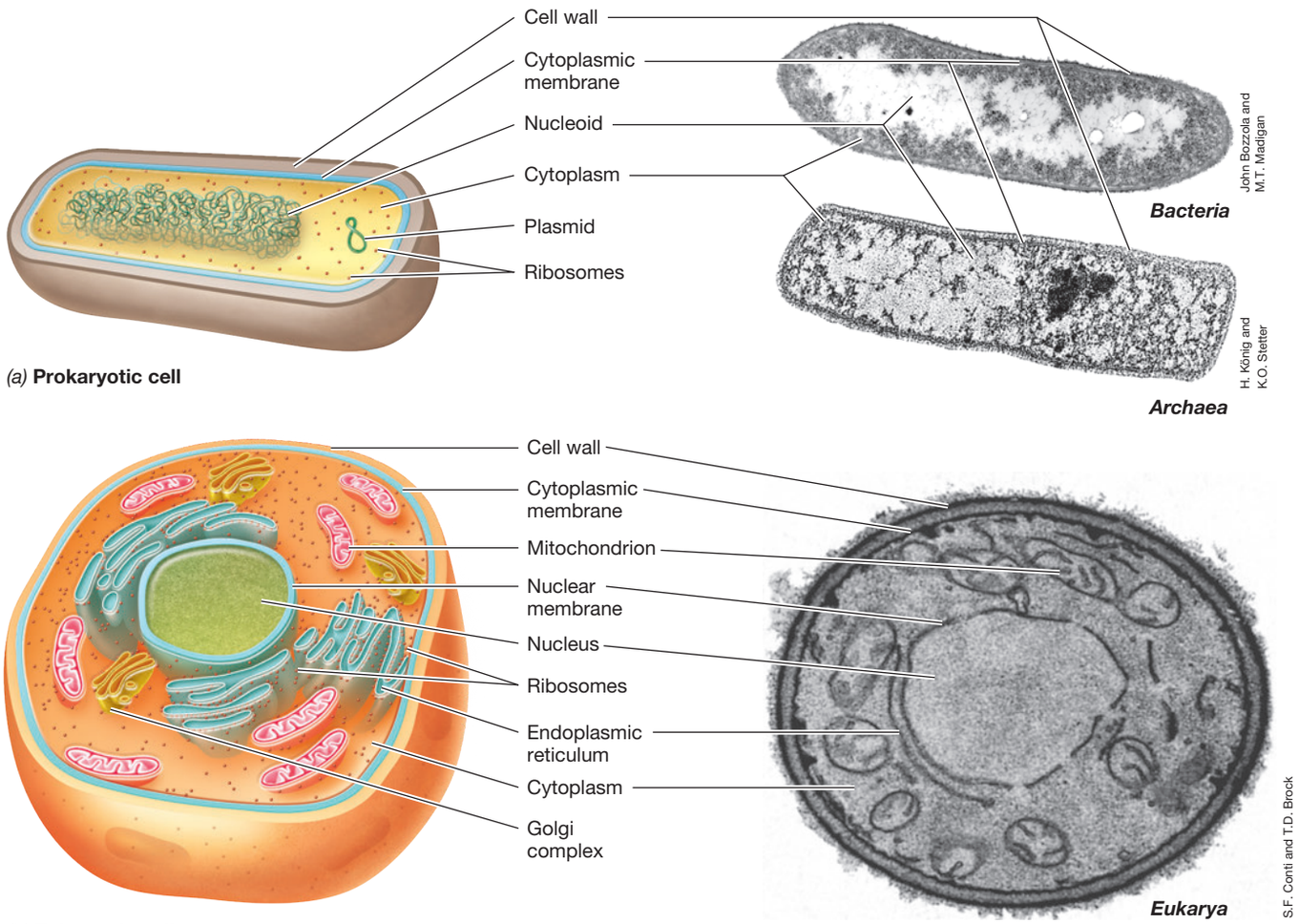


Figure 1.4 **Microbial cell structure.** *(a)* (Left) Diagram of a prokaryotic cell. (Right) Electron micrograph of *Heliobacterium modesticaldum* (*Bacteria*, cell is about 1 μm in diameter) and *Thermoproteus neutrophilus* (*Archaea*, cell is about 0.5 μm in diameter). *(b)* (Left) Diagram of a eukaryotic cell. (Right) Electron micrograph of a cell of *Saccharomyces cerevisiae* (*Eukarya*, cell is about 8 μm in diameter). In terms of relative scale, the bacterial cell in *a* is about the same size as the mitochondria of *Saccharomyces* in *b*.

in the formation of modified cells specialized for growth, dispersal, or survival. Cells respond to chemical signals in their environment, including those produced by other cells of either the same or different species, and these signals often trigger new cellular activities. Microbial cells thus exhibit **intercellular communication**; that is, they are "aware" of their neighbors and can respond accordingly. Many prokaryotic cells can also exchange genes with neighboring cells, regardless of their species, in the process of **horizontal gene transfer**.

Evolution (Figure 1.5) results when genes in a population of cells change in sequence and frequency over time, leading to descent with modification. The evolution of microorganisms can be very rapid relative to the evolution of plants and animals. For example, the indiscriminate use of antibiotics in human and veterinary medicine has selected for the proliferation of antibiotic resistance in pathogenic bacteria. The rapid pace of microbial evolution can be attributed in part to the ability of microorganisms to grow very quickly and to acquire new genes though the process of horizontal gene transfer. Not all of the processes depicted in Figure 1.5 occur in all cells. Metabolism, growth, and evolution, however, are universal and will be major areas of emphasis throughout this text.

We now move on to consider the diversity of cell shapes and sizes found in the microbial world.

Check Your Understanding

- What structures are universal to all type of cells?
- What processes are universal to all types of cells?
- What structures can be used to distinguish between prokaryotic cells and eukaryotic cells?

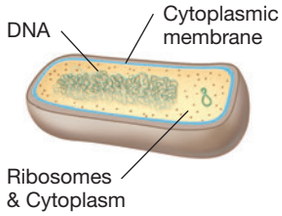
1.3 Cell Size and Morphology

Microscopic examination of microorganisms immediately reveals their **morphology**, which is defined by cell size and shape. A variety of cell shapes pervade the microbial world, and although microscopic by their very nature, microbial cells come in a variety of sizes.

Properties of *all* cells:

Structure

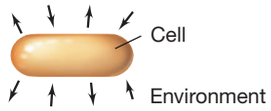
All cells have a cytoplasmic membrane, cytoplasm, a genome made of DNA, and ribosomes.



Metabolism

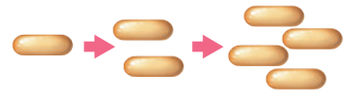
All cells use information encoded in DNA to make RNA and protein. All cells take up nutrients, transform them, conserve energy, and expel wastes.

1. **Catabolism** (transforming molecules to produce energy and building blocks)
2. **Anabolism** (synthesizing macromolecules)



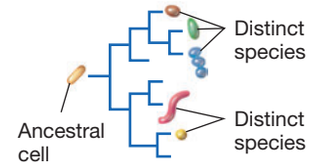
Growth

Information from DNA is converted into proteins, which do work. Proteins are used to convert nutrients from the environment into new cells.



Evolution

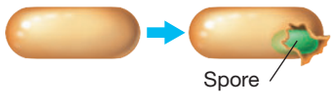
Chance mutations in DNA cause new cells to have new properties, thereby promoting evolution. Phylogenetic trees built from DNA sequences capture evolutionary relationships between species.



Properties of *some* cells:

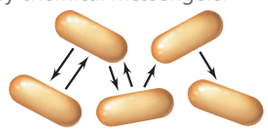
Differentiation

Some cells can form new cell structures such as a spore.



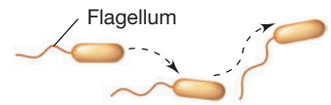
Communication

Cells interact with each other by chemical messengers.



Motility

Some cells are capable of self-propulsion.



Horizontal gene transfer

Cells can exchange genes by several mechanisms.

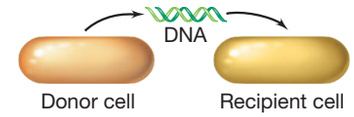


Figure 1.5 The properties of microbial cells. While cells are tremendously diverse in form and function, certain properties are shared by all cells.

Cell shape can be useful for distinguishing different microbial cells and often has ecological significance. Moreover, the very small size of most microbial cells has a profound effect on their ecology and dictates many aspects of their biology. We begin by considering cell size and then consider cell shape.

The Small World

A micrometer (μm or micron) is one-millionth of a meter in length. The unaided human eye has difficulty resolving objects that are less than 100 μm in diameter, but this is the scale of the microbial world. Most prokaryotic cells are small, ranging between 0.5 and 10 μm in length, but prokaryotic cells can vary widely in size. For example, the smallest prokaryotic cells are about 0.2 μm in diameter and the largest can be more than 600 μm long (**Table 1.1**). In contrast, most eukaryotic cells are larger on average than prokaryotic cells, being between 5 and 100 μm in length, but eukaryotic cells can vary widely in size too. For example, the smallest eukaryotic microorganism known is about 0.8 μm in diameter and the largest eukaryotic cells can be many centimeters in length (Section 1.4).

Cell size is influenced fundamentally by cell structure. Eukaryotic cells, owing to their complex intracellular structure and organelles (Figure 1.4), can actively transport molecules and macromolecules within the cytoplasm. Prokaryotic cells, in contrast, rely on diffusion for transport through the cytoplasm and this limits their size. While diffusion is very fast at small distances, the rate of diffusion increases as the square of the distance traveled. Hence, the metabolic rate in a prokaryotic cell varies inversely with the square of its size. This relationship means that, as cell size increases, it becomes advantageous to have cellular structures that facilitate transport and compartmentalize cellular activities as seen in eukaryotic cells. In contrast, since diffusion is rapid at small spatial scales, high metabolic rates can be maintained in small prokaryotic cells without a need for complex cellular structures.

It is possible, though unusual, for prokaryotic cells to be visible to the human eye; the largest are more than 600 μm (0.6 mm) long. To achieve this size, these bacteria must have traits that allow them to overcome diffusional limitation. The bacterium *Epulopiscium fishelsoni* (**Figure 1.6*a***; Figure 1.9), which is found in the gut of the surgeonfish, can be more than 75 μm wide and 600 μm long (Table 1.1). One of the traits that allows this bacterium to get so large is that it can have more than 10,000 copies of its genome distributed throughout its cytoplasm, thereby preventing diffusional limitation between the genome and any region of the cytoplasm. Cells of the largest known bacterium, the sulfur-oxidizing chemolithotroph *Thiomargarita* (Figure 1.6*b*, Table 1.1), are even larger than those of *Epulopiscium*, about 750 μm in diameter. *Thiomargarita* achieves this enormous size by having a large vacuole that fills the center of the cell. Hence, the cytoplasm of *Thiomargarita* occurs as a thin layer squeezed between the cytoplasmic membrane and this central vacuole. In this way, the cytoplasm is never more than 1 μm from the membrane. In addition, *Thiomargarita*, like *Epulopiscium*, also has many copies of its genome, which are distributed throughout its cytoplasm.

TABLE 1.1 Cell size and volume of some cells of *Bacteria*, from the largest to the smallest

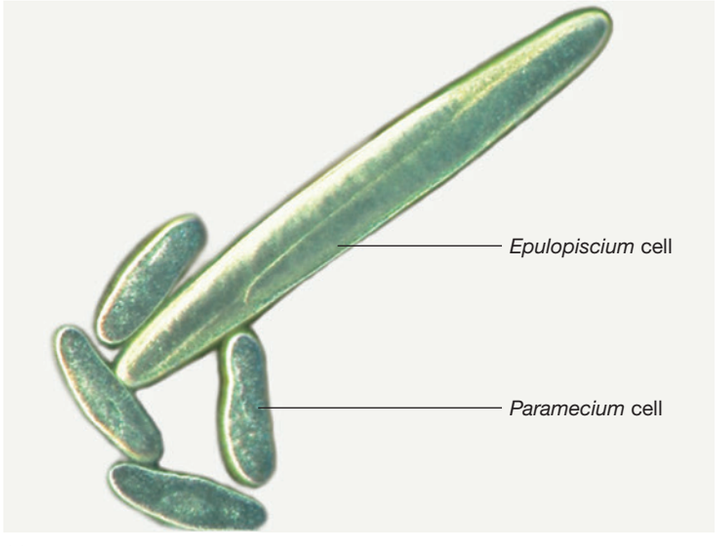
Organism	Characteristics	Morphology	Size[a] (μm^3)	Cell volume (μm^3)	Volumes compared to *E. coli*
Thiomargarita namibiensis	Sulfur chemolithotroph	Cocci in chains	750	200,000,000	100,000,000×
Epulopiscium fishelsoni[a]	Chemoorganotroph	Rods with tapered ends	80 × 600	3,000,000	1,500,000×
Beggiatoa species[a]	Sulfur chemolithotroph	Filaments	50 × 160	1,000,000	500,000×
Achromatium oxaliferum	Sulfur chemolithotroph	Cocci	35 × 95	80,000	40,000×
Lyngbya majuscula	Cyanobacterium	Filaments	8 × 80	40,000	20,000×
Thiovulum majus	Sulfur chemolithotroph	Cocci	18	3,000	1,500×
Staphylothermus marinus[a]	Hyperthermophile	Cocci in irregular clusters	15	1,800	900×
Magnetobacterium bavaricum	Magnetotactic bacterium	Rods	2 × 10	30	15×
Escherichia coli	Chemoorganotroph	Rods	1 × 2	2	1×
Pelagibacter ubique[a]	Marine chemoorganotroph	Rods	0.2 × 0.5	0.014	0.007×
Ultra-small bacteria[a]	Uncultured, from groundwater	Variable	<0.2	0.009	0.0045×
Mycoplasma pneumoniae	Pathogenic bacterium	Pleomorphic[b]	0.2	0.005	0.0025×

[a]Where only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200 μm in diameter. But on occasion, giant cells of 750 μm are observed. Likewise, an average cell of *S. marinus* is about 1 μm in diameter. The species of *Beggiatoa* here is unclear, and *E. fishelsoni*, *M. bavaricum*, and *P. ubique* are not formally recognized names in taxonomy. For more on ultra-small bacteria, see Explore the Microbial World "Tiny Cells."
[b]*Mycoplasma* is a bacterium that lacks a cell wall and can thus take on many shapes (*pleomorphic* means "many shapes").
Source: Data obtained from Schulz, H.N., and B.B. Jørgensen. 2001. *Annu. Rev. Microbiol. 55*: 105–137, and Luef, B., et al. 2015. *Nat. Commun.* doi:10.1038/ncomms7372.

At the opposite end of the spectrum from these large prokaryotic cells are very small prokaryotic cells. Exactly how small a cell can be is not precisely known. However, cells 0.2 μm in diameter exist (see Explore the Microbial World, "Tiny Cells"), and the lower limit is probably only a bit smaller than this. Ultimately, the lower limit to cell size is likely a function of the amount of space needed to house the essential biochemical components—proteins, nucleic acids, ribosomes and so on (Section 1.2)—that all cells need to survive and reproduce.

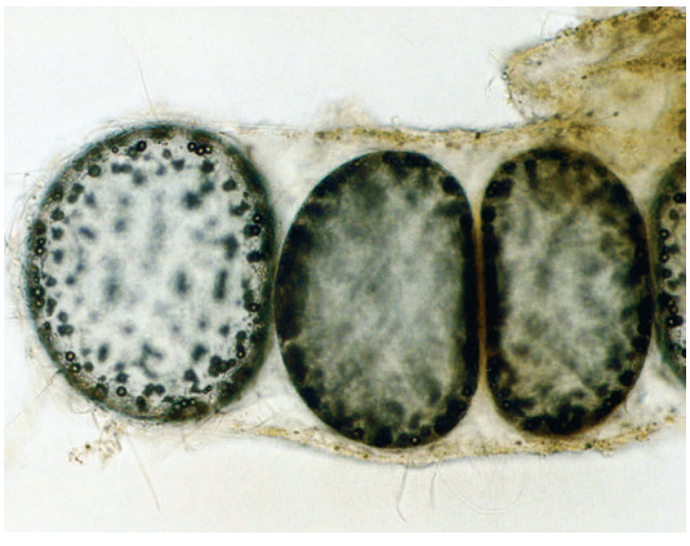
Surface-to-Volume Ratios, Growth Rates, and Evolution

For a cell, there are advantages to being small. Small cells have more surface area relative to cell volume and thus have a higher *surface-to-volume ratio* than larger cells. To understand this principle, consider a spherical cell. The volume of a sphere is a function of the cube of its radius ($V = \frac{4}{3}\pi r^3$), whereas its surface area is a function of the square of the radius ($S = 4\pi r^2$). Therefore, the S/V ratio of a coccus is $3/r$ (**Figure 1.7**). As cell size *increases*, its S/V ratio *decreases*.



Esther R. Angert, Harvard University

(a)



Heide Schulz-Vogt

(b)

Figure 1.6 Two very large *Bacteria*. *(a) Epulopiscium fishelsoni.* The rod-shaped cell is about 600 μm (0.6 mm) long and 75 μm wide and is shown with four cells of the protist *Paramecium* (a microbial eukaryote), each of which is about 150 μm long. *(b) Thiomargarita namibiensis*, a large sulfur chemolithotroph and currently the largest known of all prokaryotic cells. Cell widths vary from 400 to 750 μm.

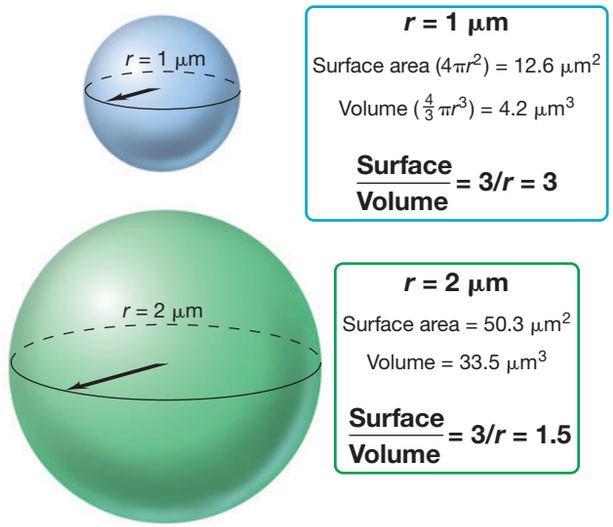


Figure 1.7 Surface area and volume relationships in cells. As a cell increases in size, its *S*/*V* ratio decreases.

To illustrate this, consider the *S*/*V* ratio for some of the cells of different sizes listed in Table 1.1: *Pelagibacter ubique*, 22; *Escherichia coli*, 4.5; and *E. fishelsoni* (Figure 1.6*a*), 0.05. The *S*/*V* of a rod-shaped organism can be estimated as if it were a cylinder; hence, the *S*/*V* of the cell will *decrease* as its radius *increases*.

The *S*/*V* ratio of a cell controls many of its properties, including how fast it grows (its *growth rate*) and shape. Cellular growth rate depends in part on the rate at which cells exchange nutrients and waste products with their environment. As cell size decreases, the *S*/*V* ratio of the cell increases, and this means that small cells can exchange nutrients and wastes more rapidly (per unit cell volume) than can large cells. As a result, free-living cells that are smaller tend to be more efficient than those that are larger, and any given mass of nutrients will support the synthesis of more small cells than large cells. We will see that cell morphology is also often predicated on the effect of cell shape on *S*/*V* ratio. For example, cell shapes that increase the overall membrane area of the cell, such as those having long thin appendages or invaginations, allow bacteria to increase their *S*/*V* ratio for a given mass of cytoplasm. We will see that prokaryotic cell morphology is remarkably diverse and different cell shapes can convey different benefits upon the cell.

Major Morphologies of Prokaryotic Cells

Common morphologies of prokaryotic cells are shown in **Figure 1.8**. A cell that is spherical or ovoid in morphology is called a *coccus* (plural, cocci). A cylindrically shaped cell is called a *rod* or a *bacillus* (plural, bacilli). A spiral-shaped cell is called a *spirillum* (plural, spirilla). A cell that is slightly curved and comma-shaped is called a *vibrio*. A *spirochete* is a special kind of organism (▶ Section 15.17) that has a spiral shape but which differs from spirilla because the cells of spirochetes are flexible, whereas cells of spirilla are rigid. Some bacteria are irregular in shape. Appendages, such as stalks and hyphae, are used by some cells for attachment or to increase surface area. In addition, asymmetrical cell division such as budding can result in irregular and asymmetrical cell shapes.

Cell division has a major impact on morphology because cells that remain attached to each other can form distinctive shapes. For instance, some cocci occur in pairs (diplococci), some form long chains (streptococci), others occur in three-dimensional cubes (tetrads or sarcinae), and still others occur in grapelike clusters (staphylococci). Filamentous bacteria are long, thin, rod-shaped bacteria that divide terminally and then form long filaments composed of many cells attached end to end.

The cell morphologies described here are representative but certainly not exhaustive; many variations of these morphologies are known. For example, there can be fat rods, thin rods, short rods, and long rods, rods that occur as single cells, as pairs of cells, or rods that

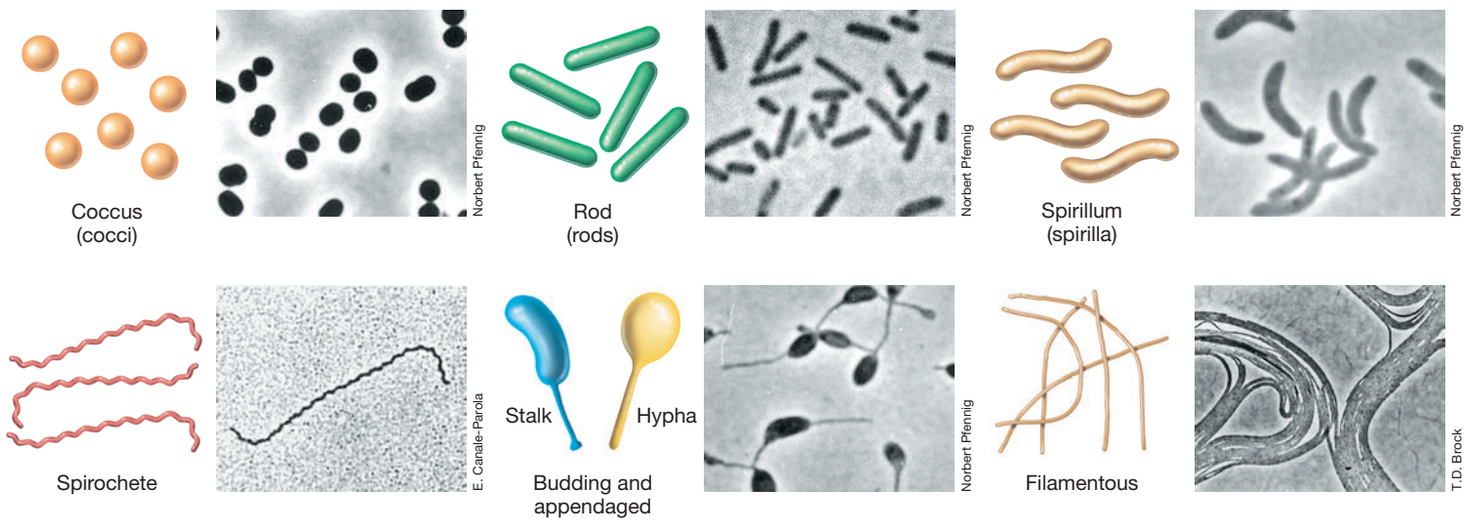


Figure 1.8 Cell morphologies. Beside each drawing is a phase-contrast photomicrograph of cells showing that morphology. Coccus (cell diameter in photomicrograph, 1.5 μm); rod (1 μm); spirillum (1 μm); spirochete (0.25 μm); budding (1.2 μm); filamentous (0.8 μm). All photomicrographs are of species of *Bacteria*. Not all of these morphologies are known among the *Archaea*, but cocci, rods, and spirilla are common.

Tiny Cells

Viruses are very small microbes and range in diameter from as small as 20 nm to almost 750 nm. Although no cells exist that are as small as most viruses, the recent discovery of ultra-small bacterial cells[1,2] has pushed the lower limits of cell size to what microbiologists feel must be very close to the minimal value. And, because microbiologists today can deduce amazing amounts of information about cells in nature without culturing them, the lack of laboratory cultures of these tiny cells has been only a minor impediment to understanding their biology in detail.

Microbiologists collected groundwater, which travels through Earth's deep subsurface, from a Colorado (USA) aquifer (**Figure 1**) and passed it through a membrane filter whose pores were only 0.2 μm in diameter. The liquid that passed through the filter was then subjected to microbiological analyses. Surprisingly, since filters with 0.2-μm pores have been used for decades to remove bacterial cells from solutions to generate "sterile solutions," prokaryotic cells were present in the groundwater filtrate. In fact, a diverse array of *Bacteria* were present in the filtrate, revealing that the groundwater was inhabited by a microbial community of tiny cells[1] that microbiologists have come to call ultramicrobacteria.

Figure 1 **Sampling the anoxic groundwater aquifer that parallels the Colorado River near Rifle, Colorado.**

Electron cryotomography, a microscopic technique in which a specimen is examined at extremely cold temperatures without fixation (chemical treatment that can alter a cell's morphology, see Section 1.10), showed the groundwater ultramicrobacteria to consist primarily of oval-shaped cells about 0.2 μm in diameter (**Figure 2**). The volume of these cells was calculated to be about 1/200 that of a cell of the bacterium *Escherichia coli* (see Table 1.1) such that more than 200 of the small cells could fit into one *E. coli* cell! Each of the tiny cells contained about 50 ribosomes, which is also about 1/100 of the number present in a slowly growing (100-min generation time) cell of *E. coli*. The very small size of the groundwater ultramicrobacteria gives them an enormous surface-to-volume ratio, and it is hypothesized that this advantage benefits them in extracting resources from their nutrient-deficient habitat.

Despite the fact that the tiny groundwater bacteria have yet to be cultured in the laboratory, much is already known about them because their small genomes—less than 1 megabase (Mb) in size—were obtained and analyzed.[2] From a phylogenetic perspective, the different species detected were distantly related to major phyla of *Bacteria* known from environmental analyses of diverse environments but which have thus far defied laboratory culture. Further analyses showed that genes encoding the enzymes for several core metabolic pathways widely distributed among microorganisms were absent from the genomes of the groundwater ultramicrobacteria. This suggests a metabolically minimalist lifestyle for these tiny cells and a survival strategy of cross-feeding essential nutrients with neighboring species in their microbial community.

A strategy of obtaining nutrients from other organisms is one widely used in the microbial world. As we will see later in this book, many disease-causing (pathogenic or parasitic) bacteria have very small genomes that are missing many key genes otherwise necessary for a free-living lifestyle. However, the pathogenic or parasitic way of life of these microbes lets them "get away" with a minimal genomic complement because any essential molecules they are unable to biosynthesize are supplied by the host.

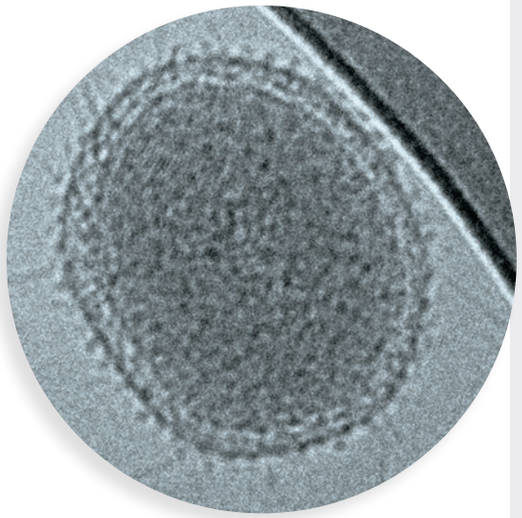


Figure 2 **A tiny bacterial cell from anoxic groundwater that passed through a filter with 0.2-μm pores.** The cell is not quite 0.2 μm in diameter.

Although we do not yet know exactly how small a microbial cell can be, microbiologists are closing in on this number from environmental analyses such as the Colorado groundwater study. From the same samples that yielded ultra-small *Bacteria* in this study, ultra-small *Archaea* were also detected and found to contain small and highly reduced genomes.[2]

It is thus likely that a large diversity of very small prokaryotic cells occurs in nature, and from the continued study of these tiny cells, more precise values for both the lower limits to cell size and the minimal genomic requirements for life should emerge. Moreover, theoretical considerations of cell size have shown that DNA and proteins dominate the volume of very small cells and that the theoretical lower limit to cell size agrees closely with the smallest bacteria observed in nature thus far.[3]

[1]Luef, B., et al. 2015. *Nat. Commun.* doi:10.1038/ncomms7372.
[2]Castelle, C.J., et al. 2015. *Curr. Biol. 25:* 1–12.
[3]Kempes, C.P., et al. 2016. *ISME J. 10:* 2145–2157.

form into filaments. As we will see, there are even square bacteria, hexagon-shaped bacteria, and star-shaped bacteria! Cell morphologies thus form a continuum, with some shapes, such as rods and cocci, being very common, whereas others, such as spiral, budding, and filamentous shapes, are less common.

Check Your Understanding

- What properties of the cell change as it gets smaller?
- Why is it that eukaryotic cells are typically larger than prokaryotic cells?
- What traits have allowed the bacteria *Epulopiscium* and *Thiomargarita* to have such large cells?

1.4 An Introduction to Microbial Life

As we have seen, microorganisms vary dramatically in size, shape, and structure. In this section we will learn more about different evolutionary (phylogenetic) lineages of cells. All cells fall into one of three major groups: *Bacteria*, *Archaea*, or *Eukarya*. These three major cell lineages are called **domains**, and all known cellular organisms belong to one of these three domains. In addition, while much of our focus in this chapter is on cellular forms of life, not all microbes form cells. In this section, we will also consider viruses, which are a group of microorganisms that lack a cellular structure. All known microorganisms can be classified into one of these four groups.

Bacteria

Bacteria have a prokaryotic cell structure (Figure 1.4*a*). *Bacteria* are often thought of as undifferentiated single cells with a length that ranges from 0.5 to 10 μm. While bacteria that fit this description are common, the *Bacteria* are actually tremendously diverse in appearance, size, and function (**Figure 1.9**). Although most bacteria are unicellular, some bacteria can differentiate to form multiple cell types and others are even multicellular (for example, *Magnetoglobus*, Figure 1.9).

Among the *Bacteria*, 30 major phylogenetic lineages (called *phyla*) have at least one species that has been grown in culture, though many more phyla exist which remain largely uncharacterized. Some of these phyla contain thousands of described species while others contain only a few. More than 90% of cultivated bacteria belong to one of only four phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*. The analyses of environmental DNA sequences provide evidence for the existence of at least 80 bacterial phyla (Section 1.15).

Archaea

Like *Bacteria*, *Archaea* also have a prokaryotic cell structure (Figure 1.4*a*). The domain *Archaea* consists of five described phyla: *Euryarchaeota*, *Crenarchaeota*, *Thaumarchaeota*, *Nanoarchaeota*, and *Korarchaeota*. *Archaea* have historically been associated with extreme environments; the first isolates came from hot, salty, or acidic sites. But not all *Archaea* are extremophiles. *Archaea* are indeed common in

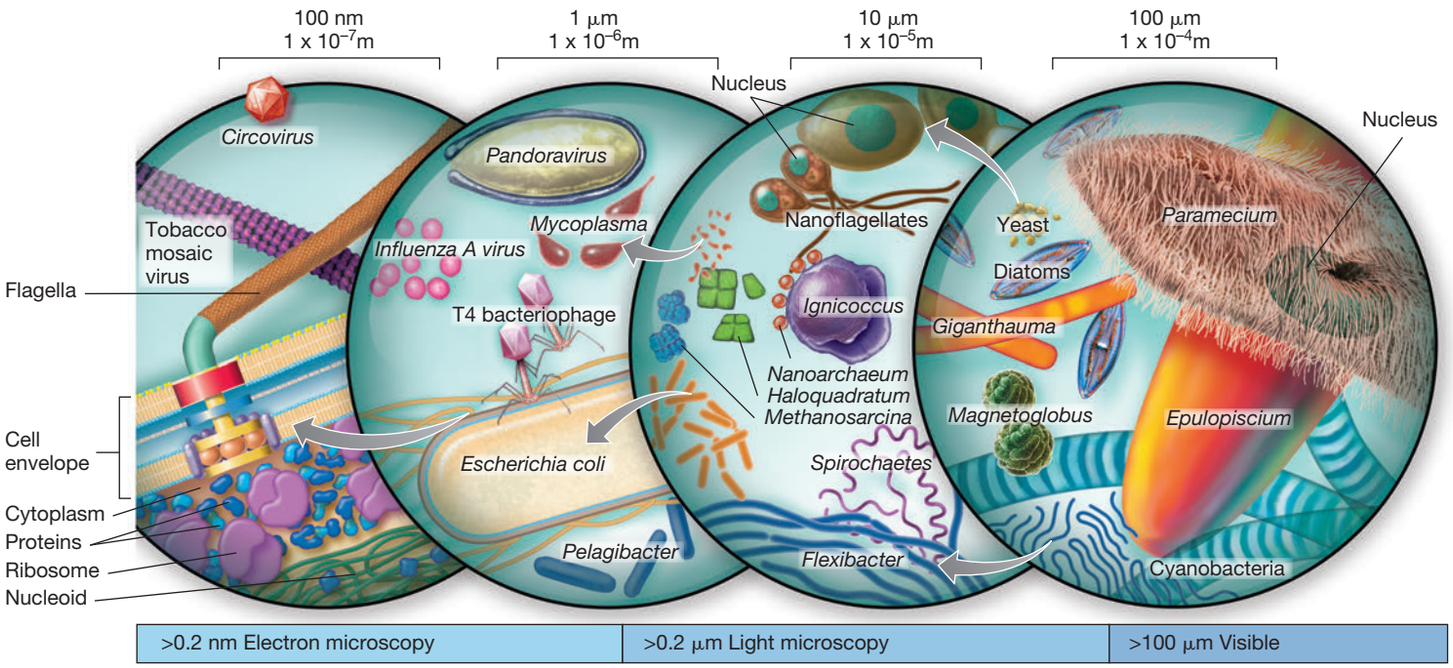


Figure 1.9 Microorganisms vary greatly in size and shape. The smallest known microbe is the circovirus (20 nm) and the largest shown here is the bacterium *Epulopiscium* (700 μm), which represents a 35,000-fold difference in length! Certain protozoa can be even larger than *Epulopiscium* (>2 mm long) and are visible to the unaided eye. Included in the figure are *Eukarya*: *Paramecium* (300 μm × 85 μm), diatoms (*Navicula*, 50 μm × 12 μm), yeast (*Saccharomyces*, 5 μm), and nanoflagellates (*Cafeteria*, 2 μm); *Bacteria*: *Epulopiscium* (700 μm × 80 μm), cyanobacteria (*Oscillatoria*, 10-μm-diameter multicellular filaments), *Magnetoglobus* (multicellular aggregate, 20 μm diameter), *Spirochaetes* (2–10 μm × 0.25 μm), *Flexibacter* (5–100 μm × 0.5 μm filaments), *Escherichia coli* (2 μm × 0.5 μm), *Pelagibacter* (0.4 μm × 0.15 μm), and *Mycoplasma* (0.2 μm); *Archaea*: *Giganthauma* (10-μm-diameter multicellular filament), *Ignicoccus* (6 μm), *Nanoarchaeum* (0.4 μm), *Haloquadratum* (2 μm), *Methanosarcina* (2 μm per cell in packet); and viruses: *Pandoravirus* (1 μm × 0.4 μm), T4 bacteriophage (200 nm × 90 nm), *Influenza A virus* (100 nm), *Tobacco mosaic virus* (300 nm × 20 nm), *Circovirus* (20 nm).

the most extreme environments that support life, such as those associated with volcanic systems, and species of *Archaea* hold many of the records that define the chemical and physical limits of life as we know them. However, in addition to these, *Archaea* are found widely in nature in nonextreme environments. For example, methane-producing *Archaea* (methanogens) are common in wetlands and in the guts of animals (including humans) and have a major impact on the greenhouse gas composition of our atmosphere. In addition, species of *Thaumarchaeota* inhabit soils and oceans worldwide and are important contributors to the global nitrogen cycle.

Archaea are also notable in that this domain lacks any known disease-causing (pathogenic or parasitic) species of plants or animals. Most described species of *Archaea* fall within the phyla *Crenarchaeota* and *Euryarchaeota* while only a handful of species have been described for the *Nanoarchaeota*, *Korarchaeota*, and *Thaumarchaeota*. Analysis of environmental DNA sequences indicate more than 12 archaeal phyla likely exist. We discuss *Archaea* in detail in Chapter 17.

Eukarya

Plants, animals, and fungi are the most well-known groups of *Eukarya*. These groups are phylogenetically relatively young compared with *Bacteria* and *Archaea*, originating during an evolutionary burst called the *Cambrian explosion*, which began about 600 million years ago. The first eukaryotes, however, were unicellular microbes. Microbial eukaryotes, which include diverse algae and protozoa, may have first appeared as early as 2 billion years ago, well before the origin of plants, animals, and fungi (Section 1.5). The major lineages of *Eukarya* are traditionally called *kingdoms* instead of phyla. There are at least six kingdoms of *Eukarya*, and this diverse domain contains microorganisms as well as the plants and animals.

Microbial eukaryotes vary dramatically in size, shape, and physiology (Figure 1.9). Among the smallest are the nanoflagellates, which are microbial predators that can be as small as 2 μm long. In addition, *Ostreococcus*, a genus of green algae that contains species whose cells are only 0.8 μm in diameter, are smaller than many bacteria. The largest single-celled organisms are eukaryotes, but they are hardly microbial. Xenophyophores are amoeba-like, single-celled organisms that live exclusively in the deep oceans and can be up to 10 *centimeters* in length. In addition, plasmodial slime molds consisting of a single cytoplasmic compartment can be up to 30 cm in diameter. In Chapter 18 we consider microbial eukaryotes in detail.

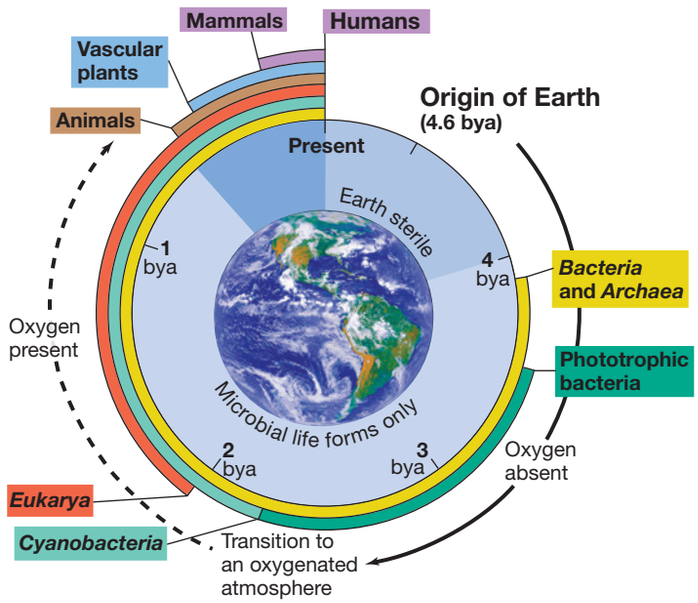
Viruses

Viruses are not found on the tree of life, and for a variety of reasons, it can be argued that they are not truly alive. Although viruses can replicate—a hallmark of cells—viruses are obligate parasites that can only replicate within the cytoplasm of a host cell. Viruses are not cells, and they lack the cytoplasmic membrane, cytoplasm, and ribosomes found in all forms of cellular life. Viruses do not carry out metabolic processes; instead, they take over the metabolic systems of infected cells and turn them into vessels for producing more viruses. Unlike cells, which all have genomes composed of double-stranded DNA, viruses have genomes composed of DNA *or* RNA that can be either double- or single-stranded. Viral genomes are often quite small, with the smallest having only three genes. The small size of most viral genomes means that no genes are conserved among all viruses, or between all viruses and all cells.

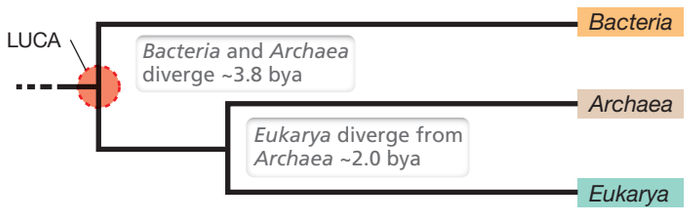
Although they are not cells, viruses are as diverse as the cells they infect, and different viruses are known to infect cells from all three domains of life. Viruses are often classified on the basis of their structure, genome composition, and host specificity. Viruses that infect bacteria are called *bacteriophages* (or *phages*, for short). Bacteriophages have been used as model systems to explore many aspects of viral biology. While most viruses are considerably smaller than bacterial cells (Figure 1.9), there are also unusually large viruses such as the Pandoraviruses, which can be more than 1 micrometer long and have a genome that contains as many as 2500 genes, larger than that of many bacteria! We will learn much more about viruses in Chapters 5 and 11.

Check Your Understanding

- How are viruses different from *Bacteria*, *Archaea*, and *Eukarya*?
- What four bacterial phyla contain the largest number of well-characterized species?
- What phylum of *Archaea* is common worldwide in soils and in the oceans?



(a)



(b)

Figure 1.10 A summary of life on Earth through time and origin of the cellular domains. *(a)* At its origin, Earth was sterile and anoxic. Cellular life, in the form of *Bacteria* and *Archaea*, was present on Earth by 3.8 billion years ago (bya). The evolution of phototrophic bacteria called *Cyanobacteria* caused Earth's atmosphere to become oxygenated over time. While the first evidence for oxygen in Earth's atmosphere appears 2.4 bya, current levels of atmospheric O_2 were not achieved until 500–800 million years ago. *(b)* The three domains of cellular organisms are *Bacteria*, *Archaea*, and *Eukarya*. *Bacteria* and *Archaea* appeared first and *Eukarya* evolved later, diverging from the *Archaea*. LUCA, last universal common ancestor.

1.5 Microorganisms and the Biosphere

Microbes are the oldest form of life on Earth, and they have evolved to perform critical functions that sustain the biosphere. In this section we will learn how microbes have changed our planet and how they continue to do so.

A Brief History of Life on Earth

Earth is about 4.6 billion years old, and microbial cells first appeared between 3.8 and 4.3 billion years ago (**Figure 1.10**). During the first 2 billion years of Earth's existence, its atmosphere was anoxic (O_2 was absent), and only nitrogen (N_2), carbon dioxide (CO_2), and a few other gases were present. Only microorganisms capable of anaerobic metabolism (that is, metabolisms that do not require O_2) could survive under these conditions.

The evolution of phototrophic microorganisms—organisms that harvest energy from sunlight—occurred within 1 billion years of the formation of Earth (Figure 1.10*a*). The first phototrophs were anoxygenic (non-oxygen-producing), such as the purple sulfur bacteria and green sulfur bacteria we know today (**Figure 1.11**). *Cyanobacteria*—oxygen-producing (oxygenic) phototrophs (Figure 1.11*f*)—evolved nearly a billion years later (Figure 1.10*a*) and began the slow process of oxygenating Earth's atmosphere. These early phototrophs lived in structures called *microbial mats*, which are still found on Earth today (Figure 1.11*a–c*). After the oxygenation of Earth's atmosphere, multicellular life forms eventually evolved, culminating in the plants and animals we know today. But plants and animals have only existed for about half a billion years. The timeline of life on Earth (Figure 1.10*a*) shows that *80% of life's history was exclusively microbial*, and thus in many ways, Earth can be considered a microbial planet.

As evolutionary events unfolded, three major lineages of microbial cells—the *Bacteria*, the *Archaea*, and the *Eukarya* (Figure 1.10*b*)—were distinguished. All cellular organisms share certain characteristics (Figure 1.5) and as a result, certain genes are found in all cells. For example, approximately 60 genes are universally present in cells of all three domains. Examination of these genes reveals that all three domains have descended from a common ancestor, the *last universal common ancestor* (LUCA, Figure 1.10*b*). Over enormous periods of time, microorganisms derived from these three domains have evolved to fill every habitable environment on Earth.

Microbial Abundance and Activity in the Biosphere

Microorganisms are present everywhere on Earth that will support life. They constitute a major fraction of global biomass and are key reservoirs of nutrients essential for life. There are an estimated 2×10^{30} microbial cells on Earth. To put this number in context, the universe in all its vast extent is estimated to contain merely 7×10^{22} stars. The total amount of carbon present in all microbial cells is a significant fraction of Earth's biomass (**Figure 1.12**). Moreover, the total amount of nitrogen and phosphorus (essential nutrients for life) within microbial cells is almost four times that in all plant and animal cells combined. Microbes also represent a major fraction of the total DNA in the biosphere (about 31%), and their genetic diversity far exceeds that of plants and animals.

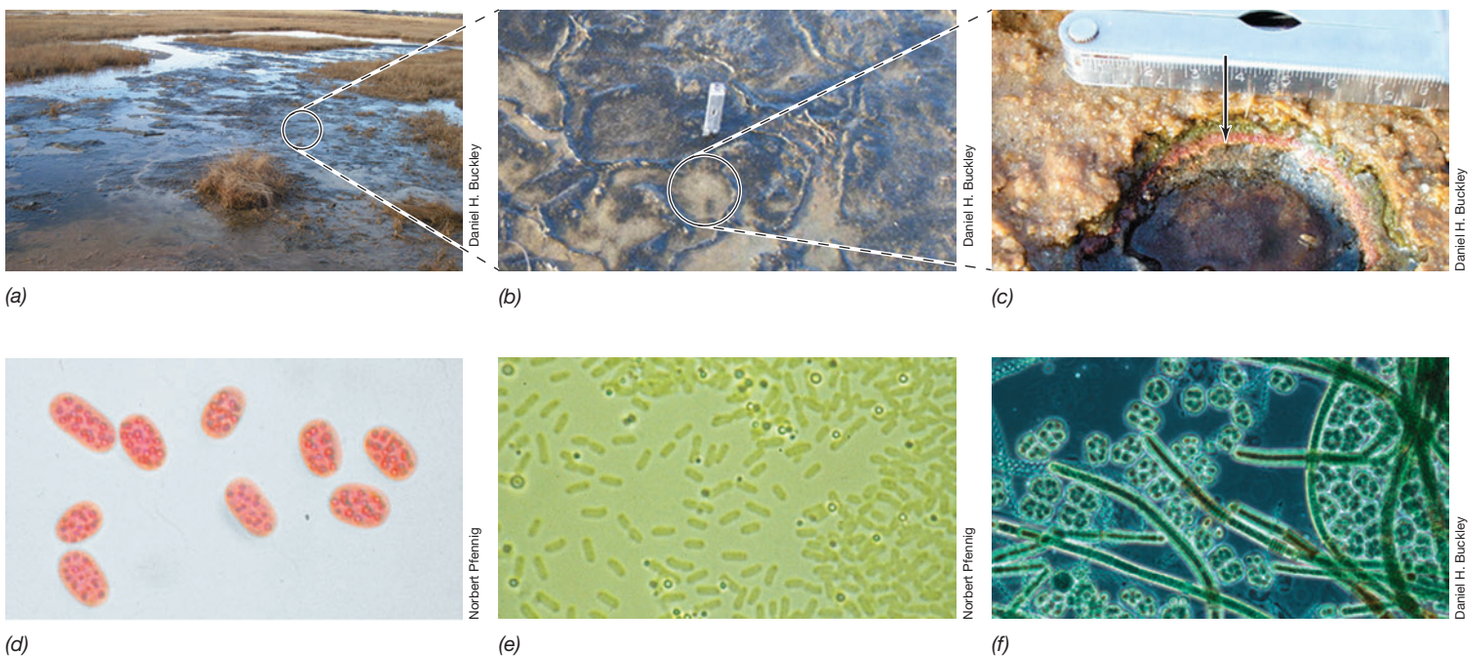


Figure 1.11 Phototrophic microorganisms. The earliest phototrophs lived in microbial mats. *(a)* Microbial mats in the Great Sippewissett Marsh, a salt marsh in Massachusetts, USA. *(b)* Mats develop a cohesive structure that forms at the sediment surface. *(c)* A slice through the mat shows colored layers that form due to the presence of photopigments. Cyanobacteria form the green layer nearest the surface, purple sulfur bacteria form the purple and yellow layers below, and green sulfur bacteria form the bottommost green layer. The scale on the knife is in cm. *(d)* Purple sulfur bacteria, *(e)* green sulfur bacteria, and *(f)* cyanobacteria imaged by bright-field and phase-contrast microscopy. Purple and green sulfur bacteria are anoxygenic phototrophs that appeared on Earth long before oxygenic phototrophs (that is, *Cyanobacteria*) evolved (see Figure 1.10*a*).

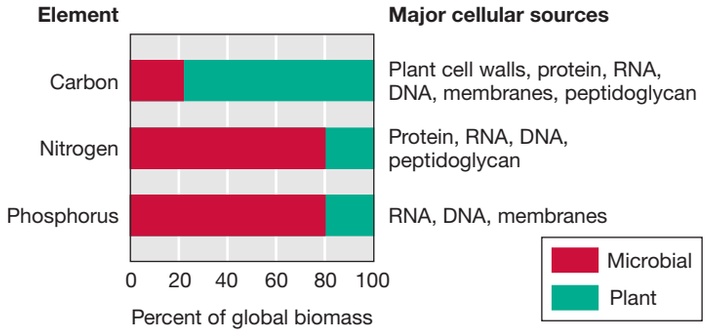


Figure 1.12 Contribution of microbial cells to global biomass. Microorganisms comprise a significant fraction of the carbon (C) and a majority of the nitrogen (N) and phosphorus (P) in the biomass of all organisms on Earth. C, N, and P are the macronutrients required in the greatest quantity by living organisms. Animal biomass is a minor fraction (<0.1%) of total global biomass and is not shown.

Microbes are even abundant in habitats that are much too harsh for other forms of life, such as volcanic hot springs, glaciers and ice-covered regions, high-salt environments, extremely acidic or alkaline habitats, and deep in the sea or deep in the earth at extremely high pressure. Such microorganisms are called **extremophiles** and their properties define the physiochemical limits to life as we know it (Table 1.2). We will revisit many of these organisms in later chapters and discover the special structural and biochemical properties that allow them to thrive under extreme conditions.

All ecosystems are influenced to one extent or another by microbial activities. The metabolic activities of microorganisms can change the habitats in which they live, both chemically and physically, and these changes can affect other organisms. For example, excess nutrients added to a habitat can cause aerobic (O_2-consuming) microorganisms to grow rapidly and consume O_2, rendering the habitat anoxic (O_2-free). Many human activities release nutrients into the coastal oceans, thereby stimulating excessive microbial growth, which can cause enormous anoxic zones in these waters. These "dead zones" cause massive mortality of fish and shellfish in coastal oceans worldwide, because most aquatic animals require O_2 and die if it is not available. Only by understanding microorganisms and microbiology can we predict and minimize the effects of human activity on the biosphere that sustains us.

Though diverse habitats are influenced strongly by microorganisms, their contributions are easy to overlook because of their small sizes. Within the human body, for example, more microbial cells can be present than human cells, and more than 200 microbial genes are present for every human gene. These microbes provide benefits and services that are essential to human health. In later chapters, we will return to a consideration of the ways in which microorganisms affect animals, plants, and the entire global ecosystem. This is the science of **microbial ecology**, perhaps the most exciting subdiscipline of microbiology today. We will see that microbes are important to myriad issues of global importance to humans including climate change, agricultural productivity, and even energy policy.

We focus now on the effects of microbes on humans and human activities.

Check Your Understanding

- How old is Earth and when did cells first appear on Earth?
- Name the three domains of life. Which of these contain eukaryotic life forms?
- Why were cyanobacteria so important in the evolution of life on Earth?

1.6 The Impact of Microorganisms on Human Society

Microbiologists have made great strides in discovering how microorganisms function, and application of this knowledge has greatly advanced human health and welfare. Besides understanding microorganisms as agents of disease, microbiology has made great

TABLE 1.2 Classes and examples of extremophiles[a]

Extreme	Descriptive term	Genus, species	Domain	Habitat	Minimum	Optimum	Maximum
Temperature							
High	Hyperthermophile	*Methanopyrus kandleri*	*Archaea*	Undersea hydrothermal vents	90°C	106°C	122°C[b]
Low	Psychrophile	*Psychromonas ingrahamii*	*Bacteria*	Sea ice	−12°C[c]	5°C	10°C
pH							
Low	Acidophile	*Picrophilus oshimae*	*Archaea*	Acidic hot springs	−0.06	0.7[d]	4
High	Alkaliphile	*Natronobacterium gregoryi*	*Archaea*	Soda lakes	8.5	10[e]	12
Pressure	Barophile (piezophile)	*Moritella yayanosii*	*Bacteria*	Deep ocean sediments	500 atm	700 atm[f]	>1000 atm
Salt (NaCl)	Halophile	*Halobacterium salinarum*	*Archaea*	Salterns	15%	25%	32% (saturation)

[a]The organisms listed are the current "record holders" for growth in laboratory culture at the extreme condition listed.
[b]Anaerobe showing growth at 122°C only under several atmospheres of pressure.
[c]The permafrost bacterium *Planococcus halocryophilus* can grow at −15°C and metabolize at −25°C. However, the organism grows optimally at 25°C and grows up to 37°C and thus is not a true psychrophile.
[d]*P. oshimae* is also a thermophile, growing optimally at 60°C.
[e]*N. gregoryi* is also an extreme halophile, growing optimally at 20% NaCl.
[f]*M. yayanosii* is also a psychrophile, growing optimally near 4°C.

advances in understanding the important roles microorganisms play in food and agriculture, and microbiologists have exploited microbial activities to produce valuable human products, generate energy, and clean up the environment.

Microorganisms as Agents of Disease

The statistics summarized in **Figure 1.13** show how microbiologists and clinical medicine have combined to conquer infectious diseases in the past 120 years. At the beginning of the twentieth century, more than half of all humans died from infectious diseases caused by bacterial and viral **pathogens**. Today, however, infectious diseases are largely preventable due to advances in our understanding of microbiology. Microbiology has fueled advances in medicine such as vaccination and antibiotic therapy, advances in engineering such as water and wastewater treatment, advances in food safety such as pasteurization, and a better understanding of how microorganisms are transmitted. Infectious diseases now cause fewer than 5% of all deaths in countries where these interventions, made possible by microbiology, are readily available. However, while infectious diseases are preventable, the World Health Organization has documented that they still account for more than a third of all deaths in countries where microbial interventions are less available, such as those having low-income economies. As we will see later in this chapter, the development of microbiology as a science can be traced to pioneering studies of infectious disease.

While pathogens and infectious disease remain a major threat to humanity, and combating these harmful organisms remains a major focus of microbiology, most microorganisms are not harmful to humans. In fact, most microorganisms are beneficial, and in many cases are even essential to human welfare and the functioning of the planet. We turn our attention to these microorganisms and microbial activities now.

Microorganisms, Agriculture, and Human Nutrition

Agriculture benefits from nutrient cycling performed by microorganisms, in particular, the cycling of nitrogen, sulfur, and carbon compounds. For example, legumes are a diverse family of plants that include major crop species such as soybeans, peas, and lentils, among others. Legumes live in close association with bacteria that form structures called *nodules* on their roots. In the nodules, these bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) through the process of *nitrogen fixation*. NH_3 is the major nutrient found in fertilizer and is used as a nitrogen source for plant growth (**Figure 1.14**). In this way bacteria allow legumes to make their own fertilizer, thereby reducing the need for farmers to apply fertilizers produced industrially. When plants die they are decomposed by bacteria in the soil, and this process produces the nutrients that form the basis of soil fertility. Bacteria regulate nutrient cycles (Figure 1.14), in soils and throughout the biosphere, transforming and recycling the nutrients required by plants and animals.

Also of major agricultural importance are microorganisms that inhabit the rumen of ruminant animals, such as cattle and sheep. Ruminants, like most animals, lack enzymes for breaking down the polysaccharide cellulose, the major component of plant cell walls. The digestive tract of ruminants has a large specialized chamber called the *rumen* in which cellulose is digested. The rumen contains a dense and diverse community of microorganisms that digest and ferment cellulose. Without these symbiotic microorganisms, ruminants could not digest plant matter like grass and hay, most of which consists of cellulose. Ruminants ultimately get their nutrition by metabolizing the waste products of microbial fermentation and by digesting dead microbial cells. Many domesticated and wild herbivorous mammals—including deer, bison, camels, giraffes, and goats—are also ruminants.

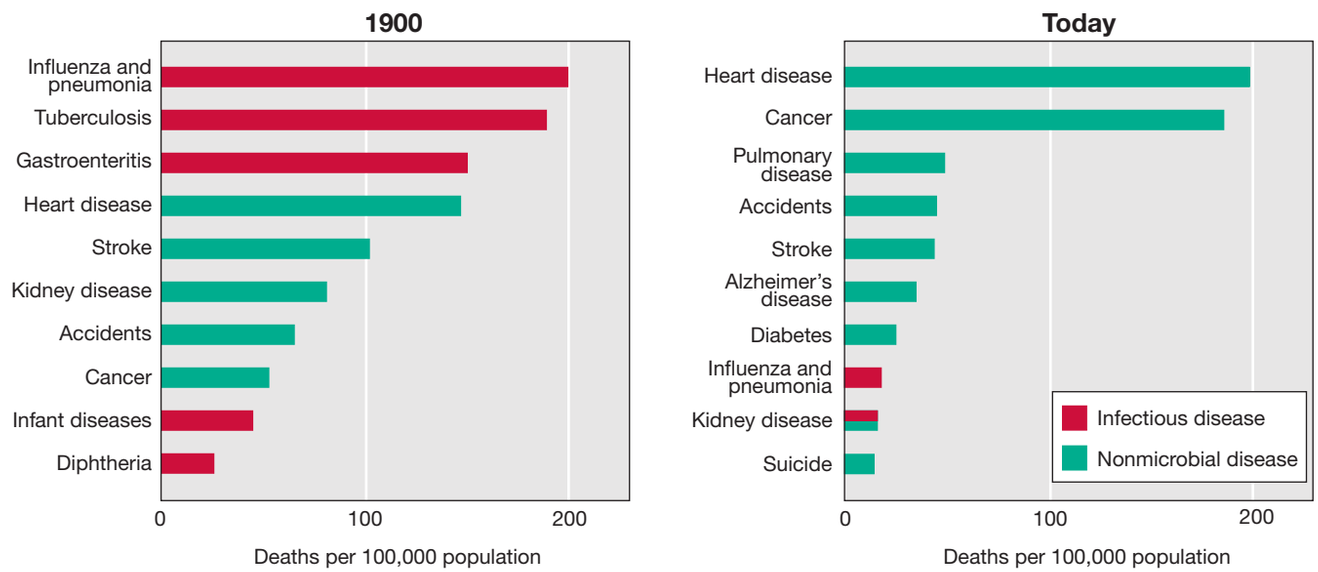


Figure 1.13 Death rates for the leading causes of death in the United States: 1900 and 2016. Infectious diseases were the leading causes of death in 1900, whereas today they account for relatively few deaths. Kidney diseases can be caused by microbial infections or systemic sources (diabetes, cancers, toxicities, metabolic diseases, etc.). Data are from the United States National Center for Health Statistics and the Centers for Disease Control and Prevention.

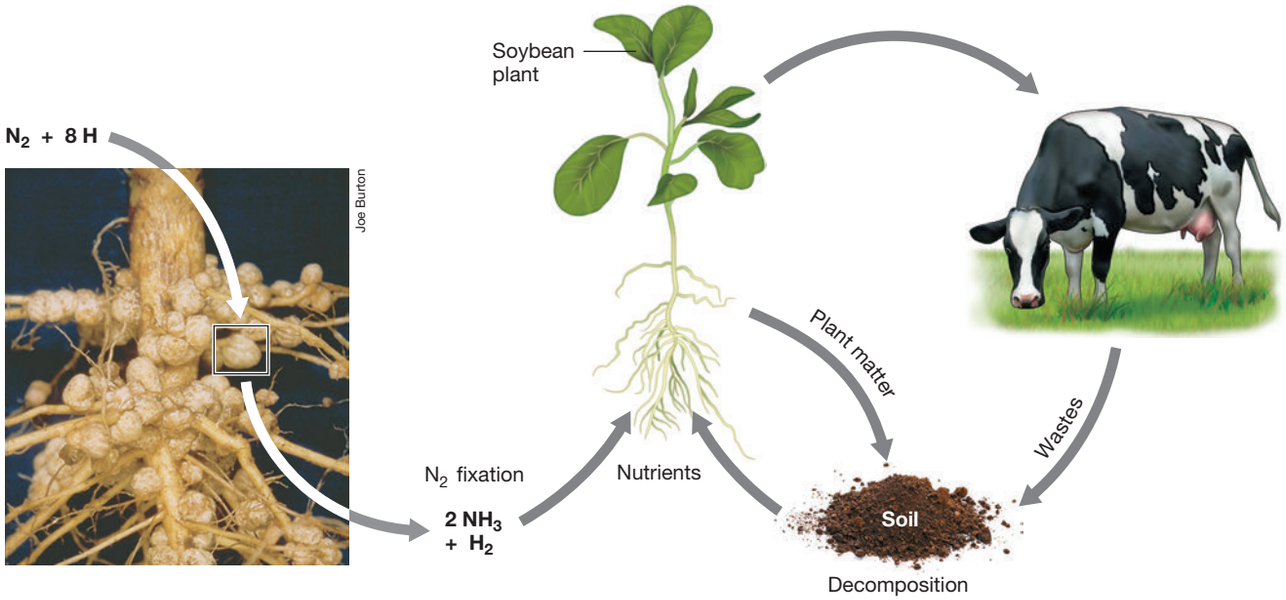


Figure 1.14 Microorganisms in modern agriculture. Root nodules on this soybean plant contain bacteria that fix atmospheric nitrogen (N_2) to form nitrogenous compounds used by the plant. Ruminant animals such as cows and sheep require rumen microbes to digest cellulose from plants. Plant matter and animal wastes are decomposed in soil to produce nutrients that are the basis of soil fertility and which are required for plant growth.

The human gastrointestinal (GI) tract lacks a rumen, but we too rely on microbial partners for our nutrition. Human enzymes lack the ability to break down complex carbohydrates (which can represent 10–30% of food energy) and so we rely on our **gut microbiome** for this purpose. The colon, or large intestine (**Figure 1.15**), follows the stomach and small intestine in the human digestive tract, and it contains about 10^{11} microbial cells per gram of colonic contents. Microbial cell numbers are low in the very acidic (pH 2) stomach (about 10^4 per gram) but increase to about 10^8 per gram near the end of the small intestine (pH 4–5) and then reach maximal numbers in the colon (pH 7) (Figure 1.15). The colon contains diverse microbial species that assist in the digestion of complex carbohydrates, and that synthesize vitamins and other nutrients essential to host nutrition. The gut microbiome develops from birth, but it can change over time with the human host. The composition of the gut microbiome has major effects on GI function and human health as we will see in Chapter 24.

Microorganisms and Food

Microbes are intimately associated with the foods we eat. Microbial growth in food can cause food spoilage and foodborne disease. The manner in which we harvest and store food (for example, canning, refrigeration, drying, salting, etc.), the ways in which we cook it, and even the spices we use, have all been fundamentally influenced by the goal of eliminating harmful organisms from our food. Microbial food safety and prevention of food spoilage is a major focus of the food industry and a major cause of economic loss every year.

While some microbes can cause foodborne disease and food spoilage, not all microorganisms in foods are harmful. Indeed, beneficial microbes have been used for thousands of years to improve food safety and to preserve foods (**Figure 1.16**). For example, cheeses, yogurt, and buttermilk are all produced by microbial fermentation of dairy products. Microbial production of lactic acid in these foods improves their shelf life and prevents the growth of foodborne pathogens. Lactic acid–producing bacteria are used to produce a variety of sour-tasting foods, including sauerkraut, kimchi, pickles, and even certain sausages. Even the production of chocolate and coffee rely on microbial fermentation. Moreover, the fermentative activities of yeast are essential for baking (by generating carbon dioxide—CO_2—to raise the dough), and for the production of alcoholic beverages (by generating alcohol). The products of microbial fermentation affect the flavor and taste of foods and can prevent spoilage as well as the growth of deleterious organisms.

Microorganisms and Industry

Microorganisms play important roles in all manner of human activity. The field of *industrial microbiology* is focused on the use of microorganisms as tools for major industries such as pharmaceuticals and brewing (**Figure 1.17**). For example, in large industrial settings, naturally occurring microorganisms are grown on a massive scale in bioreactors called *fermentors* to make large amounts of products, such as antibiotics, enzymes, alcohol, and certain other chemicals, at relatively low cost. By contrast, *biotechnology* employs genetically engineered microorganisms to synthesize products of high commercial value, such as insulin or other human proteins, usually on a small scale.

Microorganisms can also be used to produce *biofuels* (▶ Section 12.19 and Figure 12.33). For example, as previously discussed,

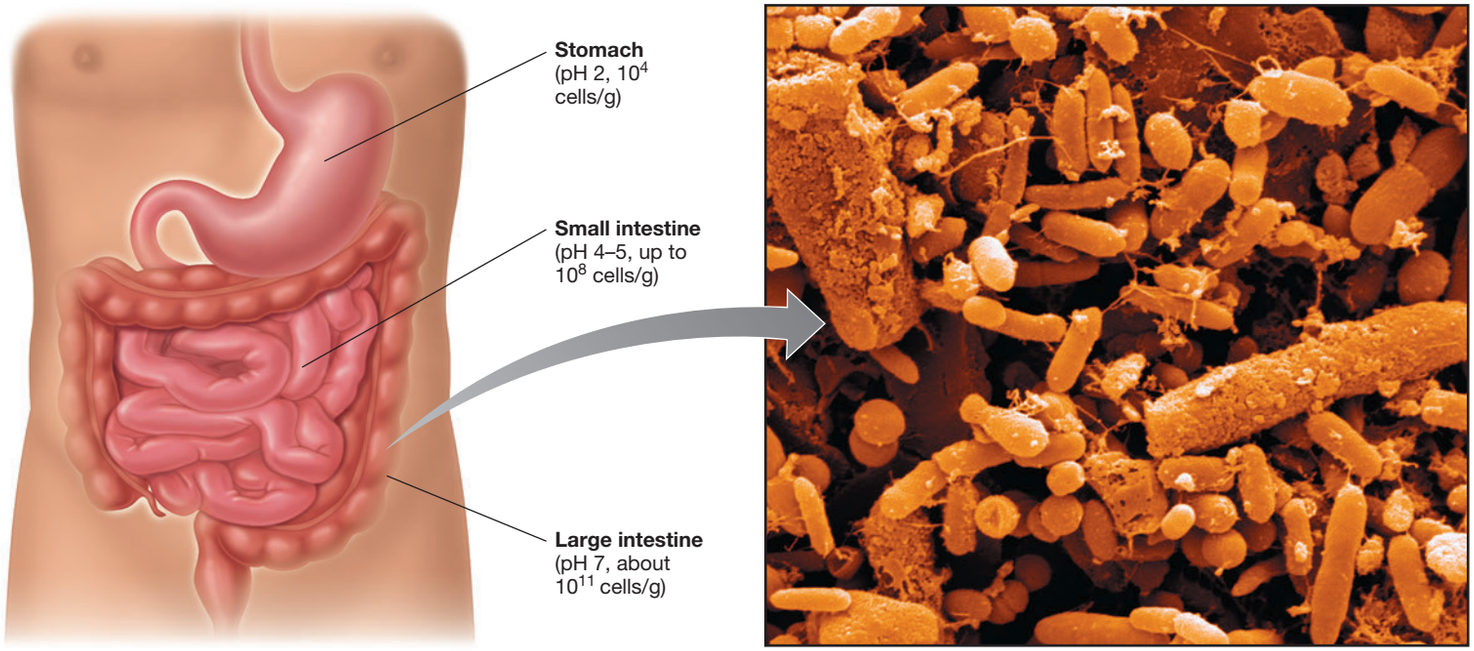


Figure 1.15 **The human gastrointestinal tract.** *(a)* Diagram of the human GI tract showing the major organs. *(b)* Scanning electron micrograph of microbial cells in the human colon (large intestine). Cell numbers in the colon can reach as high as 10^{11} per gram. As well as high *numbers* of cells, the microbial *diversity* in the colon is also quite high.

natural gas (methane, CH_4) is a product of the anaerobic metabolism of methanogenic *Archaea*. Ethyl alcohol (ethanol) is a major fuel supplement, which is produced by the microbial fermentation of glucose obtained from carbon-rich feedstocks such as sugarcane, corn, or rapidly growing grasses. Microorganisms can even convert waste materials, such as domestic refuse, animal wastes, and cellulose, into ethanol and methane. In producing these biofuels, humans are simply exploiting the metabolic features of particular microbes, but at the same time, are reducing the use of fossil fuels. As we will document in Chapter 21, CO_2 levels have been rising

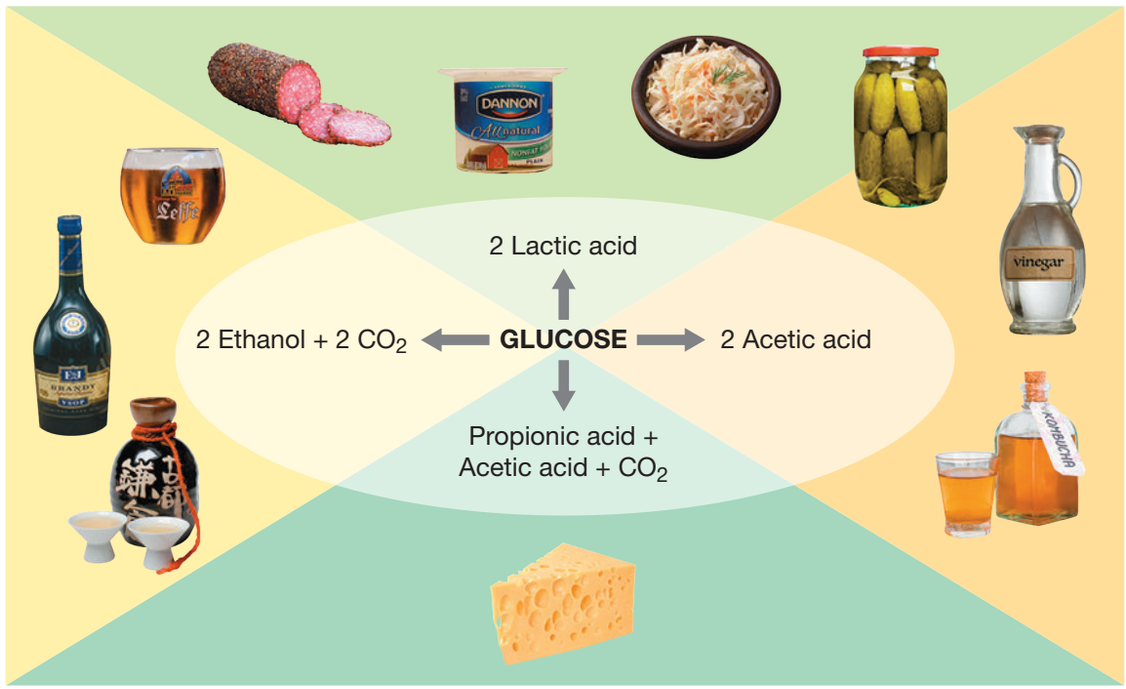


Figure 1.16 **Fermented foods.** Major fermentations in various fermented foods. It is the fermentation product (ethanol, or lactic, propionic, or acetic acids) that both preserves the food and renders in it a characteristic flavor.

Wastewater Treatment: Microbes are used to clean wastewater.

Bioremediation: Microbes are used to clean contaminated environments.

Biofilms: Microbes grow on surfaces and can foul pipes and pipelines.

Biotechnology: Microbes can be genetically modified to produce high-value products such as pharmaceuticals and enzymes.

Fermentation: Microbes are used at industrial scale to make chemicals, solvents, enzymes, and pharmaceuticals.

Biofuels: Microbes are used to convert biomass into ethanol and wastes into natural gas (methane).

Figure 1.17 Industrial microbiology. Microbes have major impacts on human industry. Microbes can be used to produce valuable products and biofuels and they can also be used to clean up our wastes. Microbial biofilms have major impacts on industry because biofilms can clog and corrode pipelines and holding tanks in factories, in ships, and in the oil industry.

rapidly on Earth in the industrial era, and the link between this "greenhouse gas" and Earth's rising temperatures is firm. Thus, as a sustainable fuel source, biofuels should help cool our planet and are one facet of the "green revolution" many countries support today.

Microorganisms are also used to clean up wastes. Wastewater treatment is essential to sanitation and human health. *Wastewater treatment* relies on microbes to treat water contaminated with human waste so that it can be reused or returned safely to the environment. Waterborne diseases such as cholera and typhoid (major killers before the blossoming of microbiology: see gastroenteritis, Figure 1.13) can proliferate in the absence of proper wastewater treatment. Microbes can also be used to clean up industrial pollution in a process called *bioremediation*. In bioremediation, microorganisms are used to transform spilled oil, solvents, pesticides, heavy metals, and other environmentally toxic pollutants into nontoxic forms. Bioremediation accelerates the cleanup process either by adding special microorganisms to a polluted environment or by adding nutrients that stimulate indigenous microorganisms to degrade the pollutants. In either case the goal is to accelerate disappearance of the pollutant.

Microbes can grow in almost any environment containing liquid water, including structures made by humans. For example, microbes often grow on submerged surfaces, forming *biofilms*. Biofilms that grow in pipes and drains can cause fouling and blockages in factory settings and pipelines, in sewers, and even in water distribution systems. In addition, biofilms that grow on ships' hulls can cause marked reductions in speed and efficiency. Biofilms can even grow in tanks that store oil and fuel, leading to spoilage of these products. We will learn that biofilms are also of great importance in medicine, as biofilms that form on implanted medical devices (▶ Section 4.9) can cause infections that are extremely difficult to treat.

As these examples show, the influence of microorganisms on humans is great and their activities are essential for the functioning of the planet. Or, as the famous French chemist and early microbiologist Louis Pasteur so aptly put it: "The role of the infinitely small in nature is infinitely large." Microscopes provide an essential portal through which microbiologists such as Pasteur gazed into the world of microbes. We therefore continue our introduction to the microbial world with an overview of microscopy.

Check Your Understanding

- How do microbes contribute to the nutrition of animals such as humans and cows?
- Describe several ways in which microorganisms are important in the food and agricultural industries.
- What is wastewater treatment and why is it important?

II • Microscopy and the Origins of Microbiology

The microscope first revealed the microbial world, and the several different types of microscopes available today remain among the microbiologist's foremost tools.

Historically, the science of microbiology has taken its greatest leaps forward as new tools are developed and old tools improve. The microscope is the microbiologist's oldest and most fundamental tool for studying the microbial world. Indeed, microbiology did not exist before the invention of the microscope. Many forms of microscopy are available, and some are extremely powerful. Throughout this text you will see images of microorganisms that were taken through the microscope using a variety of different techniques. So let's take a moment to explore how microscopy can be used to visualize microbial cells, starting at the very beginning with the invention of the microscope.

1.7 Light Microscopy and the Discovery of Microorganisms

Although the existence of creatures too small to be seen with the naked eye had been suspected for centuries, their discovery had to await invention of the microscope. The English mathematician and natural historian Robert Hooke (1635–1703) was an excellent microscopist. In his famous book *Micrographia* (1665), the first book devoted to microscopic observations, Hooke illustrated many microscopic images including the fruiting structures of molds (**Figure 1.18**). This was the first known description of microorganisms.

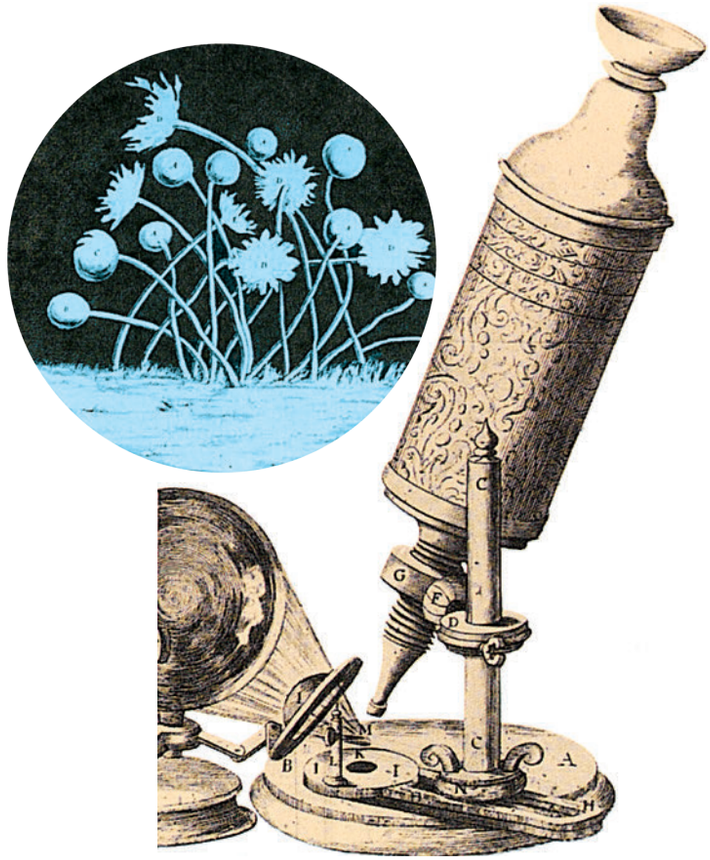


Figure 1.18 Robert Hooke and early microscopy. A drawing of the microscope used by Robert Hooke in 1664. The lens was fitted at the end of an adjustable bellows (G) and light focused on the specimen by a separate lens (1). Inset: Hooke's drawing of a bluish mold he found degrading a leather surface; the round structures contain spores of the mold.

The first person to see bacteria, the smallest microbial cells, was the Dutch draper and amateur microscopist Antoni van Leeuwenhoek (1632–1723). Van Leeuwenhoek constructed extremely simple microscopes containing a single lens to examine various natural substances for microorganisms (**Figure 1.19**). These microscopes were crude by today's standards, but by careful manipulation and focusing, van Leeuwenhoek was able to see bacteria. He discovered bacteria in 1676 while studying pepper–water infusions and reported his observations in a series of letters to the prestigious Royal Society of London, which published them in English translation in 1684. Drawings of some of van Leeuwenhoek's "wee animalcules," as he referred to them, are shown in Figure 1.19*b*, and a photo taken through a van Leeuwenhoek microscope is shown in Figure 1.19*c*.

Van Leeuwenhoek's microscope was a *light* microscope, and his design used a simple lens that could magnify an image

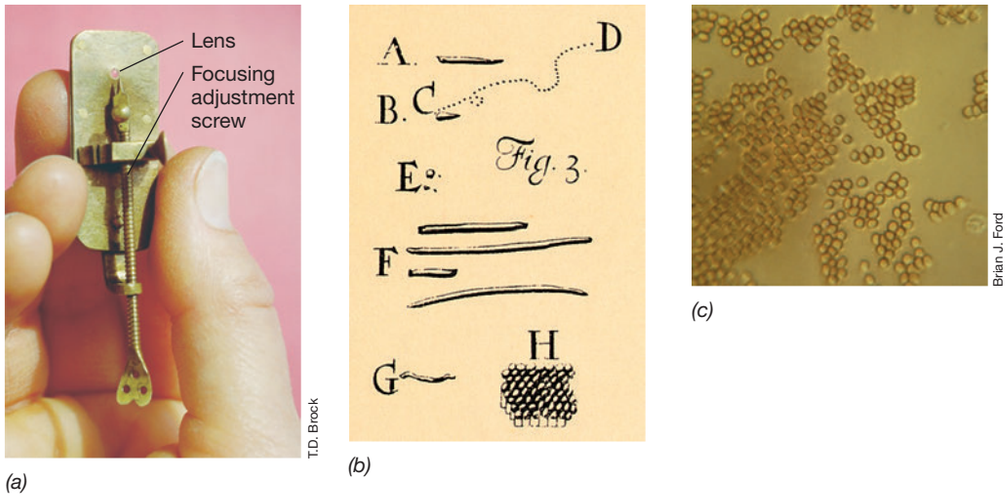


Figure 1.19 The van Leeuwenhoek microscope. *(a)* A replica of Antoni van Leeuwenhoek's microscope. *(b)* Van Leeuwenhoek's drawings of bacteria, published in 1684. Even from these simple drawings we can recognize several shapes of common bacteria: A, C, F, and G, rods; E, cocci; H, packets of cocci. *(c)* Photomicrograph of a human blood smear taken through a van Leeuwenhoek microscope. Red blood cells are clearly apparent.

at least 266 times. In a light microscope the sample is illuminated with visible light. **Magnification** describes the capacity of a microscope to enlarge an image. All microscopes employ lenses that provide magnification. Magnification, however, is not the limiting factor in our ability to see small objects. It is *resolution* that governs our ability to see the very small. **Resolution** is the ability to distinguish two adjacent objects as distinct and separate. The limit of resolution for a light microscope is about 0.2 μm (μm is the abbreviation for micrometer, 10^{-6} m). What this means is that two objects that are closer together than 0.2 μm cannot be resolved as distinct and separate.

Microscopy has improved remarkably since the days of van Leeuwenhoek. Several types of light microscopy are now available, including *bright-field*, *phase-contrast*, *differential interference contrast*, *dark-field*, and *fluorescence*. With the modern compound light microscope, light is focused on the specimen by the condenser (**Figure 1.20**) and this light passes through the sample and is collected by the lenses. The modern compound light microscope contains two types of lenses, *objective* and *ocular*, that function in combination to magnify the image. Microscopes used in microbiology have ocular lenses that magnify 10–30× and objective lenses that magnify 10–100× (Figure 1.20*b*). The total magnification of a compound light microscope is the *product* of the magnification of its objective and ocular lenses (Figure 1.20*b*). Magnification of 1000× is required to resolve objects 0.2 μm in diameter, which is the limit of resolution for most light microscopes (increasing magnification beyond 1000× provides little improvement in the resolution of a light microscope).

In addition to magnification, the limit of resolution for a light microscope is a function of the wavelength of light used and the light-gathering ability of the objective lens, a property known as its *numerical aperture*. There is a correlation between the magnification of a lens and its numerical aperture; lenses with higher magnification typically have higher numerical apertures. The diameter of the smallest object resolvable by any lens is equal to 0.5λ/numerical aperture, where λ is the wavelength of light used. With objectives that have a very high numerical aperture (such as the 100× objective), an optical-grade oil is placed between the microscope slide and the objective. Lenses on which oil is used are called *oil-immersion* lenses. Immersion oil increases the light-gathering ability of a lens, that is, it increases the amount of light that is collected and viewed by the lens.

Mastering Microbiology
Microbiology Animation: Light Microscopy

In light microscopy, specimens are visualized because of differences in **contrast** that exist between them and their surroundings (**Figure 1.21**). In bright-field microscopy, contrast results when cells absorb or scatter light differently from their surroundings. Bacterial cells typically lack contrast, that is, their optical properties are similar to the surrounding liquid, and hence they are difficult to see well with the bright-field microscope. Pigmented microorganisms are an exception because the color of the organism adds contrast, thus improving visualization by bright-field optics (Figure 1.21). For cells lacking pigments there are several ways to boost contrast, and we consider these methods in the next section.

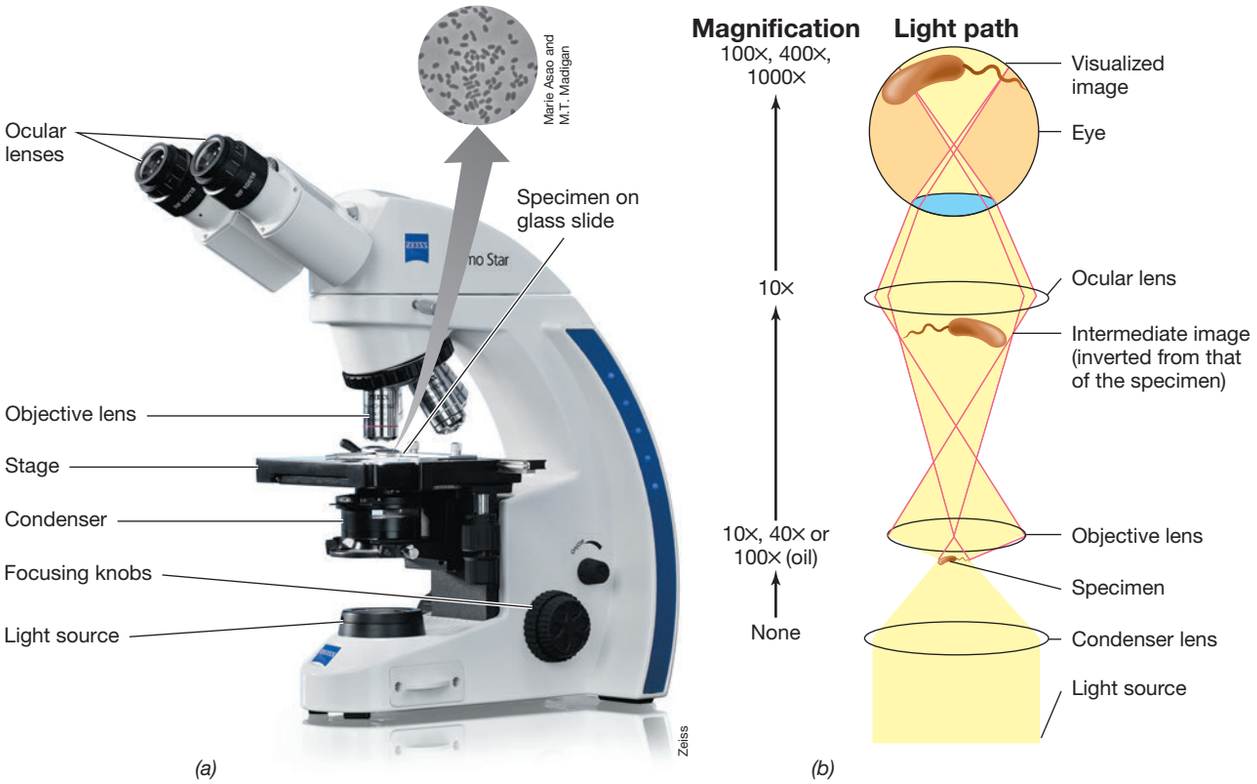
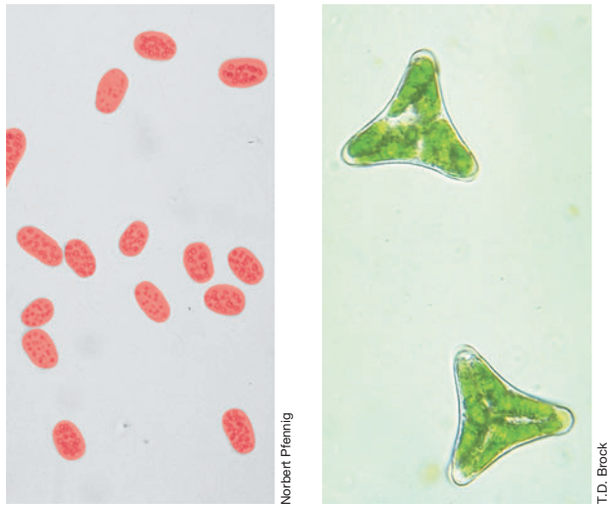


Figure 1.20 Microscopy. *(a)* A compound light microscope (inset photomicrograph of unstained cells taken through a phase-contrast light microscope). *(b)* Path of light through a compound light microscope. Figure 1.24 compares cells visualized by bright field with those visualized by phase contrast.



(a) (b)

Figure 1.21 Bright-field photomicrographs of pigmented microorganisms. *(a)* Purple phototrophic bacteria (*Bacteria*). The bacterial cells are about 5 μm wide. *(b)* A green alga (eukaryote). The green structures are chloroplasts. The algal cells are about 15 μm wide. Purple bacteria are anoxygenic phototrophs, whereas algae are oxygenic phototrophs. Both groups contain photosynthetic pigments, but only oxygenic phototrophs produce O_2 (Section 1.5 and Figure 1.10*a*).

Check Your Understanding

- Define the terms magnification and resolution.
- What is the limit of resolution for a bright-field microscope? What defines this limit?

1.8 Improving Contrast in Light Microscopy

Contrast is necessary in light microscopy to distinguish microorganisms from their surroundings. Cells can be stained to improve contrast, and staining is commonly used to visualize bacteria with bright-field microscopy. In addition to staining, other methods of light microscopy have been developed to improve contrast with or without staining, and we consider all of these methods here.

Staining: Increasing Contrast for Bright-Field Microscopy

Dyes can be used to stain cells and increase their contrast so that they can be more easily seen in the bright-field microscope. Each class of dye has an affinity for specific cellular materials. Many dyes used in microbiology are positively charged, and for this reason, they are called *basic dyes*. Examples of basic dyes include methylene blue, crystal violet, and safranin. Basic dyes bind strongly to negatively charged cell components, such as nucleic acids and acidic polysaccharides. These dyes also stain the surfaces of cells because cell surfaces tend to be negatively charged. These properties make basic dyes useful general-purpose stains that nonspecifically stain most bacterial cells.

To perform a *simple* stain, one begins with dried preparations of cells (Figure 1.22). A clean glass slide containing a dried suspension of cells is flooded for a minute or two with a dilute solution of a basic dye, rinsed several times in water, and blotted dry. Because bacterial cells are so small, it is common to observe dried, stained preparations of those cells with a high-power (oil-immersion) lens.

Differential Stains: The Gram Stain

Stains that render different kinds of cells different colors are called *differential* stains. An important differential-staining procedure used in microbiology is the **Gram stain** (Figure 1.23). On the basis of their reaction in the Gram stain, bacteria can be divided into two major groups: **gram-positive** and **gram-negative**. After Gram staining, gram-positive bacteria appear purple-violet and gram-negative bacteria appear pink (Figure 1.23*b*). The color difference in the Gram stain arises because of differences in the cell wall structure of gram-positive and gram-negative cells (▶ Section 2.3). Staining with a basic dye such as crystal violet renders cells purple in color. Cells are then treated with ethanol, which decolorizes gram-negative cells but

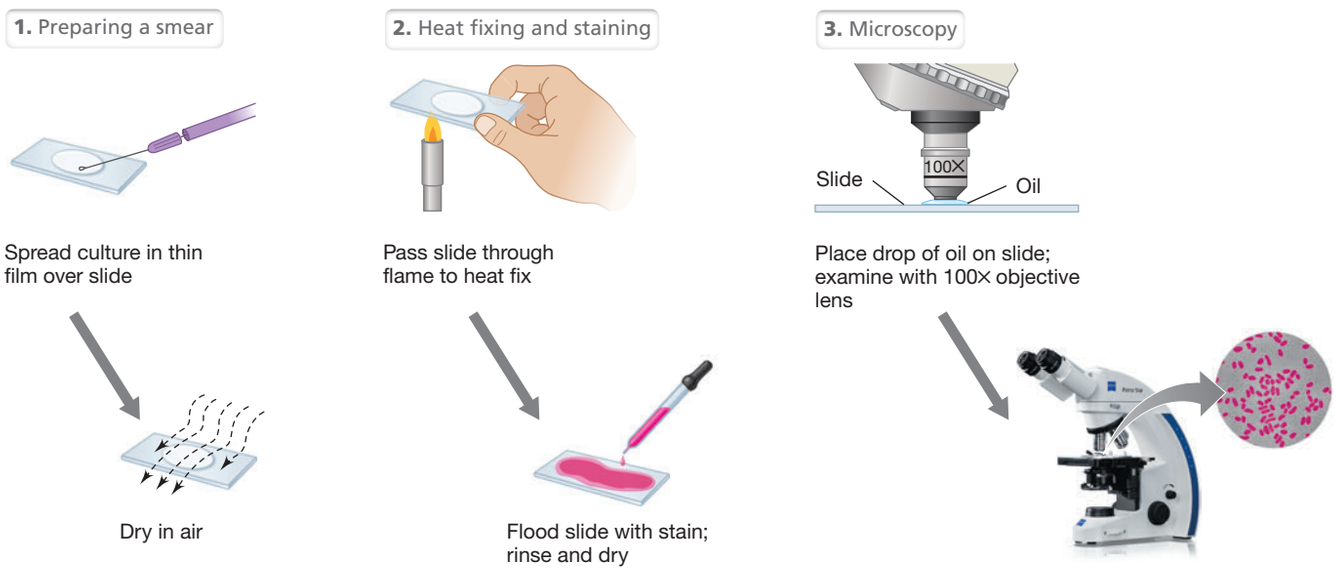


Figure 1.22 Staining cells for microscopic observation. Stains improve the contrast between cells and their background. Step 3 lower right: Same cells as shown in Figure 1.20*a* inset but stained with a basic dye.

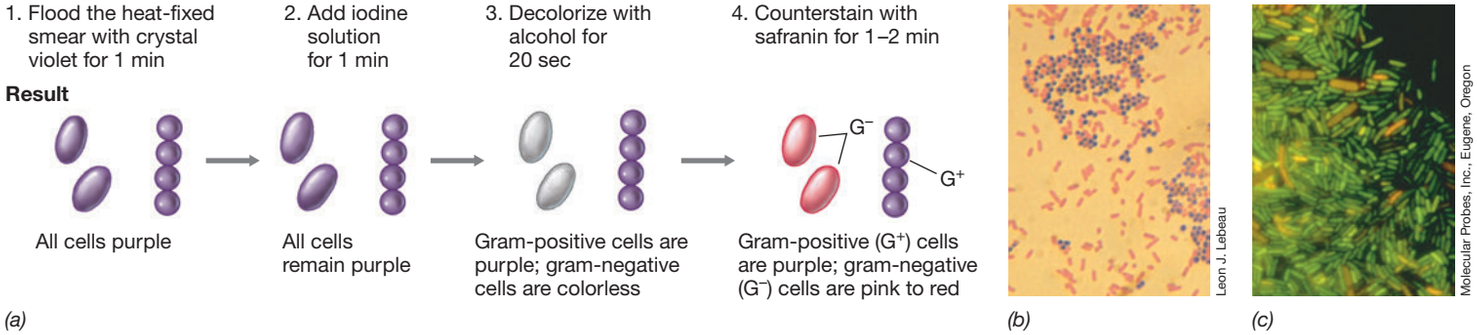


Figure 1.23 **The Gram stain.** *(a)* Steps in the procedure. *(b)* Microscopic observation of gram-positive (purple) and gram-negative (pink) bacteria. The organisms are *Staphylococcus aureus* and *Escherichia coli*, respectively. *(c)* Cells of *Pseudomonas aeruginosa* (gram-negative, green) and *Bacillus cereus* (gram-positive, orange) stained with a one-step fluorescent staining method. This method allows for differentiating gram-positive from gram-negative bacteria in a single staining step.

not gram-positive cells. Finally, cells are counterstained with a different-colored stain, typically the red stain safranin. As a result, gram-positive and gram-negative cells can be distinguished microscopically by their different colors (Figure 1.23*b*).

The Gram stain is the most common staining procedure used in microbiology, and it is often performed to begin the characterization of a new bacterium. If a fluorescence microscope is available, the Gram stain can be reduced to a one-step procedure; gram-positive and gram-negative cells fluoresce different colors when treated with a special chemical (Figure 1.23*c*).

Phase-Contrast and Dark-Field Microscopy

Although staining is widely used in light microscopy, staining often kills cells and can distort their features. Two forms of light microscopy improve image contrast of unstained (and thus live) cells. These are phase-contrast microscopy and dark-field microscopy (**Figure 1.24**). The phase-contrast microscope in particular is widely used in teaching and research for the observation of living preparations.

Phase-contrast microscopy is based on the principle that cells differ in refractive index (that is, the ability of a material to alter the speed of light) from their surroundings. Light passing through a cell thus differs in phase from light passing through the surrounding liquid. This subtle difference is amplified by a device in the objective lens of the phase-contrast microscope called the *phase ring*, resulting in a dark image on a light background (Figure 1.24*b*; see also inset to Figure 1.20*a*). The ring consists of a phase plate that amplifies the variation in phase to produce the higher-contrast image.

In the dark-field microscope, light does not pass through the specimen. Instead, light is directed from the sides of the specimen and only light that is scattered when it hits the specimen reaches the lens. Thus, the specimen appears light on a dark background (Figure 1.24*c*). Dark-field microscopy often has better resolution than light microscopy, and some objects can be resolved by dark-field that cannot be resolved by bright-field or even by phase-contrast microscopes. Dark-field microscopy is a particularly good way to observe microbial motility, as bundles of flagella (the structures responsible for swimming motility) are often resolvable with this technique.

Fluorescence Microscopy

The fluorescence microscope visualizes specimens that *fluoresce* (emit light). In fluorescence microscopy, cells are made to fluoresce by illuminating them from above with light of a single color. Filters are used so that only fluorescent light is seen, and thus cells appear to glow in a black background (**Figure 1.25**).

Cells fluoresce either because they contain naturally fluorescent substances such as chlorophyll (autofluorescence, Figure 1.25*b*, *d*)

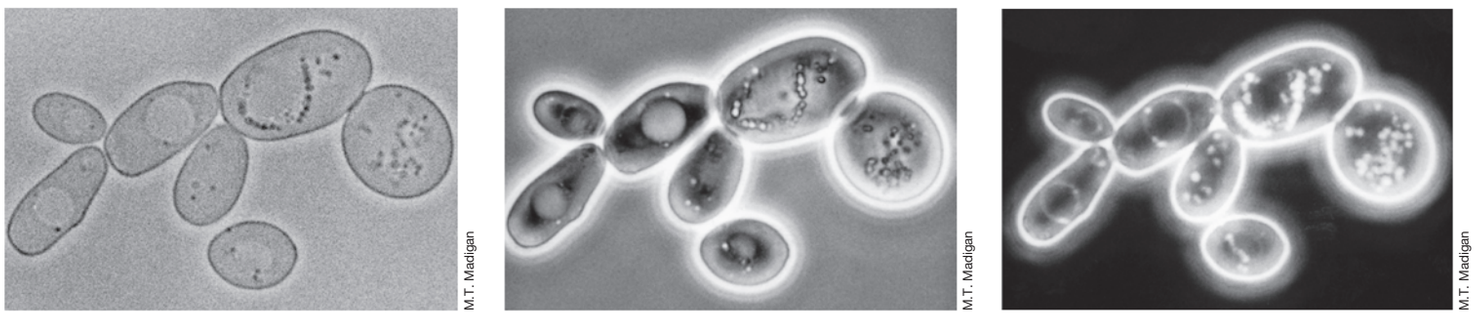


Figure 1.24 **Cells visualized by different types of light microscopy.** The same field of cells of the yeast *Saccharomyces cerevisiae* visualized by *(a)* bright-field microscopy, *(b)* phase-contrast microscopy, and *(c)* dark-field microscopy. Cells average 8–10 μm wide.

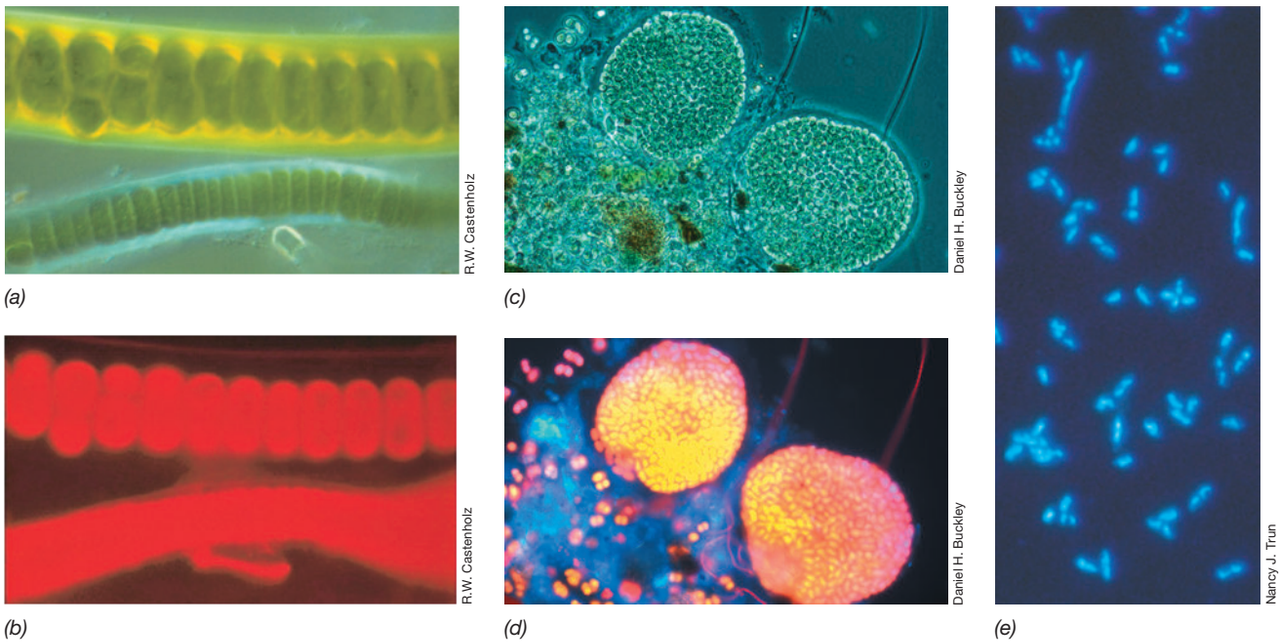


Figure 1.25 **Fluorescence microscopy.** *(a, b, c, d)* Cyanobacteria. The same cells are observed in *a* and *b*, and likewise in *c* and *d*. The top photos are taken with phase-contrast microscopy and the bottom photos with fluorescence microscopy. The cells fluoresce because they contain chlorophyll *a* and other pigments. The image in *b* was generated using a filter specific for the fluorescence of chlorophyll *a*, while the image in *d* was generated using a permissive filter that shows fluorescence from a range of pigments that occur naturally in cyanobacteria. *(e)* Fluorescence photomicrograph of cells of *Escherichia coli* made fluorescent by staining with the fluorescent dye DAPI, which binds to DNA.

or because they have been stained with a fluorescent dye (Figure 1.25*e*). DAPI (4′,6-*dia*midino-2-*p*heny*l*indole) is a widely used fluorescent dye that stains cells bright blue because it complexes with the cell's DNA (Figure 1.25*e*). DAPI can be used to visualize cells in their natural habitats, such as soil, water, food, or a clinical specimen. Fluorescence microscopy using DAPI is widely used in clinical diagnostic microbiology and also in microbial ecology for enumerating bacteria in a natural environment or in a cell suspension (Figure 1.25*e*).

Check Your Understanding

- What color will a gram-negative cell be after Gram staining by the conventional method?
- What major advantage does phase-contrast microscopy have over staining?
- How can cells be made to fluoresce?

1.9 Imaging Cells in Three Dimensions

Thus far we have only considered forms of microscopy in which the rendered images are two-dimensional. Two methods of light microscopy can render a more three-dimensional image, and in this section we explore these forms of microscopy.

Differential Interference Contrast Microscopy

Differential interference contrast (DIC) microscopy is a form of light microscopy that employs a polarizer in the condenser to produce polarized light (light in a single plane). The polarized light then passes through a prism that generates two distinct beams. These beams pass through the specimen and enter the objective lens, where they are recombined into one. Because the two beams pass through substances that differ in refractive index, the combined beams are not totally in phase but instead interfere with each other. This optical effect provides a three-dimensional perspective, which enhances subtle differences in cell structure.

Using DIC microscopy, cellular structures such as the nucleus of eukaryotic cells (**Figure 1.26**), or various types of inclusions present in some bacterial cells, appear more three-dimensional than in

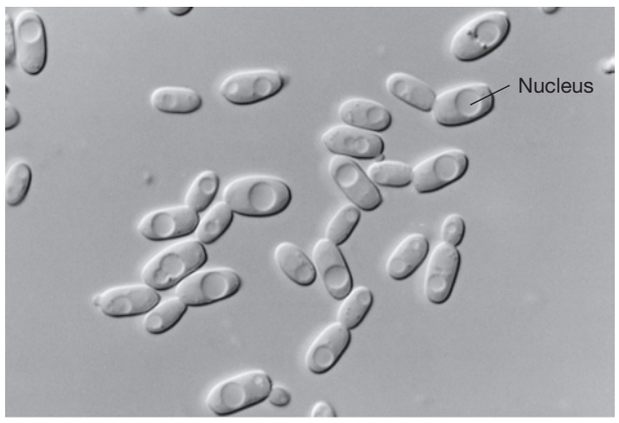


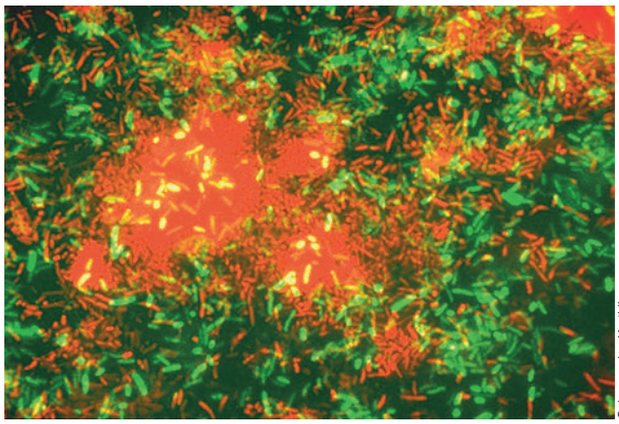
Figure 1.26 **Differential interference contrast microscopy.** The yeast cells are about 8 μm wide. Note the clearly visible nucleus and compare to the bright-field image of yeast cells in Figure 1.24*a*.

other forms of light microscopy. DIC microscopy is typically used on unstained cells as it can reveal internal cell structures that are nearly invisible by bright-field microscopy without the need for staining (compare Figure 1.24*a* with Figure 1.26).

Confocal Scanning Laser Microscopy

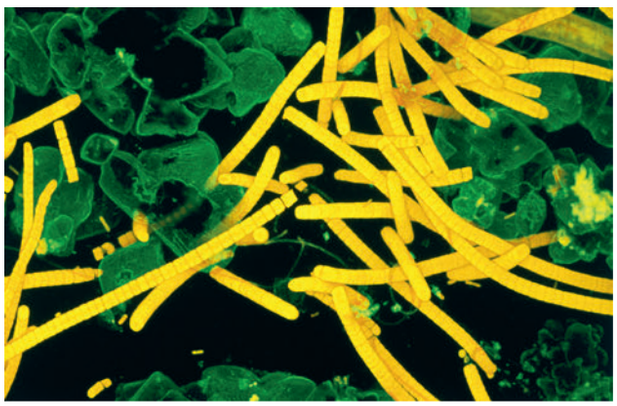
A confocal scanning laser microscope (CSLM) is a computer-controlled microscope that couples a laser to a fluorescence microscope. The laser generates a high-contrast, three-dimensional image and allows the viewer to access several planes of focus in the specimen (**Figure 1.27**). The laser beam is precisely adjusted such that only a particular layer within a specimen is in perfect focus at one time. Cells struck by the laser fluoresce to generate the image as in fluorescence microscopy (Section 1.8).

Cells in CSLM preparations can also be stained with fluorescent dyes to make them more distinct (Figure 1.27*a*). The laser then scans up and down through the layers of the sample, generating an image for each layer. A computer assembles the images to compose the many layers into a single high-resolution, three-dimensional image. Thus, for a relatively thick specimen (such as a bacterial biofilm, Figure 1.27*a*), not only can cells on the surface of the biofilm be observed, as with conventional light microscopy, but cells in the various layers are also observed by adjusting the plane of focus of the laser beam. CSLM is particularly useful when thick specimens need to be examined.



Subramanian Karthikeyan

(a)



Gernot Arp and Christian Boeker, Carl Zeiss, Jena

(b)

Figure 1.27 Confocal scanning laser microscopy. *(a)* Confocal image of a microbial biofilm community. The green, rod-shaped cells are *Pseudomonas aeruginosa* experimentally introduced into the biofilm. Cells of different colors are present at different depths in the biofilm. *(b)* Confocal image of a filamentous cyanobacterium growing in a soda lake. Cells are about 5 μm wide.

Check Your Understanding

- What structure in eukaryotic cells is more easily seen in DIC than in bright-field microscopy? (*Hint:* Compare Figures 1.24*a* and 1.26).
- Why is CSLM able to show different layers in a thick preparation while bright-field microscopy cannot?

1.10 Probing Cell Structure: Electron Microscopy

Electron microscopes use electrons instead of visible light (photons) to image cells and cell structures. In the electron microscope, electromagnets function as lenses, and the whole system operates in a vacuum (**Figure 1.28**). Electron microscopes are fitted with cameras to allow a photograph, called an *electron micrograph*, to be taken. Two types of electron microscopy are in routine use in microbiology: transmission and scanning.

Transmission Electron Microscopy

The *transmission electron microscope* (TEM) is used to examine cells and cell structure at very high magnification and resolution. The resolving power of a TEM is much greater than that of the light

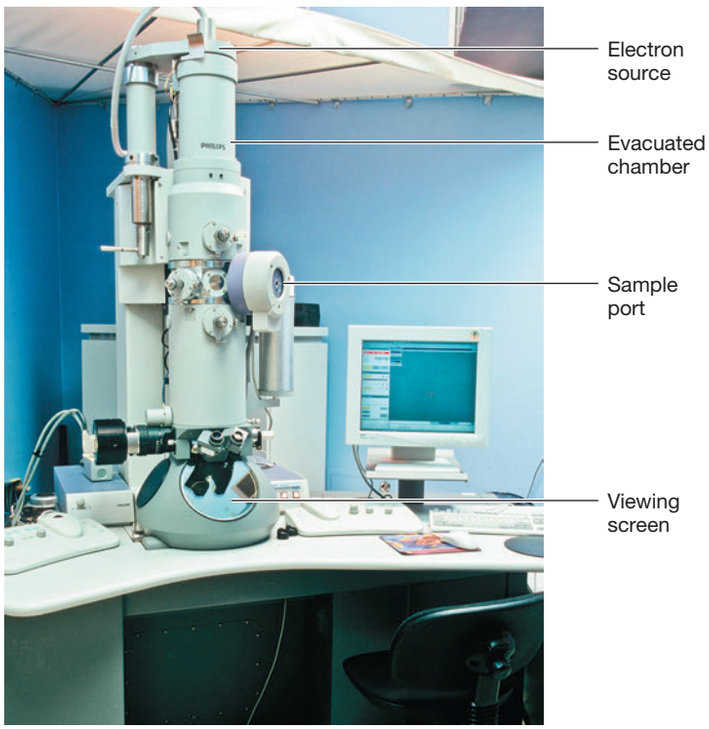
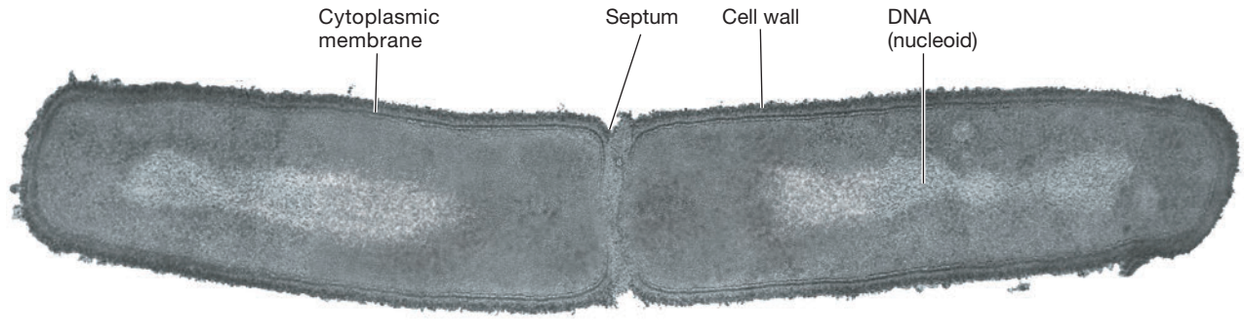
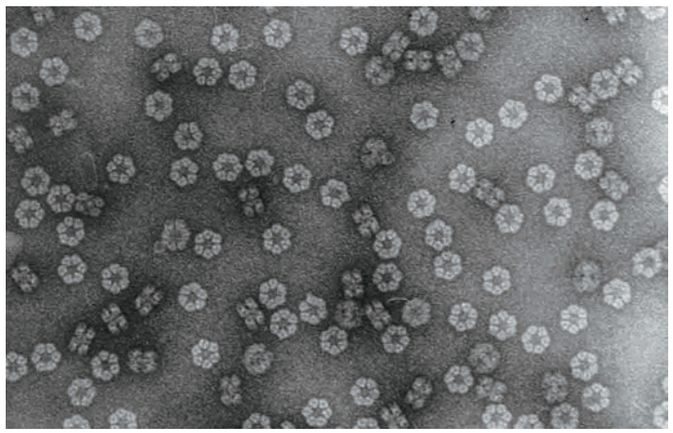


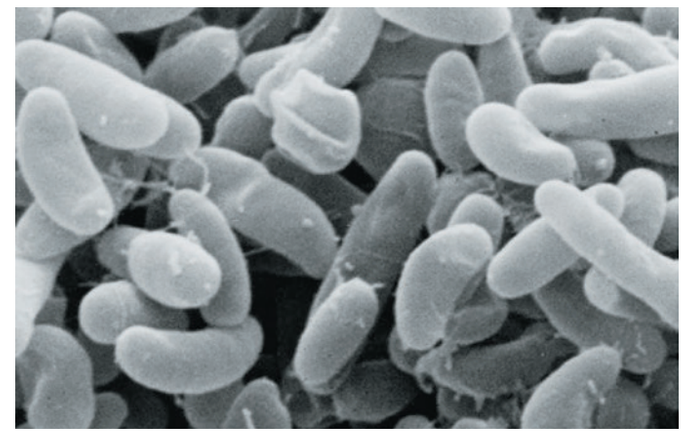
Figure 1.28 The electron microscope. This instrument encompasses both transmission and scanning electron microscope functions.



(a)



(b)



(c)

Figure 1.29 **Electron micrographs.** *(a)* Micrograph of a thin section of a dividing bacterial cell, taken by transmission electron microscopy (TEM). The cell is about 0.8 μm wide. *(b)* TEM of negatively stained molecules of hemoglobin. Each hexagonal-shaped molecule is about 25 nanometers (nm) in diameter and consists of two doughnut-shaped rings, a total of 15 nm thick. *(c)* Scanning electron micrograph (SEM) of bacterial cells. A single cell is about 0.75 μm wide.

microscope, even allowing one to view structures at the molecular level (**Figure 1.29**). This is because the wavelength of electrons is much shorter than the wavelength of visible light, and, as we have learned, wavelength affects resolution (Section 1.7). For example, whereas the resolving power of a light microscope is about 0.2 *micrometer*, the resolving power of a TEM is about 0.2 *nanometer*, a thousandfold improvement. With such powerful resolution, objects as small as individual protein and nucleic acid molecules can be visualized by transmission electron microscopy (Figure 1.29*b*).

Unlike photons, electrons are very poor at penetrating; even a single cell is too thick to penetrate with an electron beam. Consequently, to view the internal structure of a cell, *thin sections* of the cell are needed, and the sections must be stabilized and stained with various chemicals to make them visible. A single bacterial cell, for instance, is cut into extremely thin (20- to 60-nm) slices, which are then examined individually by TEM (Figure 1.29*a*). To obtain sufficient contrast, the sections are treated with stains such as osmic acid, or permanganate, uranium, lanthanum, or lead salts. Because these substances are composed of atoms of high atomic weight, they scatter electrons well and thus improve contrast. If only the *external* features of an organism are to be observed, thin sections are unnecessary. Intact cells or cell components can be observed directly in the TEM by a technique called *negative staining* (Figure 1.29*b*).

Electron cryotomography (cryoET) is an imaging technique in which TEM is used to obtain three-dimensional images. In cryoET, samples are prepared by rapid freezing to very low temperatures so that they are immobilized in noncrystalline vitreous ice. They are then imaged at very low temperatures ($<-150°C$), thereby preserving cell structures in their native states. Finally, samples are tilted as they are being imaged and this series of tilted images is assembled computationally to generate a three-dimensional image of the interior of the cell with a resolution of 4 nm.

Scanning Electron Microscopy

For optimal three-dimensional imaging of cells, a *scanning electron microscope* (SEM) is used. In scanning electron microscopy, the specimen is coated with a thin film of a heavy metal, typically gold. An electron beam then scans back and forth across the specimen. Electrons scattered from the metal coating are collected and projected on a monitor to produce an image (Figure 1.29*c*). In the SEM, even fairly large specimens can be observed, and the depth of field (the portion of the image that remains in sharp focus) is extremely good. A wide range of magnifications can be obtained with the SEM, from as low as 15× up to about 100,000×, but only the *surface* of an object is typically visualized.

Electron micrographs taken by either TEM or SEM are black-and-white images. Although the original image contains the maximum amount of scientific information that is available, color is often added to scanning electron micrographs by manipulating them in a computer. However, such false color does not improve resolution of a micrograph. In this text, false color will be used sparingly in electron micrographs so as to present the micrographs in their original scientific context.

Check Your Understanding

- What is an electron micrograph? Why do electron micrographs have greater resolution than light micrographs?
- What type of electron microscope would be used to view a cluster of cells? What type would be used to observe internal cell structure?

III • Microbial Cultivation Expands the Horizon of Microbiology

Microbes can be cultured in the laboratory, and microbial cultures have played a major role in unraveling the metabolic diversity and medical importance of the microbial world.

Following the discovery of microorganisms through microscopic methods, advances in microbial cultivation fueled major discoveries in microbiology. Important advances included the development of **aseptic technique**, a collection of practices that allow for the preparation and maintenance of **sterile** (that is, without the presence of living organisms) nutrient media and solutions (Chapter 4). Aseptic technique is essential for the isolation and maintenance of pure cultures of bacteria. **Pure cultures** are those that contain cells from only a single type of microorganism and are of great value for the study of microorganisms. Finally, **enrichment culture** techniques, which allow for the isolation from nature of microbes having particular metabolic characteristics, facilitate the discovery of diverse microorganisms.

Advances in microbial cultivation are directly responsible for success in fighting infectious disease, the discovery of microbial diversity, and the use of microbes as model systems to discover the fundamental properties of all living cells. Important advances in microbial cultivation occurred in the nineteenth century as microbiologists sought to answer two major questions of that time: (1) Does spontaneous generation occur? (2) What is the nature of infectious disease? Answers to these seminal questions emerged from the work of two giants in the field of microbiology: the French chemist Louis Pasteur and the German physician Robert Koch. We begin with the work of Pasteur.

1.11 Pasteur and Spontaneous Generation

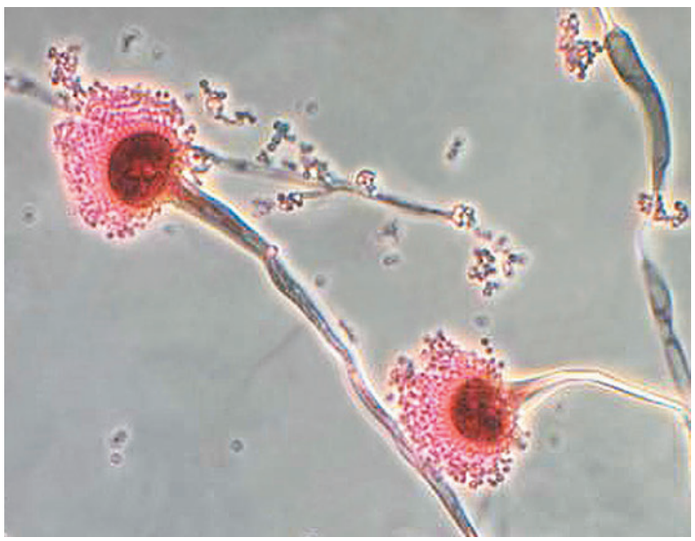
Pasteur was a chemist by training and was one of the first to recognize that many of what were thought to be strictly chemical reactions were actually catalyzed by microorganisms. Pasteur studied the chemistry of crystal formation and he used microscopes to examine crystal structure. His training in chemistry and microscopy prepared him to make a series of foundational discoveries to further the science of microbiology.

The Microbial Basis of Fermentation

Early in his career, Pasteur studied crystals formed during the production of alcohol. Through careful microscopic observation of tartaric acid crystals formed in wine, he observed two types of crystals that had mirror-image structures. He separated these by hand and observed that each type of crystal bent a beam of polarized light in a different direction. In this way he discovered that chemically identical substances can have *optical isomers*, which have different molecular structures that can influence their properties. Pasteur went on to discover that microorganisms could discriminate between optical isomers. For example, cultures of the mold *Aspergillus* (Figure 1.30) metabolized exclusively D-tartrate but not its optical isomer, L-tartrate. The fact that a living organism could discriminate between optical isomers led Pasteur to strongly suspect that many reactions previously thought to be abiotic were actually catalyzed by microbes.

While a professor of chemistry, Pasteur encountered a local businessman who produced alcohol industrially from beet juice. The businessman was losing money because many of his vats produced, instead of alcohol, a product that smelled like sour milk, which Pasteur determined to be lactic acid. In the mid-nineteenth century the production of alcohol was thought to be solely a chemical process. Pasteur studied the broth with his microscope, but instead of crystals he observed cells. Pasteur observed that the vats that produced alcohol were full of yeast, but the sour vats were full of rod-shaped bacteria. He hypothesized that these were living organisms whose growth produced either alcohol or lactic acid.

Pasteur needed to grow these organisms to prove his hypothesis. He prepared a hot-water extract of yeast cells, deducing that this would contain all of the nutrients that yeast need to grow. He then used a porcelain filter to remove all cells from this yeast extract



CDC/PHIL

Figure 1.30 Louis Pasteur and his observation of isomeric discrimination by *Aspergillus*. Light micrograph of cells of the mold *Aspergillus*.

nutrient medium, rendering it sterile. If he introduced living yeast back into this sterile yeast extract medium, he could observe their growth and show the production of alcohol, but if he instead introduced the small rods, he then observed lactic acid formation. Heating of these cultures eliminated growth *and* the production of either alcohol or lactic acid. In this way he proved that fermentation is carried out by microorganisms and that different microorganisms perform different fermentation reactions.

During his work on fermentation, Pasteur observed that other organisms would often grow in his yeast extract medium. He deduced that these organisms were being introduced from the air. Pasteur's work on fermentation had prepared him to conduct a series of classic experiments on spontaneous generation, experiments that are forever linked to his name and which helped establish microbiology as a modern science.

Spontaneous Generation

The concept of **spontaneous generation** existed for thousands of years and its basic tenet can be easily grasped. If food or some other perishable material is allowed to stand for some time, it putrefies. When examined microscopically, the putrefied material is teeming with microorganisms. From where do these organisms arise? Prior to Pasteur it was common belief that life arose spontaneously from nonliving materials, that is, by *spontaneous generation*.

Mastering Microbiology
Art Activity: Figure 1.26 Pasteur's swan-necked flask experiments

Pasteur became a powerful opponent of spontaneous generation. He predicted that microorganisms in putrefying materials were descendants of cells that entered from the air or cells that had initially been present on the decaying materials. Pasteur reasoned that if food were treated in such a way as to destroy all living organisms present—that is, if it were rendered sterile—and if it were kept sterile, it would not putrefy.

Pasteur used heat to kill contaminating microorganisms, and he found that extensive heating of a nutrient solution followed by sealing kept it from putrefying. Proponents of spontaneous generation criticized these experiments by declaring that "fresh air" was necessary for the phenomenon to occur. In 1864 Pasteur countered this objection simply and brilliantly by constructing a swan-necked flask, now called a *Pasteur flask* (**Figure 1.31**). In such a flask, nutrient solutions could be heated to boiling and sterilized. After the flask cooled, air could reenter, but the bend in the neck prevented particulate matter (including microorganisms) from entering the nutrient solution and initiating putrefaction. Nutrient solutions in such flasks remained sterile indefinitely. Microbial growth was observed only after particulate matter from the neck of the flask was allowed to enter the liquid in the flask (Figure 1.31*c*). This experiment settled the spontaneous generation controversy forever.

Pasteur's work on spontaneous generation demonstrated the importance of sterilization and led to the development of effective sterilization procedures that were eventually standardized and applied widely in microbiology, medicine, and industry. For example, the British physician Joseph Lister (1827–1912) deduced from Pasteur's discoveries that surgical infections were caused by microorganisms. He implemented a range of techniques designed to kill microorganisms and to prevent microbial infection of surgical patients. Lister is credited with the introduction of aseptic techniques for surgeries (1867), and his methods were adopted worldwide; these greatly reduced postoperative infections and greatly improved the survival rate of surgical patients. The food industry

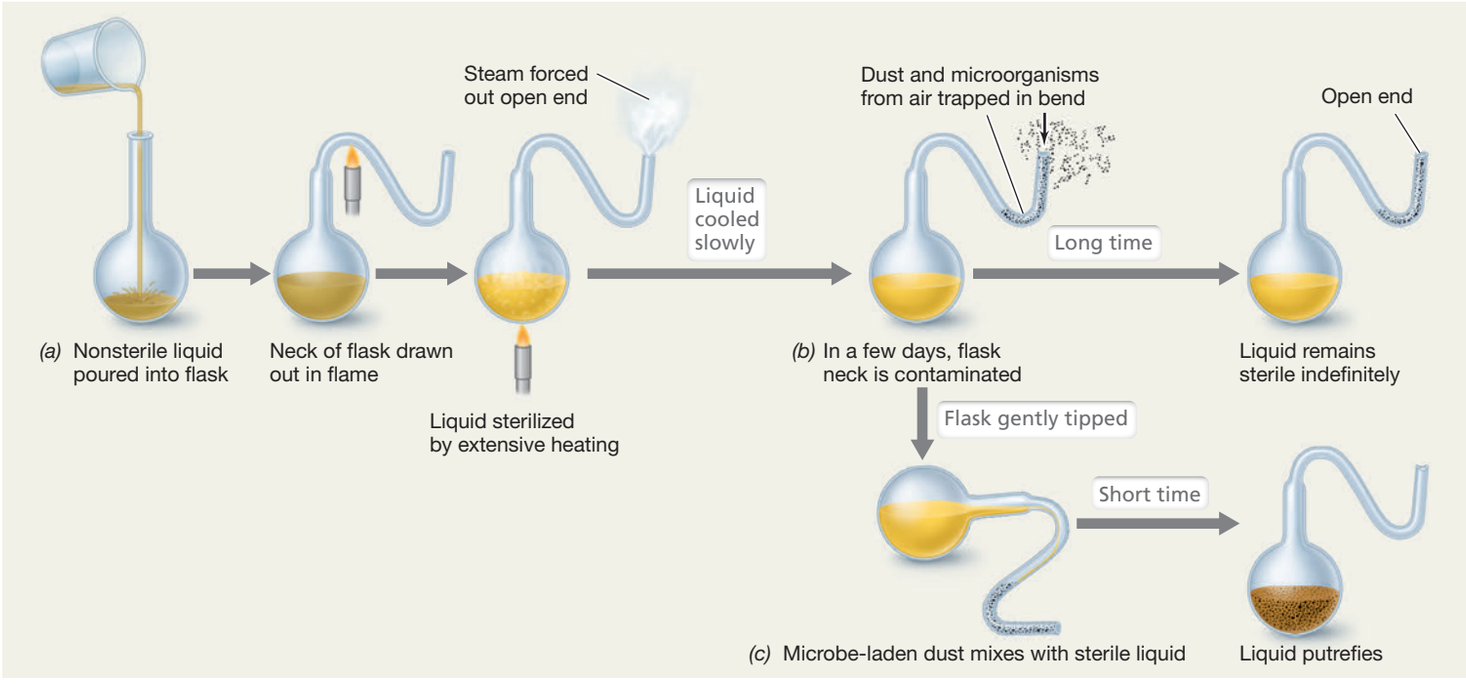


Figure 1.31 The defeat of spontaneous generation: Pasteur's swan-necked flask experiment. In *(c)* the liquid putrefies because microorganisms enter with the dust. The bend in the flask allowed air to enter (a key objection to Pasteur's sealed flasks) but prevented microorganisms from entering.

also benefited from the work of Pasteur, as his principles were quickly adapted for the preservation of milk and many other foods by heat treatment, which we now call *pasteurization*.

Other Major Accomplishments of Pasteur

Pasteur went on to many other triumphs in microbiology and medicine. Some highlights include his development of vaccines for the diseases anthrax, fowl cholera, and rabies. Pasteur's work on rabies was his most famous success, culminating in July 1885 with the first administration of a rabies vaccine to a human, a young French boy named Joseph Meister who had been bitten by a rabid dog. In those days, a bite from a rabid animal was invariably fatal. News spread quickly of the success of Meister's vaccination, and of one administered shortly thereafter to a young shepherd boy, Jean-Baptiste Jupille (**Figure 1.32*a***). Within a year several thousand people bitten by rabid animals had traveled to Paris to be treated with Pasteur's rabies vaccine.

Pasteur's fame was legendary and led the French government to establish the Pasteur Institute in Paris in 1888 (Figure 1.32*b*).

(a)

M.T. Madigan

(b)

Figure 1.32 Louis Pasteur and some symbols of his contributions to microbiology. *(a)* A French 5-franc note honoring Pasteur. The shepherd boy Jean-Baptiste Jupille is shown killing a rabid dog that had attacked children. Pasteur's rabies vaccine saved Jupille's life. In France, the franc preceded the euro as a currency. *(b)* Part of the Pasteur Institute, Paris, France. Today this structure, built for Pasteur by the French government, houses a museum that displays some of the original swan-necked flasks used in his experiments and a chapel containing Pasteur's crypt.

Originally established as a clinical center for the treatment of rabies and other contagious diseases, the Pasteur Institute today is a major biomedical research center focused on antiserum and vaccine research and production. The medical and veterinary breakthroughs of Pasteur not only were highly significant in their own right but helped solidify the concept of the germ theory of disease, whose principles were being developed at about the same time by a second giant of this era, Robert Koch.

Check Your Understanding

- Define the term sterile. What two methods did Pasteur use to make solutions sterile?
- How did Pasteur's experiments using swan-necked flasks defeat the theory of spontaneous generation?
- Besides ending the controversy over spontaneous generation, what other accomplishments do we credit to Pasteur?

1.12 Koch, Infectious Diseases, and Pure Cultures

Proof that some microorganisms can cause disease provided the greatest impetus for the development of microbiology as an independent biological science. As early as the sixteenth century it was suspected that some agent of disease could be transmitted from a diseased person to a healthy person. After microorganisms were discovered, a number of individuals proposed that they caused infectious diseases, but skepticism prevailed, and definitive proof was lacking. As early as 1847, the Hungarian physician Ignaz Semmelweis promoted sanitary methods including hand washing as a method for preventing infections. His methods are credited with saving many lives, but he could not prove why these methods worked and his advice was met with scorn by most of the medical community. The work of Pasteur and Lister provided strong evidence that microbes were the cause of infectious disease, but it was not until the work of the German physician Robert Koch (1843–1910) that the *germ theory* of infectious disease had direct experimental support.

The Germ Theory of Disease and Koch's Postulates

In his early work Koch studied anthrax, a disease of cattle and occasionally of humans. Anthrax is caused by the bacterium *Bacillus anthracis.* By careful microscopy and staining, Koch established that the bacteria were always present in the blood of an animal that was succumbing to the disease. However, Koch reasoned that the mere *association* of the bacterium with the disease was not actual proof of *cause and effect*, and he seized the opportunity to study cause and effect experimentally using anthrax and laboratory animals. The results of this study formed the standard by which infectious diseases have been studied ever since.

Koch used mice as experimental animals. Using appropriate controls, Koch demonstrated that when a small drop of blood from a mouse with anthrax was injected into a healthy mouse, the latter quickly developed anthrax. He took blood from this second animal, injected it into another, and again observed the characteristic disease symptoms. However, Koch carried this experiment a critically important step further. He discovered that the anthrax bacteria could be

Mastering Microbiology
Art Activity: Figure 1.29 Koch's Postulates for proving cause and effect in infectious diseases

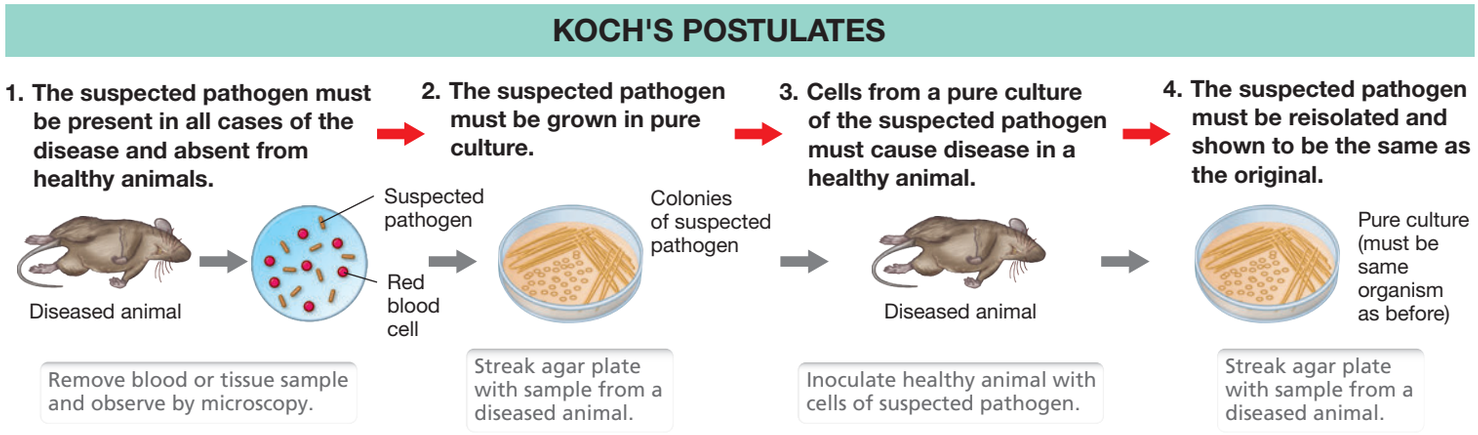


Figure 1.33 Koch's postulates for proving cause and effect in infectious diseases. Note that following isolation of a pure culture of the suspected pathogen, the cultured organism must both initiate the disease and be recovered from the diseased animal. Establishing the correct conditions for growing the pathogen is essential.

grown in a nutrient medium *outside the host* and that even after many transfers in laboratory culture, the bacteria still caused the disease when inoculated into a healthy animal.

On the basis of these experiments and others on the causative agent of tuberculosis, Koch formulated a set of rigorous criteria, now known as **Koch's postulates**, for definitively linking cause and effect in an infectious disease. Koch's postulates, summarized in **Figure 1.33**, stressed the importance of *laboratory culture* of the putative infectious agent followed by introduction of the suspected agent into virgin animals and recovery of the pathogen from diseased or dead animals. With these postulates as a guide, Koch, his students, and those that followed them discovered the causative agents of most of the important infectious diseases of humans and domestic animals. These discoveries also led to the development of successful treatments for the prevention and cure of many of these diseases, greatly improving the scientific basis of clinical medicine and human health and welfare (Figure 1.13).

Koch, Pure Cultures, and Microbial Taxonomy

The second of Koch's postulates states that the suspected pathogen must be isolated and grown away from other microorganisms in laboratory culture (Figure 1.33); in microbiology we say that such a culture is *pure*. To accomplish this important goal, Koch and his associates developed several simple but ingenious methods of obtaining and growing bacteria in pure culture, and many of these methods are still used today.

Koch started by using natural surfaces such as a potato slice to obtain pure cultures, but he quickly developed more reliable and reproducible growth media employing liquid nutrient solutions solidified with gelatin, and later with agar, an algal polysaccharide with excellent properties for this purpose. Along with his associate Walther Hesse, Koch observed that when a solid surface was incubated in air, masses of microbial cells called colonies developed, each having a characteristic shape and color (**Figure 1.34**). He inferred that each colony had arisen from a single bacterial cell that had grown to yield the mass of cells (see also Figure 1.3). Koch reasoned that each colony harbored a pure culture (a population of identical cells), and he quickly realized that solid media provided an easy way to obtain pure cultures. Richard Petri, another associate of Koch, developed the transparent double-sided "Petri dish" in 1887, and this quickly became the standard tool for obtaining pure cultures.

Koch was keenly aware of the implications his pure culture methods had for classifying microorganisms. He observed that colonies that differed in color and size (Figure 1.34) bred true and that cells from different colonies typically differed in size and shape and often in their nutrient requirements as well. Koch realized that these differences were analogous to the criteria taxonomists had established for the classification of larger organisms, such as plant and animal species, and he suggested that the different types of bacteria should be considered as "species, varieties, forms, or other suitable designation." Such insightful thinking was important for the rapid acceptance of microbiology as a new biological science, rooted as biology was in classification during Koch's era.

Koch and Tuberculosis

Koch's crowning scientific accomplishment was his discovery of the causative agent of tuberculosis. At the time Koch began this work (1881), one-seventh of all reported human deaths were caused by tuberculosis (Figure 1.13). There was a strong suspicion that

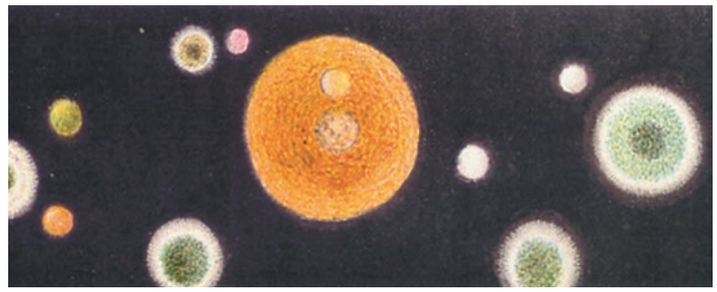


Figure 1.34 A hand-colored photograph taken by Walther Hesse of colonies formed on agar. The colonies include those of molds and bacteria obtained during Hesse's studies of the microbial content of air in Berlin, Germany, in 1882. From Hesse, W. 1884. "Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte. 2*: 182–207.

tuberculosis was a contagious disease, but the suspected agent had never been seen, either in diseased tissues or in culture. Following his successful studies of anthrax, Koch set out to demonstrate the cause of tuberculosis, and to this end he brought together all of the methods he had so carefully developed in his previous studies with anthrax: microscopy, staining, pure culture isolation, and an animal model system (Figure 1.33).

The bacterium that causes tuberculosis, *Mycobacterium tuberculosis*, is very difficult to stain because *M. tuberculosis* cells contain large amounts of a waxlike lipid in their cell walls. Nevertheless, Koch devised a staining procedure for *M. tuberculosis* cells in lung tissue samples. Using this method, he observed the blue, rod-shaped cells of *M. tuberculosis* in tubercular tissues but not in healthy tissues (**Figure 1.35**). Obtaining cultures of *M. tuberculosis* was not easy, but eventually Koch succeeded in growing colonies of this organism on a solidified medium containing blood serum. Under the best of conditions, *M. tuberculosis* grows slowly in culture, but Koch's persistence and patience eventually led to pure cultures of this organism from human and animal sources.

From this point Koch used his postulates (Figure 1.33) to obtain definitive proof that the organism he had isolated was the cause of the disease tuberculosis. Guinea pigs can be readily infected with *M. tuberculosis* and eventually succumb to systemic tuberculosis. Koch showed that tuberculous guinea pigs contained masses of *M. tuberculosis* cells in their lungs and that pure cultures obtained from such animals transmitted the disease to healthy animals. In this way, Koch successfully satisfied all four of his postulates, and the cause of tuberculosis was understood. Koch announced his discovery of the cause of tuberculosis in 1882, and for this accomplishment he was awarded the 1905 Nobel Prize for Physiology or Medicine. Koch had many other triumphs in the growing field of infectious diseases, including the discovery of the causative agent of cholera (the bacterium *Vibrio cholerae*) and the development of methods to diagnose infection with *M. tuberculosis* (the tuberculin skin test).

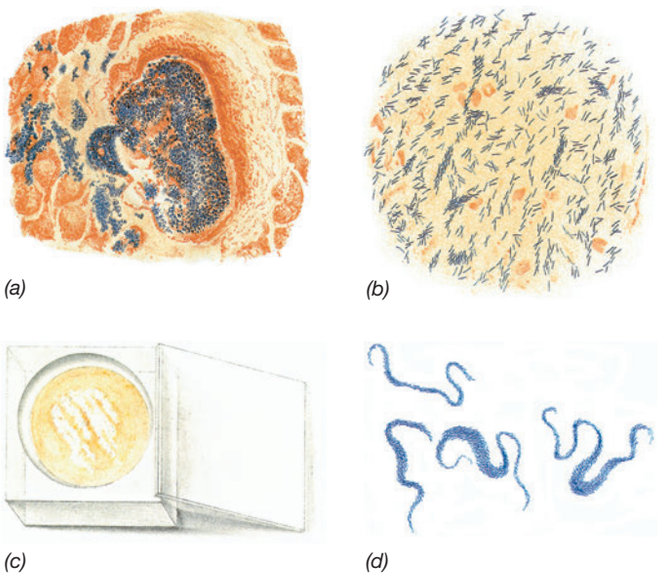


Figure 1.35 **Robert Koch's drawings of *Mycobacterium tuberculosis*.** *(a)* Section through infected lung tissue showing cells of *M. tuberculosis* (blue). *(b) M. tuberculosis* cells in a sputum sample from a tubercular patient. *(c)* Growth of *M. tuberculosis* on a glass plate of coagulated blood serum stored inside a glass box to prevent contamination. *(d) M. tuberculosis* cells taken from the plate in *c* and observed microscopically; cells appear as long, cordlike forms (▶ Section 16.11 and Figures 16.32 and 16.33). Original drawings from Koch, R. 1884. "Die Aetiologie der Tuberkulose." *Mittheilungen aus dem Kaiserlichen Gesundheitsamte 2*: 1–88.

Check Your Understanding

- How do Koch's postulates ensure that cause and effect of a given disease are clearly differentiated?
- What advantages do solid media offer for the isolation of microorganisms?
- What is a pure culture?

1.13 Discovery of Microbial Diversity

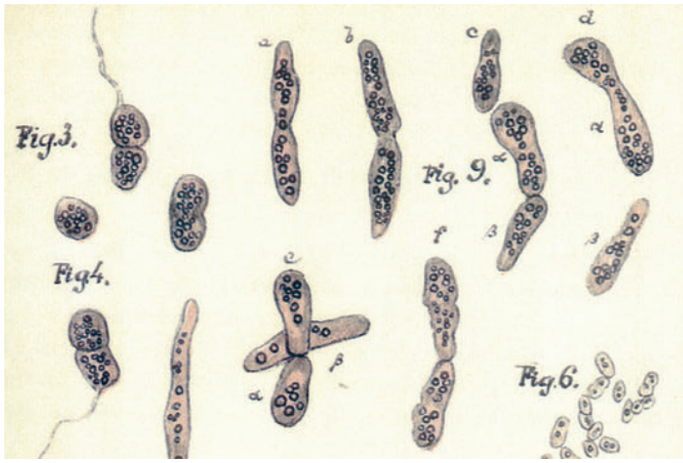
As microbiology entered the twentieth century, its initial focus on basic principles, methods, and medical aspects broadened to include studies of the microbial diversity of soil and water and the metabolic processes that microorganisms carried out in these habitats. Major contributors of this era included the Dutchman Martinus Beijerinck and the Russian Sergei Winogradsky.

Sergei Winogradsky and Chemolithotrophy

Sergei Winogradsky (1856–1953) was interested in the bacterial diversity of soils and waters and was highly successful in isolating several notable bacteria from natural samples. Winogradsky was particularly interested in bacteria that cycle nitrogen and sulfur compounds, such as the nitrifying bacteria and the sulfur bacteria (Chapters 14 and 15). He studied *Beggiatoa*, which are large sulfur-oxidizing bacteria found in marine sediments. *Beggiatoa* are morphologically distinctive and readily identified under the microscope (**Figure 1.36**), but Winogradsky found that they would not grow on the rich nutrient media used by Koch and Pasteur. In order to grow these *Beggiatoa*, Winogradsky designed a medium that chemically imitated the environment in which *Beggiatoa* lived. He showed that *Beggiatoa* are able to grow in the absence of organic nutrients, and that their growth requires only inorganic substances (compounds lacking carbon–carbon bonds). In this way, Winogradsky was the first to define **chemolithotrophy**, which is any metabolic process in which energy for growth is produced using only *inorganic* chemical compounds. Winogradsky also revealed that these chemolithotrophic bacteria obtain their carbon from CO_2, much like plants, though they get their energy from chemical reactions rather than from light. Winogradsky further showed that these organisms, which he called *lithotrophs* (meaning, literally, "stone eaters"), are widespread in nature.

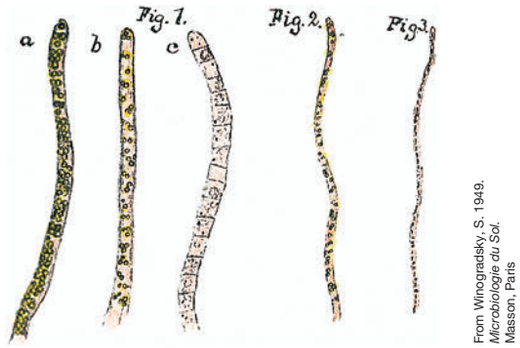
Martinus Beijerinck, the Enrichment Culture Technique, and Nitrogen Fixation

Martinus Beijerinck (1851–1931) was a professor at the Delft Polytechnic School in Holland and a contemporary of Winogradsky. Beijerinck's greatest contribution to the field of microbiology was his clear formulation of the *enrichment culture technique*. Enrichment cultures were used by both Beijerinck and Winogradsky to discover many unique forms of metabolism that we now know to be essential to nutrient cycling in nature.



From *Microbiologie du Sol*, used with permission

(a)



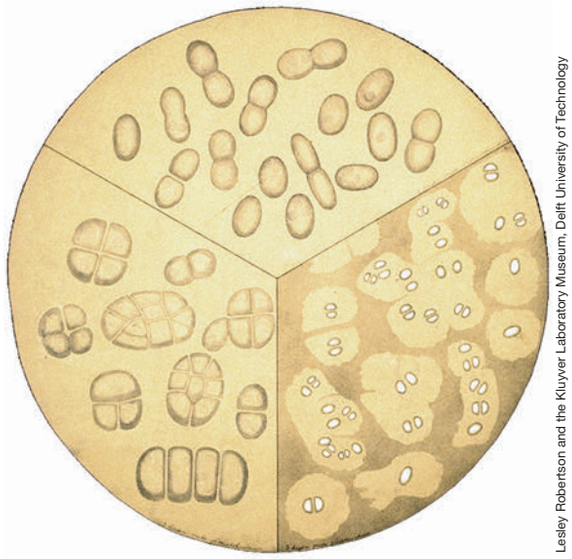
From Winogradsky, S. 1949. *Microbiologie du Sol.* Masson, Paris

(b)

Figure 1.36 Sulfur bacteria. The original drawings were made by Sergei Winogradsky in the late 1880s and then copied and hand-colored by his wife Hèléne. *(a)* Purple sulfur phototrophic bacteria. Figures 3 and 4 show cells of *Chromatium okenii* (compare with photomicrographs of *C. okenii* in Figures 1.1*a* and 1.11*d*, and 1.21*a*). *(b) Beggiatoa*, a sulfur chemolithotroph (compare with ▶ Figure 15.32*a*).

The growth media used by Pasteur and Koch (for example, yeast extract media) were rich in a wide variety of nutrients. These media support the growth of many microorganisms and are often suitable for growing pathogenic bacteria, but as Winogradsky discovered, most bacteria found in nature do not grow well on such media. Enrichment culture techniques are used to selectively encourage the growth of *specific* microorganisms. To do this, enrichment cultures employ culture media and selective incubation conditions that are deliberately designed to favor only certain types of microbes.

For example, Winogradsky used enrichment culture to isolate the first nitrogen-fixing bacterium. He devised a liquid growth medium that lacked a source of nitrogen, inoculated this medium with soil, and incubated it in the presence of air. He knew that all living things require nitrogen, and so when he observed bacteria growing in the medium he knew that they must be getting their nitrogen from N_2 in the air. In this way he enriched for and ultimately isolated the anaerobic nitrogen-fixing bacterium *Clostridium pasteurianum*, becoming the first to demonstrate the process of nitrogen fixation. The growth of nitrogen-fixing bacteria was favored in the enrichment culture because only those bacteria that can use nitrogen from the air can grow on media that lack a nitrogen source such as ammonia or nitrate. Beijerinck used a similar technique shortly thereafter to isolate the first aerobic nitrogen-fixing bacterium, *Azotobacter* (**Figure 1.37**). Beijerinck also devised a medium to isolate rhizobia and proved that these bacteria cause root nodules to form on legumes and that they perform nitrogen fixation within the nodules (Figure 1.14).



Lesley Robertson and the Kluyver Laboratory Museum, Delft University of Technology

Figure 1.37 Martinus Beijerinck and *Azotobacter*. The first aerobic nitrogen-fixing bacterium, *Azotobacter chroococcum*, was isolated by M. Beijerinck in 1900. The image is a painting by M. Beijerinck's sister, Henriëtte Beijerinck, showing cells of *Azotobacter chroococcum*. Beijerinck used such paintings to illustrate his lectures. Compare these drawings of *A. chroococcum* cells with the photomicrograph of cells of *Azotobacter* in Figure 15.24.

Using the enrichment culture technique, Beijerinck and Winogradsky isolated the first pure cultures of many important soil and aquatic microorganisms. For example, Winogradsky isolated the first nitrifying bacteria using an enrichment medium that contained ammonium salts and CO_2 since these chemolithotrophic bacteria oxidize ammonium as an energy source and are autotrophic. Beijerinck was the first to isolate sulfur-cycling bacteria such as sulfate-reducing bacteria and sulfur-oxidizing bacteria, fermentative bacteria such as the lactic acid bacteria, and many other physiological types of bacteria as well as microbial eukaryotes such as green algae.

Beijerinck was also the first person to observe a virus. While studying "mosaic disease" of tobacco, Beijerinck used selective filters to show that the infectious agent in this disease was smaller than any bacterium and that it somehow became incorporated into cells of the living host plant. In this insightful work, Beijerinck described not only the first virus, but also the basic principles of virology, which we expand upon in Chapters 5 and 11.

Check Your Understanding

- What is meant by the term "enrichment culture"?
- What is meant by the term "chemolithotrophy"? In what way are chemolithotrophs like plants?

IV • Molecular Biology and the Unity and Diversity of Life

Molecular analyses of microbial cells have unveiled the biochemical principles that govern all life and the evolutionary position of microbes in the tree of life.

The development of aseptic technique and methods for the enrichment, isolation, and propagation of bacteria at the end of the nineteenth century gave rise to explosive growth in the pace of microbiological discovery. Moreover, microbiologists realized that the ability to grow bacteria rapidly and in controlled laboratory conditions made them excellent model systems for exploring the fundamental nature of life.

1.14 Molecular Basis of Life

Experiments with bacterial cultures in the twentieth century were critical in forming the foundations of molecular biology, molecular genetics, and biochemistry. Microbiologists came to realize that while microorganisms were incredibly diverse, all cells appeared to operate on similar basic principles. Thus, the search was on for the overarching molecular processes that define life itself.

Unity in Biochemistry

Albert Jan Kluyver (1888–1956) was Beijerinck's successor at what was then called the Delft Institute of Technology. Kluyver recognized that though microbial diversity was tremendous, all microorganisms used many of the same biochemical pathways and their metabolic processes were governed by similar thermodynamic constraints. Kluyver promoted the study of comparative biochemistry to identify the unifying features of all cells. He famously proclaimed, "From elephant to butyric acid bacterium—it is all the same!" This was later reformulated by Jacques Monod (1910–1976) into the expression, "What is true for *E. coli* is also true for the elephant," a statement that proclaimed the importance of working with bacteria to understand the fundamental principles that govern all living things.

The use of microbes as metabolic model systems led to the discovery that certain macromolecules and biochemical reactions are universal, and that to understand their function in one cell is to understand their function in all cells. We will learn about these fundamental macromolecules and biochemical processes in later chapters. These discoveries were of central importance to understanding microbial evolution, and none were more important than the discovery of DNA as the molecular basis of heredity, a discovery that is less than 80 years old.

Cracking the Code of Life

In the early twentieth century, it was clear that some molecule carried the hereditary information from parent to offspring, but the molecular basis of heredity remained a mystery. Most biologists thought that proteins carried this hereditary information. DNA had been discovered but it was thought to be merely a structural molecule, and too simple in its composition to encode cellular functions. The hunt for the molecular basis of heredity began in earnest with a key experiment by the British bacteriologist Frederick Griffith (1879–1941).

Griffith worked with a virulent strain of *Streptococcus pneumoniae*, a cause of bacterial pneumonia in both humans and mice. This strain, strain S, produced a polysaccharide coat (that is, a capsule, ▶ Section 2.6) that caused cells to form smooth colonies on agar media and conferred the ability to kill infected mice (**Figure 1.38*a***).

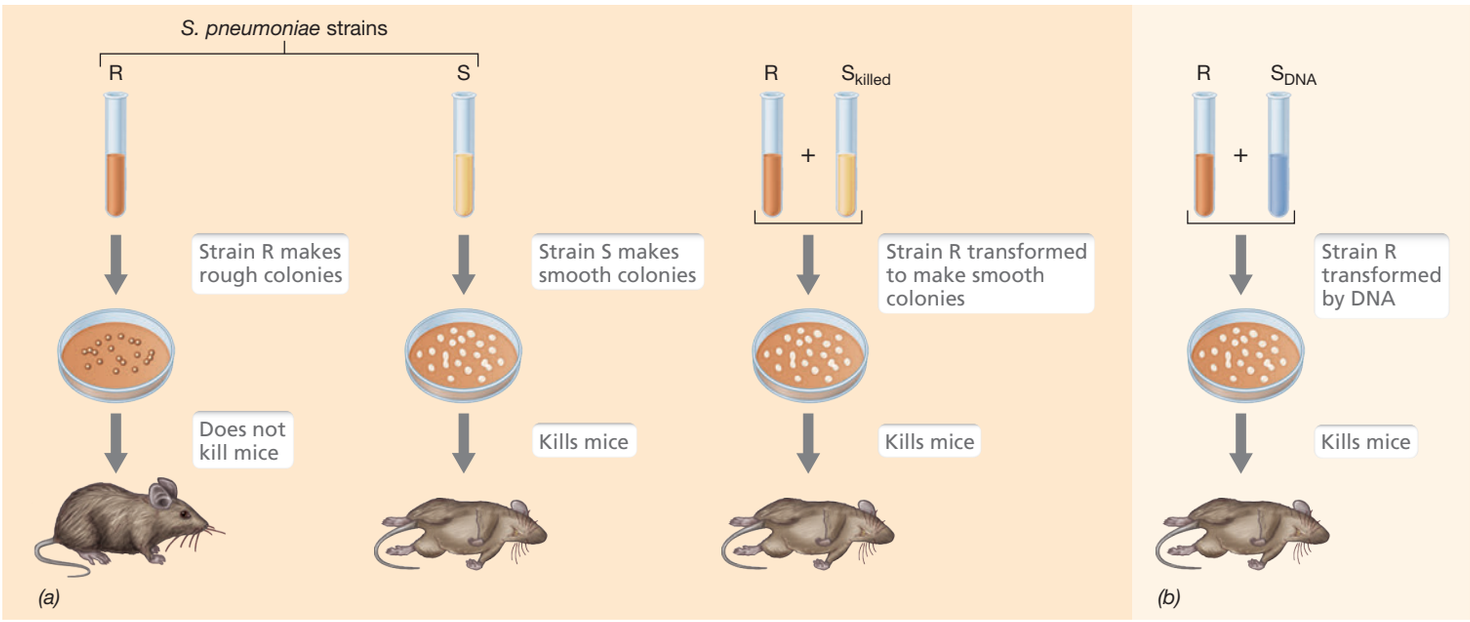


Figure 1.38 Early evidence that DNA is the molecular basis of heredity. *(a)* Griffith's experiment showed that bacteria can transfer genetic information. *Streptococcus pneumoniae* strain R makes rough colonies on agar media and does not kill mice, but strain S makes smooth colonies and does kill mice. Heat-killed cells of strain S do not cause disease, but if these killed cells are mixed with cells of strain R, then strain R is "transformed" to the S type and begins to make smooth colonies and kill mice. *(b)* The Avery–MacLeod–McCarty experiment showed that DNA contains genetic information. DNA isolated from strain S can transform strain R to cause disease, though the DNA itself does not cause disease. Degraded DNA (the *control* in the experiment) lacks the ability to transform strain R.

A related strain, strain R, lacked this polysaccharide and produced "rough" colonies that did not cause disease. However, Griffith observed that strain R could be *transformed* to type S, forming smooth colonies and causing disease, when it was mixed with the dead remains of cells of strain S (Figure 1.38*a*). He reasoned that some molecule that contained genetic information must have been transferred from strain R to strain S in this process, and this experiment showed that genetic transfer could be studied in bacteria.

Later, the Avery–MacLeod–McCarty experiment (1944), named for three scientists at the Rockefeller University, would show that this "transforming principle" was DNA. They treated the dead remains of cells of strain S with chemicals and enzymes that destroyed protein and left behind only DNA. They then repeated Griffith's experiment with the pure DNA of strain S and showed that this DNA was sufficient to cause transformation, causing strain R cells to become S-type cells and virulent (Figure 1.38*b*). They also demonstrated that transformation failed if the DNA from strain S was degraded. Collectively, these experiments proved that DNA is the genetic material of cells.

The discovery that DNA is the basis of heredity was followed by intense efforts to understand how this molecule stores genetic information. The structure of DNA was ultimately solved by James D. Watson (1928–) and Francis Crick (1916–2004) using X-ray diffraction images of DNA taken by their colleague Rosalind Franklin (1920–1958). They revealed that DNA is composed of a double helix that contains four nitrogenous bases: guanine, cytosine, adenine, and thymine, which form the genetic code (▶ Section 6.1). Later research would reveal how the genetic code is read from DNA and translated into protein, and these principles are covered in Chapter 6. Once again, however, this research to crack the code of life was enabled by a microbial model system, in this case, the bacterium *Escherichia coli*.

From DNA to Evolutionary Insight

Not long after the discovery that genetic information is encoded in the sequence of biological molecules, Emile Zuckerkandl (1922–2013) and Linus Pauling (1901–1994) proposed that molecular sequences could be used to reconstruct evolutionary relationships. They recognized that evolution, as described by Darwin, required variation in offspring and that these variations must be caused by changes in molecular sequences. They predicted that these sequence differences occur randomly in a clocklike fashion over time. This led to the conclusion that the evolutionary history of organisms is inscribed in the sequence of molecules such as DNA. Carl Woese seized upon these insights to pursue the ambitious goal of reconstructing the evolutionary history of all cells, and we explore the events that led up to this seminal discovery in evolutionary biology now.

Check Your Understanding

- Describe the experiments that proved DNA was the transforming principle described by Griffith.
- Why are microbes useful as model organisms to understand basic principles of biology?

1.15 Woese and the Tree of Life

Evolutionary relationships between microorganisms remained a mystery until it was discovered that certain molecular sequences maintain a record of evolutionary history. Here we will examine how the sequence of **ribosomal RNA (rRNA)** genes, present in all cells, revolutionized the understanding of microbial evolution and made it possible to construct the first universal tree of life. Ribosomal RNAs are components of the *ribosome*, the biosynthetic machine that all cells use to make proteins (Section 1.2).

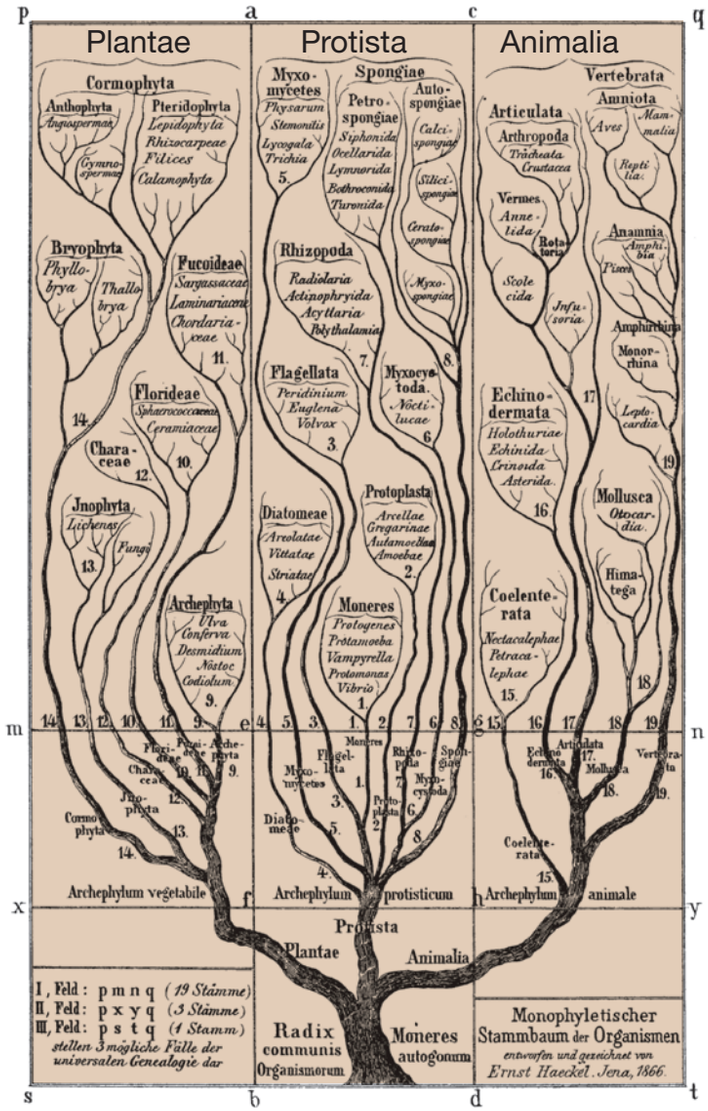
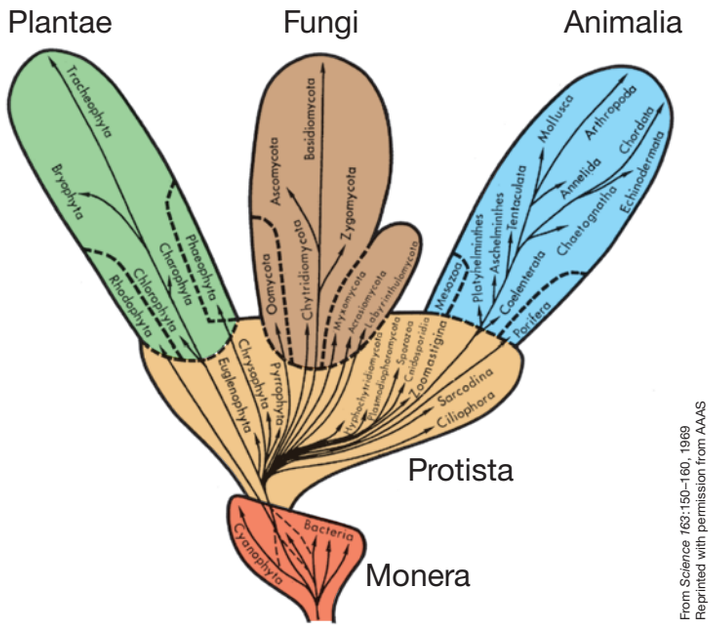


Figure 1.39 Early efforts to depict the universal tree of life. A conceptual tree of life proposed by Ernst Haeckel in *Generelle Morphologie der Organismen* (1866) shortly after Darwin published *On the Origin of Species* (1859). Haeckel proposed that four main groups of organisms (Plantae, Animalia, Protista, and Moneres) evolved from a common single-celled ancestor. The group that Haeckel proposed as Moneres included all microbes having prokaryotic cell structure, which we know today as *Bacteria* and *Archaea*.

Early Attempts to Portray the Evolutionary History of Life

For over a hundred years, following the 1859 publication of Charles Darwin's *On the Origin of Species*, evolutionary history was studied primarily with the tools of paleontology (through examining fossils) and comparative biology (through comparing the traits of living organisms). These approaches led to progress in understanding the evolution of plants and animals, but they were powerless to explain the evolution of microorganisms. The vast majority of microorganisms do not leave behind fossils, and their morphological and physiological traits provide few clues about their evolutionary history. Moreover, microorganisms do not share any morphological traits with plants and animals. Thus, it was impossible to create a robust evolutionary framework that included microorganisms.

The first attempt to depict the common evolutionary history of all living cells was published by Ernst Haeckel in 1866 (**Figure 1.39**). Haeckel correctly suggested that single-cell organisms, which he called *Monera* (labeled as Moneres near the root of the tree shown in Figure 1.39), were ancestral to other forms of life, but his scheme, which included plants, animals, and protists, did not attempt to resolve evolutionary relationships among microorganisms. The situation was little changed as late as 1969 when Robert Whittaker proposed a five-kingdom classification scheme (**Figure 1.40**). Whittaker's scheme distinguished the fungi as a distinct lineage, but it was still largely impossible to resolve evolutionary relationships among most microorganisms. Hence, our understanding of microbial evolution had made little progress since Haeckel's day.



From *Science* 163:150–160, 1969
Reprinted with permission from AAAS

Figure 1.40 Later efforts to depict the universal tree of life. A conceptual tree of life published by Robert H. Whittaker (1969). Whittaker recognized the same groups as Haeckel (Figure 1.39), but he was the first to propose adding Fungi as a distinct kingdom. Compare this tree to Haeckel's tree and consider how little our knowledge of microbial evolution had changed between 1866 and 1969, prior to the development of nucleic acid sequencing.

Molecular Sequence Data Revolutionized Microbial Phylogeny

Everything changed after the structure of DNA was discovered and it was recognized that evolutionary history is recorded in DNA sequences. Carl Woese (1928–2012), a professor at the University of Illinois (USA), realized in the 1970s that the sequence of ribosomal RNA (rRNA) molecules and the genes that encode them could be used to infer evolutionary relationships between organisms. Woese recognized that genes encoding rRNAs were excellent candidates for phylogenetic analysis because they were (1) present in all cells, (2) functionally constant, (3) highly conserved (slowly changing) in their nucleic acid sequences, and (4) of adequate length to provide a deep view of evolutionary relationships.

Woese compared the sequences of rRNA molecules from many microorganisms. Among the first microbes he examined were methanogens. To his astonishment, he found that the rRNA sequences from methanogens were distinct from those of both *Bacteria* and *Eukarya*, the only two domains recognized at that time. He named this new group of prokaryotic cells the *Archaea* (originally *Archaebacteria*) and recognized them as the third domain of life alongside the *Bacteria* and the *Eukarya* (**Figure 1.41*b***). More importantly, Woese demonstrated that the analysis of rRNA gene sequences could be used to reveal evolutionary relationships between *all cells*, providing the first effective tool for the evolutionary classification of microorganisms.

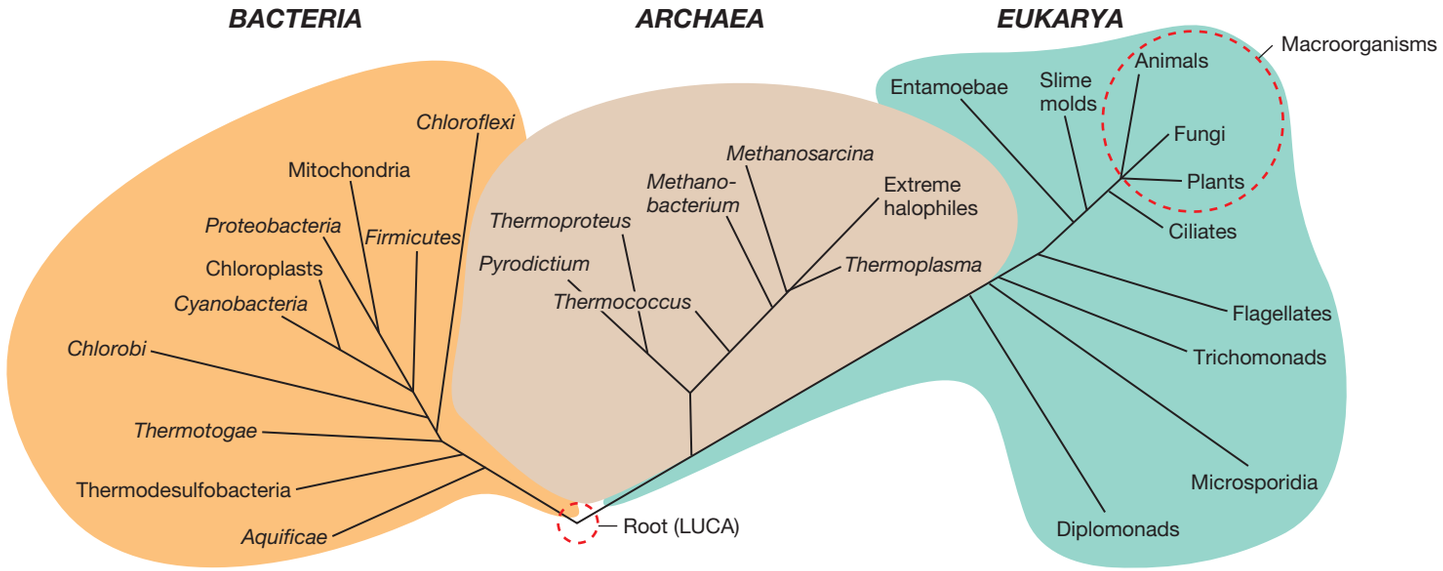
The Tree of Life Based on rRNA Gene Sequences

The universal tree of life based on rRNA gene sequences (Figure 1.41*b*) is a genealogy of all life on Earth. It is a true **phylogenetic tree**, a diagram that depicts the evolutionary history—the **phylogeny**—of all cells and clearly reveals the three domains. The root of the universal tree represents a point in time when all extant life on Earth shared a common ancestor, the last universal common ancestor, LUCA (Figures 1.10*b* and 1.41*b*). From the last universal common ancestor of all cells, evolution proceeded along two paths to form the domains *Bacteria* and *Archaea*. At some later time, the domain *Archaea* diverged to distinguish the *Eukarya* from the *Archaea* (Figures 1.10*b* and 1.41*b*). The three domains of cellular life are evolutionarily distinct and yet they share features indicative of their common descent from a universal cellular ancestor.

Revealing the Extent of Microbial Diversity

The tools Woese developed to build the tree of life were first used to determine the evolutionary history of microorganisms grown in pure culture (Figure 1.41*a*). However, Norman Pace (1942–), a professor at the University of Colorado (USA), realized that Woese's approach could be applied to rRNA molecules isolated *directly from the environment* as a way to probe the diversity of natural microbial communities without first cultivating their component organisms (Chapter 19). These *cultivation-independent* methods of rRNA gene analysis pioneered by Pace greatly improved our picture of microbial diversity (**Figure 1.42**). Despite many advances in the culturing of microorganisms from nature, many microbes have not yet been brought into laboratory culture. Hence, cultivation-independent methods provide us with valuable insights into the diversity and activities of microbes found in nature and oftentimes provide crucial hints for how they can be cultured. We also now know that although much progress has been made in describing microbial diversity, much of this diversity is yet to be explored and thus our work to describe the microbial world has only just begun.

(a)



(b)

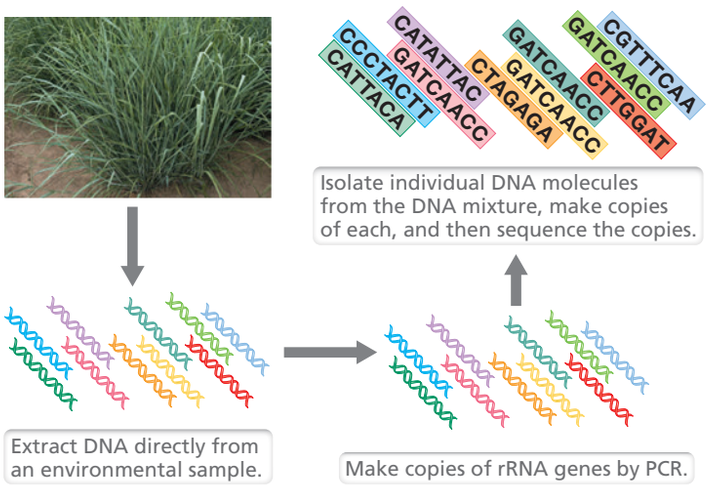
Figure 1.41 Evolutionary relationships and the phylogenetic tree of life. *(a)* The technology behind ribosomal RNA gene phylogenies. (1) DNA is extracted from cells. (2) Copies of the gene encoding rRNA are made by the polymerase chain reaction (PCR, a technique for making multiple copies of nucleic acid sequences, ▶ Section 12.1). (3) The gene is sequenced, and (4) the sequence is aligned and analyzed with sequences of the same gene from other organisms. A computer algorithm makes pairwise comparisons at each base and (5) generates a phylogenetic tree that depicts evolutionary relationships. In the example shown, the sequence differences are highlighted in yellow and are as follows: organism 1 versus organism 2, three differences; 1 versus 3, two differences; 2 versus 3, four differences. Thus organisms 1 and 3 are closer relatives than are 2 and 3 or 1 and 2. *(b)* The phylogenetic tree of life based on the analysis of rRNA gene sequences as proposed by Carl Woese (adapted from Woese, C.R., et al. *PNAS 87*: doi.org/10.1073/pnas.87.12.4576). The tree shows the three domains of organisms and a few representative groups in each domain. See detailed discussion of using molecular sequences as phylogenetic tools in Section 13.11.

Improvements in DNA sequencing technology have greatly improved our ability to study *Bacteria* and *Archaea* (Figure 1.42*b*). While the analysis of rRNA genes remains a cornerstone of microbial ecology, we now have the ability to sequence *entire microbial genomes* instead of just individual genes. Jo Handelsman (1959–), a professor at the University of Wisconsin (USA), was the first to propose *metagenomics*, a technique in which fragments of microbial genomes (or even entire genomes) can be recovered from a sample of environmental DNA. Metagenomics, the study of genomic information recovered directly from the environment, is currently providing us with profound insights on the evolution and diversity of life and new information on the metabolic potential of the microbial world.

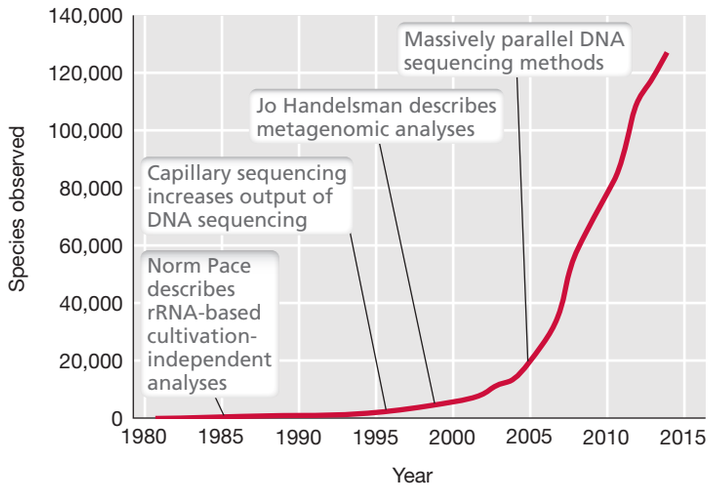
With an evolutionary framework of the microbial world in place, and with powerful new methods in hand to guide future research, advances in microbial diversity are happening quickly. Besides unveiling the previously hidden concept of three evolutionary domains of life, the contributions of Carl Woese and his associates have given microbiologists the tools they need to understand and explore the diversity of our microbial world. In the chapters that follow we will learn how microbiologists have unraveled many of the basic principles that govern living systems, how microorganisms control essential processes that sustain our biosphere, and how we can apply the lessons of microbiology to combat disease and improve our world.

Check Your Understanding

- What kinds of evidence support the three-domain concept of life?
- What is a phylogenetic tree?
- List three reasons why rRNA genes are suitable for phylogenetic analyses.



(a) **Cultivation-independent analysis of rRNA genes**



(b) **Revealing the extent of microbial diversity**

Figure 1.42 Analysis of environmental rRNA genes leads to discovery of new microbial species. *(a)* Norman Pace in 1985 described the first approach to sequence rRNA genes obtained directly from the environment without the need to grow microbes by cultivation in the laboratory. *(b)* Cultivation-independent analysis of microbial communities in environmental samples has revealed a tremendous diversity of microbial species. The application of cultivation-independent methods has been facilitated greatly by improvements in DNA sequencing technology over time. The graph shows the discovery over time of 16S rRNA gene sequences from natural samples that represent unique microbial species (data adapted from Schloss, P.D., et al. *mBio 6*: doi.org/10.1128/mBio.00201-16).

Go to **Mastering Microbiology** for videos, animations, practice tests, and more.

CHAPTER REVIEW

I • Exploring the Microbial World

1.1 Microorganisms are single-celled microscopic organisms that are essential for the well-being and functioning of other life forms and the planet. The tools of microscopy, microbial cultivation, molecular biology, and genomics are cornerstones of modern microbiology.

Q What are bacterial colonies and how are they formed?

1.2 Prokaryotic and eukaryotic cells differ in cellular architecture, and an organism's characteristics are defined by its complement of genes—its genome. All cells have a cytoplasmic membrane, a cytoplasm, ribosomes, and a double-stranded DNA genome. All cells carry out activities including metabolism, growth, and evolution.

Q What cellular structures distinguish prokaryotic and eukaryotic cells? What are some differences between a cell wall and a cytoplasmic membrane? In what types of organisms would you expect to find these structures?

1.3 Prokaryotic cells, while typically smaller than eukaryotic cells, can range widely in size and morphology. The most common shapes of prokaryotic cells are rods, cocci, and spirilla, and while most bacteria are 0.5 to 10 μm in length, some can be more than 600 μm long.

Q How do diffusion and surface-to-volume (*S*/*V*) ratio influence the size and shape of prokaryotic cells?

1.4 *Bacteria*, *Archaea*, and *Eukarya* are the major phylogenetic lineages (domains) of cells. The greatest diversity of microorganisms is found in the *Bacteria*, while many extremophiles are found within the *Archaea*. Microbial eukaryotes can vary tremendously in size, with some species being smaller than bacteria. Viruses are acellular and because of this cannot be placed on the tree of life.

Q What features (or lack of features) can be used to distinguish between viruses, *Bacteria*, *Archaea*, and *Eukarya*?

1.5 Diverse microbial populations were widespread on Earth for billions of years before plants and animals appeared. Microbes are abundant in the biosphere, and their activities greatly affect the chemical and physical properties of their habitats.

Q Why can Earth, in many ways, be considered a microbial planet? Which event in Earth's history eventually lead to the evolution of multicellular life forms?

1.6 Microorganisms can be both beneficial and harmful to humans, although many more microorganisms are beneficial (or even essential) than are harmful. Agriculture, food, energy, and the environment are all affected in major ways by microorganisms.

Q The gut microbiome directly benefits humans by digesting complex carbohydrates and synthesizing vitamins and other nutrients. In what other ways do microorganisms benefit humans?

II • Microscopy and the Origins of Microbiology

1.7 Microscopes are essential for studying microorganisms. Bright-field microscopy, the most common form of microscopy, employs a microscope with a series of lenses to magnify and resolve the image. The limit of resolution for a light microscope is about 0.2 μm.

Q What is the difference between magnification and resolution? Can either increase without the other?

1.8 An inherent limitation of bright-field microscopy is the lack of contrast between cells and their surroundings. This problem can be overcome by the use of stains or by alternative forms of light microscopy, such as phase contrast or dark field.

Q What is the function of staining in light microscopy? What is the advantage of phase-contrast microscopy over bright-field microscopy?

1.9 Differential interference contrast (DIC) microscopy and confocal scanning laser microscopy allow enhanced three-dimensional imaging or imaging through thick specimens.

Q How is confocal scanning laser microscopy different from fluorescence microscopy? In what ways are they similar? How does differential interference contrast microscopy differ from bright-field microscopy?

1.10 Electron microscopes have far greater resolving power than do light microscopes, the limits of resolution being about 0.2 nm. The two major forms of electron microscopy are transmission, used primarily to observe internal cell structure, and scanning, used to examine the surface of specimens.

Q Why does an electron microscope have a higher resolution, or greater resolving power, than a light microscope?

III • Microbial Cultivation Expands the Horizon of Microbiology

1.11 Louis Pasteur devised ingenious experiments proving that living organisms cannot arise spontaneously from nonliving matter. Pasteur introduced many concepts and techniques central to the science of microbiology, including sterilization, and developed a number of key vaccines for humans and other animals.

Q Explain the principle behind the Pasteur flask in studies on spontaneous generation. Why were the results of this experiment inconsistent with the theory of spontaneous generation?

1.12 Robert Koch developed a set of criteria called Koch's postulates for linking cause and effect in infectious diseases. Koch also developed the first reliable and reproducible means for obtaining and maintaining microorganisms in pure culture.

Q What are Koch's postulates and how did they influence the development of microbiology? Why are Koch's postulates still relevant today?

1.13 Martinus Beijerinck and Sergei Winogradsky explored soil and water for microorganisms that carry out important natural processes, such as nutrient cycling and the biodegradation of particular substances. Out of their work came the enrichment culture technique and the concepts of chemolithotrophy and nitrogen fixation.

Q What were the major microbiological interests of Martinus Beijerinck and Sergei Winogradsky? It can be said that both men discovered nitrogen fixation. Explain.

IV • Molecular Biology and the Unity and Diversity of Life

1.14 All cells share certain characteristics, and microorganisms are used as model systems to explore the fundamental processes that define life. The discoveries of DNA as the molecular basis of heredity, and of its structure and function, paved the way for progress in molecular genetics, microbial phylogeny, and genomics.

Q Describe the experiments that proved DNA to be the molecule at the basis of heredity.

1.15 Carl Woese discovered that ribosomal RNA (rRNA) sequences can be used to determine the evolutionary history of microorganisms, and in so doing, he constructed a modern phylogenetic tree of life that revealed a new domain, the *Archaea*. Analysis of rRNA sequences from the environment has shown that microbial diversity is exceptional and that the majority of microorganisms have not yet been cultivated.

Q What insights led to the reconstruction of the tree of life? Which domain, *Archaea* or *Eukarya*, is more closely related to *Bacteria*? What evidence is there to justify your answer?

APPLICATION QUESTIONS

1. Pasteur's experiments on spontaneous generation contributed to the methodology of microbiology, understanding of the origin of life, and techniques for the preservation of food. Explain briefly how Pasteur's experiments affected each of these topics.
2. Describe the lines of proof Robert Koch used to definitively associate the bacterium *Mycobacterium tuberculosis* with the disease tuberculosis. How would his proof have been flawed if any of the tools he developed for studying bacterial diseases had not been available for his study of tuberculosis?
3. Imagine that all microorganisms suddenly disappeared from Earth. From what you have learned in this chapter, why do you think that animals would eventually disappear from Earth? Why would plants disappear? By contrast, if all higher organisms suddenly disappeared, what in Figure 1.10*a* tells you that a similar fate would not befall microorganisms?

CHAPTER GLOSSARY

Aseptic technique a series of steps taken to prevent contamination of laboratory cultures and media

Cell wall a rigid layer present outside the cytoplasmic membrane; it confers structural strength on the cell

Chemolithotrophy a form of metabolism in which energy is generated from the oxidation of inorganic compounds and carbon is obtained typically from CO_2

Chromosome a genetic element, usually circular in prokaryotic cells, carrying genes essential to cellular function

Colony a macroscopically visible population of cells growing on solid medium, arising from a single cell

Contrast the ability to resolve a cell or structure from its surroundings

Culture a collection of microbial cells grown using a nutrient medium

Cytoplasm the fluid portion of a cell, enclosed by the cytoplasmic membrane

Cytoplasmic membrane a semipermeable barrier that separates the cell interior (cytoplasm) from the environment

Differentiation modification of cellular components to form a new structure, such as a spore

Domain one of the three main evolutionary lineages of cells: the *Bacteria*, the *Archaea*, and the *Eukarya*

DNA replication the process by which information from DNA is copied into a new strand of DNA

Enrichment culture a culture that employs highly selective laboratory methods for obtaining microorganisms from natural samples

Enzyme a protein (or in some cases an RNA) catalyst that functions to speed up chemical reactions

Eukaryotic having a membrane-enclosed nucleus and various other membrane-enclosed organelles; cells of *Eukarya*

Evolution a change over time in gene sequence and frequency within a population of organisms, resulting in descent with modification

Extremophiles microorganisms that inhabit environments characterized by extremes of temperature, pH, pressure, or salinity

Genome the total complement of genes contained in a cell or virus

Gram-negative a bacterial cell with a cell wall containing small amounts of peptidoglycan and an outer membrane

Gram-positive a bacterial cell whose cell wall consists chiefly of peptidoglycan; it lacks the outer membrane of gram-negative cells

Gram stain a differential staining procedure that stains cells either purple (gram-positive cells) or pink (gram-negative cells)

Growth in microbiology, an increase in cell number with time

Gut microbiome the microbial communities present in the animal gastrointestinal tract

Horizontal gene transfer the unidirectional transfer of genes between cells through a process uncoupled from reproduction

Intercellular communication interactions between cells using chemical signals

Koch's postulates a set of criteria for proving that a given microorganism causes a given disease

Macromolecules a polymer of monomeric units, for example proteins, nucleic acids, polysaccharides, and lipids

Magnification the optical enlargement of an image

Medium (plural, media) in microbiology, the liquid or solid nutrient mixture(s) used to grow microorganisms

Metabolism all biochemical reactions in a cell

Microbial community two or more populations of cells that coexist and interact in a habitat

Microbial ecology the study of the interaction of microorganisms with each other and their environment

Microorganism an organism that is too small to be seen by the unaided human eye

Morphology the physical appearance of a cell as determined by cell shape, for example: rod, coccus, or spirillum

Motility the movement of cells by some form of self-propulsion

Nucleoid the aggregated mass of DNA that makes up the chromosome(s) of prokaryotic cells

Nucleus a membrane-enclosed structure in eukaryotic cells that contains the cell's DNA genome

Organelle a bilayer-membrane-enclosed structure such as the mitochondrion, found in eukaryotic cells

Pathogen a disease-causing microorganism

Phylogenetic tree a diagram that depicts the evolutionary history of an organism; consists of nodes and branches

Phylogeny the evolutionary history of organisms

Plasmid an extrachromosomal genetic element that is usually not essential to the cell

Prokaryotic lacking a membrane-enclosed nucleus and other organelles; cells of *Bacteria* or *Archaea*

Pure culture a culture containing a single kind of microorganism

Resolution the ability to distinguish two objects as distinct and separate when viewed under the microscope

Ribosomal RNA (rRNA) the types of RNA found in the ribosome; some participate actively in protein synthesis

Ribosome a cytoplasmic particle composed of ribosomal RNA and protein, whose function is to synthesize proteins

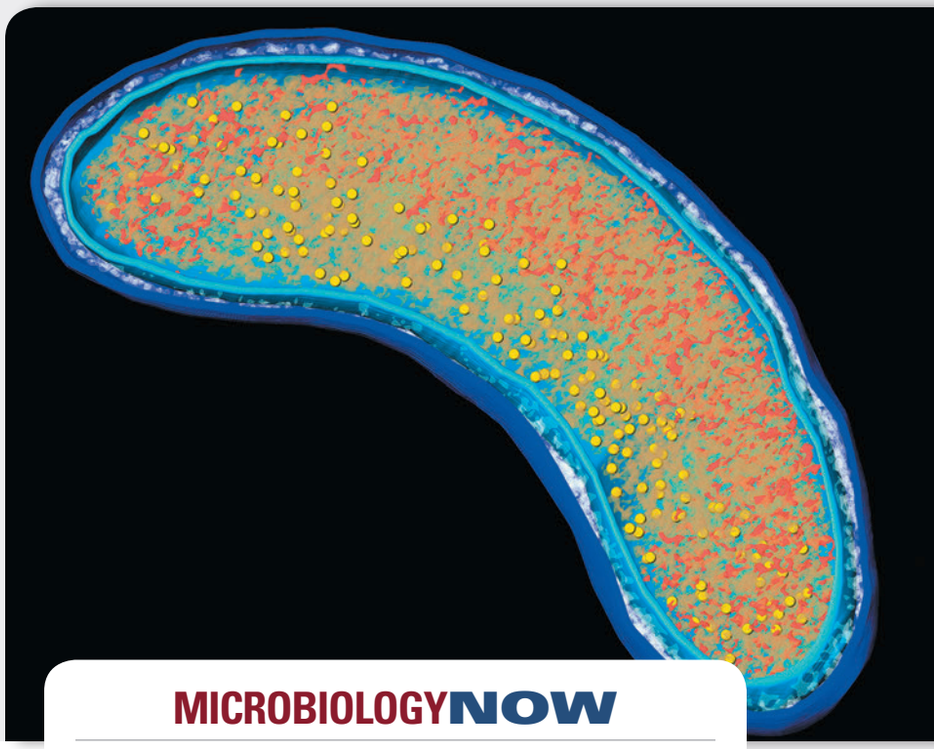
Spontaneous generation the hypothesis that living organisms can originate from nonliving matter

Sterile free of all living organisms (cells) and viruses

Transcription the synthesis of an RNA molecule complementary to one of the two strands of a double-stranded DNA molecule

Translation the synthesis of protein by a ribosome using the genetic information in a messenger RNA as a template

2 Microbial Cell Structure and Function



MICROBIOLOGYNOW

Exploring the Microbial Cell

New microscopic techniques are changing how we view microbial cells. Many cellular processes are performed by macromolecules and structures whose forms dictate their function. These structures are incredibly small and impossible to resolve with standard light microscopy. Cryogenic electron tomography (cryoET) images cells embedded within vitreous ice at ultra-low temperatures so they can be viewed in their native state without dehydration. During cryoET, samples are tilted in an electron microscope and images made at many different angles, much in the same way that a CT scan images a human body. These images are integrated using computer algorithms to generate complex, high-resolution, three-dimensional images that provide unprecedented structural detail. CryoET images have led to the discovery of new features within microbial cells and stunning new insights on well-known structures.

"*Candidatus* Pelagibacter ubique," one of the most abundant organisms on Earth, lives in microbial communities suspended in the upper waters of oceans worldwide. Cells of *Pelagibacter* are quite small (about 0.8 µm long), but their structures are readily visualized by cryoET (see image). Colors in the cryoET reconstruction indicate the outer membrane (blue), cytoplasmic membrane (cyan), peptidoglycan (white), cytoplasm (orange), nucleoid (red), and ribosomes (yellow). These new images reveal that the periplasm (the space between inner and outer membrane) in these cells is larger than expected and can occupy a remarkable 50–70% of cell volume in nongrowing cells. These images also reveal that the cytoplasm is divided into distinct regions that contain either nucleoid or ribosomes. Finally, mysterious structures such as membrane vesicles have been observed on the extracellular surface of the outer membrane and tethered to the cytoplasmic membrane (not shown). The function of these vesicles is unknown. New imaging techniques such as cryoET move microbiology forward and are revealing surprising complexity and diversity in the structures of prokaryotic cells.

Zhao, X., et al. 2017. Three-dimensional structure of the ultraoligotrophic marine bacterium "*Candidatus* Pelagibacter ubique." *Appl. Environ. Microbiol.* 83: e02807-16.

In this chapter, we consider the structure and function of microbial cells, both prokaryotic and eukaryotic. We will explore the components of microbial cells that control nutrient transport and cellular integrity but also several internal structures, which can be found in certain cells. We will also examine structures that give microbial cells the ability to move within their environment. We begin with two critical cell structures—the cytoplasmic membrane and cell wall—that collectively compose the cell envelope.

I • The Cell Envelope

The cytoplasm of the prokaryotic cell is surrounded by several layers that include a membrane, a cell wall, and other structures specific to the kind of cell. Collectively, these layers form the cell envelope and are essential for preserving the integrity and functioning of the cell.

The **cell envelope** consists of a series of layered structures that surround the cytoplasm and govern cellular interactions with the external environment. The cell envelope has many important functions: It governs transport of nutrients into the cell and wastes out of the cell, it is the site of energy conservation, it governs cell shape, it protects the cell from mechanical stress, and it can help the cell attach to surfaces and even protect the cell from attack.

The diversity of microorganisms is in part reflected in the composition of the cell envelope; but we will also learn that certain envelope structures are highly conserved and that knowledge of cell envelope structure can help us to identify and classify microorganisms. In the first few sections, we will learn about major components of cell envelope structure including the *cytoplasmic membrane*, *cell wall*, *outer membrane*, and *S-layers*. We start our tour by considering the cytoplasmic membrane, a structure found in all cells.

2.1 The Cytoplasmic Membrane

The **cytoplasmic membrane** surrounds the *cytoplasm*—the mixture of macromolecules and small molecules inside the cell—and separates it from the environment. The cytoplasmic membrane is physically rather weak but is an ideal structure for its major cellular function: selective permeability. In order for a cell to grow, nutrients must be transported inwards and waste products outwards. Both of these events occur across the cytoplasmic membrane. A variety of proteins located in the cytoplasmic membrane facilitate these reactions, and many other membrane proteins play important roles in energy metabolism.

Bacterial Cytoplasmic Membranes

The cytoplasmic membrane of all bacterial and eukaryal cells is a phospholipid bilayer containing embedded proteins. The cytoplasmic membrane is only 8–10 nanometers wide but can be resolved easily by transmission electron microscopy (**Figure 2.1*a***). Phospholipids are composed of both hydrophobic (water-repelling) and

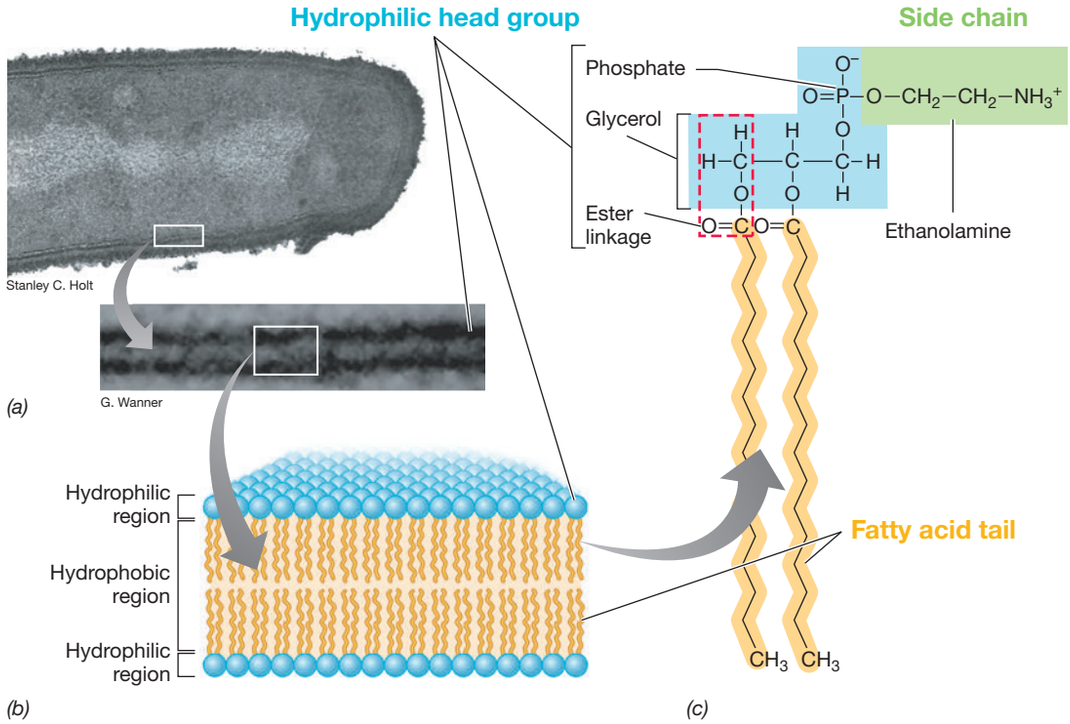


Figure 2.1 Phospholipid bilayer membrane. *(a)* Transmission electron micrograph of a cell with membrane region shown in detail. *(b)* General architecture of a bilayer membrane; phospholipids are composed of a hydrophilic head group (blue spheres) with fatty acid tails (yellow lines). The phospholipid head groups of the bilayer are visible in *a* as parallel dark lines, between which can be found a lighter region comprising the hydrophobic region of the membrane. *(c)* Structure of the phospholipid phosphatidylethanolamine. Each fatty acid side chain is connected to the head group by an ester bond (boxed with a red dashed line); ester linkages are a characteristic feature of lipids from *Bacteria* and *Eukarya* but not those of *Archaea*.

hydrophilic (water-attracting) components (Figure 2.1*b*). In *Bacteria* and *Eukarya*, the hydrophobic component consists of fatty acid "tails" and the hydrophilic component consists of a glycerophosphate (a glycerol molecule bound to a phosphate) and one of several other functional groups (such as sugars, ethanolamine, or choline) also bonded to the phosphate (Figure 2.1*c*). The membrane is comprised of two phospholipid layers in which the fatty acid tails associate together to form a hydrophobic region, leaving the hydrophilic "head groups" exposed to either the environment or the cytoplasm (Figure 2.1*b*). That is, the *outer* surface of the cytoplasmic membrane faces the environment while the *inner* surface faces the cytoplasm and interacts with the cytoplasmic milieu. This type of membrane structure is called a *lipid bilayer*, or a *unit membrane*.

Mastering Microbiology
Art Activity: Figure 2.5 Structure of the cytoplasmic membrane

A variety of proteins are attached to or integrated into the cytoplasmic membrane; membrane proteins typically have hydrophobic domains that span the membrane and hydrophilic domains that contact the environment or the cytoplasm (**Figure 2.2**). Proteins significantly embedded in the membrane are called *integral* membrane proteins. Many, though not all, integral membrane proteins extend completely across the membrane, and these are called *transmembrane* proteins. By contrast, *peripheral* membrane proteins are more loosely attached. Some peripheral membrane proteins are lipoproteins, proteins that contain a hydrophobic lipid tail that anchors the protein into the membrane. Other peripheral membrane proteins have residues that associate with the hydrophilic head groups of phospholipids, or they associate indirectly with membranes by binding to other proteins anchored in the membrane. Peripheral membrane proteins typically interact with integral membrane proteins in important cellular processes such as energy metabolism and transport.

Archaeal Cytoplasmic Membranes

The cytoplasmic membrane of *Archaea* is structurally similar to those of *Bacteria* and *Eukarya*, but the chemistry is somewhat different. In the lipids of *Bacteria* and *Eukarya* the hydrophobic *fatty acid* tails are bound to glycerol by *ester* linkages (Figure 2.1); in contrast, the lipids of *Archaea* have hydrophobic *isoprenoid* (rather than fatty acid) tails, which are bound to glycerol by *ether* bonds (**Figure 2.3**). The hydrophobic region of archaeal membranes is formed from repeating units of the five-carbon hydrocarbon *isoprene*, rather than from fatty acids (compare Figures 2.1 and 2.3).

The cytoplasmic membrane of *Archaea* is constructed from either phosphoglycerol diethers, which can have C_{20} side chains (called a *phytanyl* group), or diphosphoglycerol tetraethers (C_{40} side chains, called a *biphytanyl* group) (Figure 2.3). In the tetraether lipid structure, the ends of the inwardly pointing isoprenoid chains are covalently linked at their termini to form a *lipid monolayer* (Figure 2.3*c*) instead of a lipid bilayer (Figure 2.3*a*) membrane.

Archaeal lipids can have many different isoprenoid chains including some that contain ring structures. For example, *crenarchaeol*, a

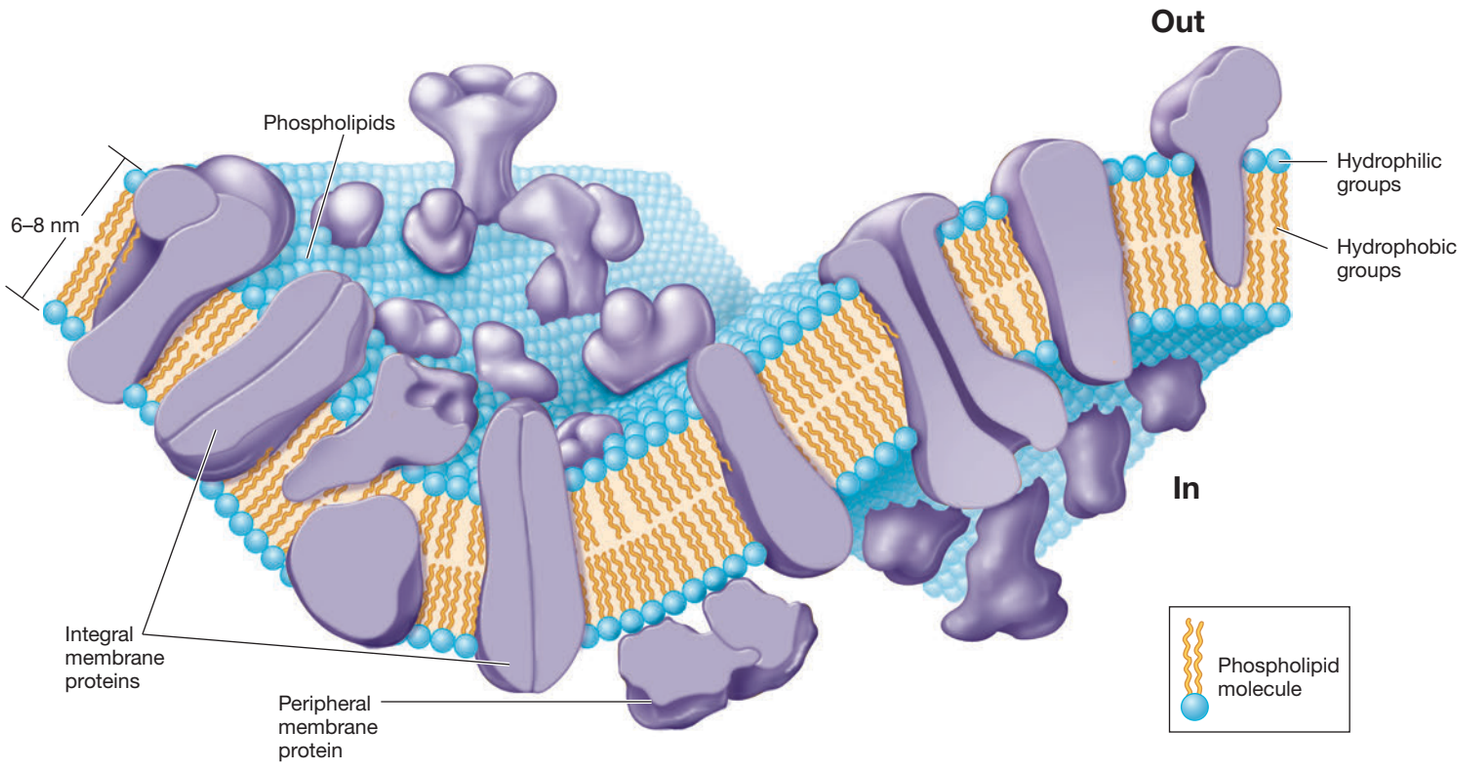


Figure 2.2 Structure of the cytoplasmic membrane. The inner surface (**In**) faces the cytoplasm and the outer surface (**Out**) faces the environment. Phospholipids compose the matrix of the cytoplasmic membrane with proteins embedded (integral) or surface associated (peripheral). The general design of the cytoplasmic membrane is similar in both prokaryotic and eukaryotic cells, although there can be differences in the chemistry between different species. Note that this membrane is shown in a relaxed shape to better illustrate its inner and outer surfaces; in a living cell, cytoplasmic turgor pressure would cause the membrane to have convex curvature.

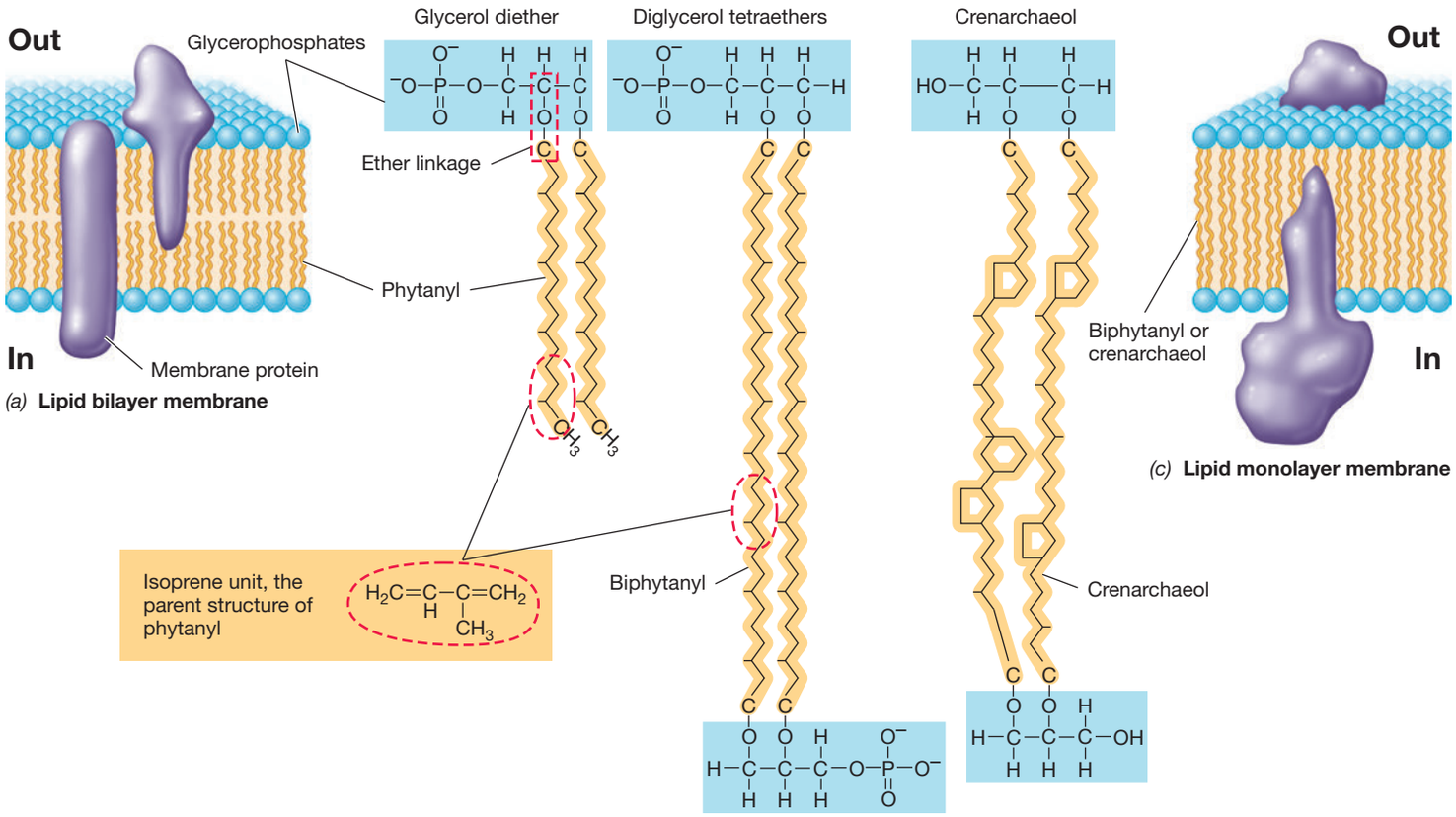


Figure 2.3 **Major lipids of *Archaea* and the architecture of archaeal membranes.** *(a, b) Archaea* can have lipid bilayers composed of phosphoglycerol diether lipids. The hydrophobic portions of archaeal lipids are comprised of isoprenoid chains synthesized from repeated units of isoprene (in dashed red ovals); this contrasts with the lipids of *Bacteria* and *Eukarya*, which have fatty acid tails (Figure 2.1). Note that these isoprenoids are bonded to glycerol by an ether linkage (in dashed red box). *(b, c)* Some *Archaea* can also have lipid monolayers composed of diphosphoglycerol tetraether lipids or other isoprenoid lipids such as crenarchaeol. The isoprenoid lipids in *b* are phytanyl (C_{20}), biphytanyl (C_{40}), and crenarchaeol. Isoprene lipids can often contain 5- and 6-carbon rings such as those present in crenarchaeol. The membrane structure in *Archaea* may form a lipid bilayer or a lipid monolayer (or a mix of both).

common membrane lipid in cells of *Thaumarchaeota* (a major phylum of *Archaea*, ▶ Section 17.5) contains four C_5 rings and one C_6 ring (Figure 2.3*b*). These rings affect the chemical properties of the lipids and thus influence membrane function. As in other organisms, the polar head groups in archaeal lipids can be sugars, ethanolamine, or a variety of other molecules.

Despite differences in chemistry between the cytoplasmic membranes of *Archaea* and organisms in the other phylogenetic domains, the fundamental construction of the archaeal cytoplasmic membrane—inner and outer hydrophilic surfaces and a hydrophobic interior—is the same as that of membranes in all cells. Obviously, evolution has selected this fundamental design as the best solution to the major functions of the cytoplasmic membrane, an issue we turn to now.

Cytoplasmic Membrane Function

The cytoplasmic membrane has at least *three* major functions (**Figure 2.4**). First, it is the cell's permeability barrier, preventing the passive leakage of solutes into or out of the cell. Second, the cytoplasmic membrane anchors several proteins that catalyze a suite of key cell functions. And third, the cytoplasmic membrane of *Bacteria* and *Archaea* plays a major role in energy conservation and consumption.

The cytoplasmic membrane is a barrier to the diffusion of most substances, especially polar or charged molecules. Because the cytoplasmic membrane is so impermeable, most substances that enter or leave the cell must be carried in or out by *transport proteins*. These are not simply ferrying proteins but instead function to *accumulate* solutes against the concentration gradient, a process that diffusion alone cannot do (**Figure 2.5**). Transport, which requires energy, ensures that the cytoplasm has sufficient concentrations of the nutrients it needs to perform biochemical reactions efficiently.

Transport proteins typically display high sensitivity and high specificity. If the concentration of a solute is high enough to saturate the transporter, which often occurs at the very low concentrations of nutrients found in nature, the rate of uptake can be near maximal (Figure 2.5). Some nutrients are transported by a low-affinity transporter when the nutrient is present at *high* external concentration and by a separate, typically higher-affinity, transporter when the nutrient is present at *low* concentration (Figure 2.5).

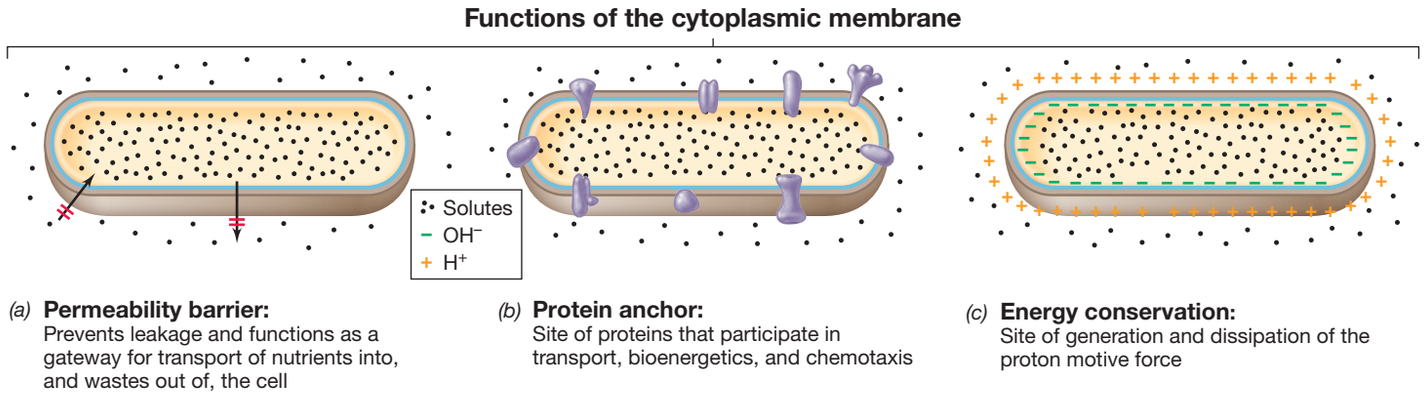


Figure 2.4 The major functions of the cytoplasmic membrane. Although physically weak, the cytoplasmic membrane controls at least three critically important cellular functions: maintaining selective permeability, anchoring proteins, and conserving energy.

Moreover, many transport proteins transport only a single kind of molecule while others carry a related class of molecules, such as different sugars or different amino acids. This economizing reduces the need for separate transport proteins for each different sugar or amino acid.

In addition to its permeability and transport functions, the cytoplasmic membrane of *Bacteria* and *Archaea* is a major site of both energy conservation and energy consumption. We discuss in Chapter 3 how the cytoplasmic membrane can be energized when protons (H^+) are separated from hydroxyl ions (OH^-) across the membrane surface (Figure 2.4*c*). This charge separation creates an energized state of the membrane called the *proton motive force*, analogous to the potential energy present in a charged battery. Dissipation of the proton motive force can be coupled to several energy-requiring reactions, such as transport, cell locomotion, and the biosynthesis of ATP. In eukaryotic microbial cells, although transport across the cytoplasmic membrane is just as necessary as it is in prokaryotic cells, energy conservation takes place in the membrane systems of the cell's key organelles, the mitochondrion (respiration) and chloroplast (photosynthesis), as we will see later in this chapter.

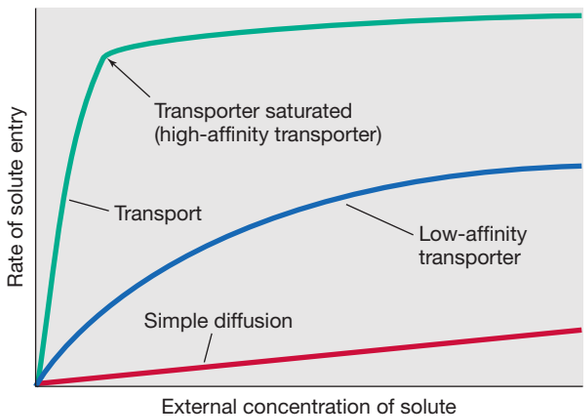


Figure 2.5 The importance of transport in membrane function. In both types of transport, the uptake rate shows saturation at relatively low external solute concentrations. Both high-affinity and low-affinity transport systems are depicted.

Check Your Understanding

- Draw the basic structure of a lipid bilayer and label the hydrophilic and hydrophobic regions. Why is the cytoplasmic membrane a good permeability barrier?
- How are the membrane lipids of *Bacteria* and *Archaea* similar, and how do they differ?
- Describe the major functions of the cytoplasmic membrane.
- Which molecule types can pass through the phospholipid bilayer directly by simple diffusion?

2.2 Transporting Nutrients into the Cell

In Section 2.1 we learned how the structure of the cytoplasmic membrane is an effective barrier to leakage; solutes leak neither into nor out of a living cell. However, selective transport is a major function of the cytoplasmic membrane. In order for cells to survive and grow, they must transport nutrients across the membrane and into the cell and they must export wastes out of the cell. Substances are transported across the cytoplasmic membrane through membrane-spanning integral membrane proteins (Figure 2.2). Some transporters also require the action of peripheral membrane proteins. We consider the most common of these transport systems here, with a focus on the well-studied transporters widespread in *Bacteria* and *Archaea*.

Active Transport and Transporters

Active transport is the process by which cells accumulate solutes against the concentration gradient. Three basic mechanisms of active transport are found in prokaryotic cells. A **simple transport system** consists only of a transmembrane transport protein, **group translocation** employs a series of proteins in the transport event, and **ABC transport systems** consist of three components: a binding protein, a transmembrane transporter, and an ATP-hydrolyzing protein (**Figure 2.6**). Each of these transport systems is energy-driven, be it from the proton motive force, ATP, or some other energy-rich compound.

The transmembrane component of virtually all transport systems is composed of a polypeptide containing 12 regions (called *domains*) that weave back and forth through the membrane to form a channel, and it is through this channel that the solute is transported into

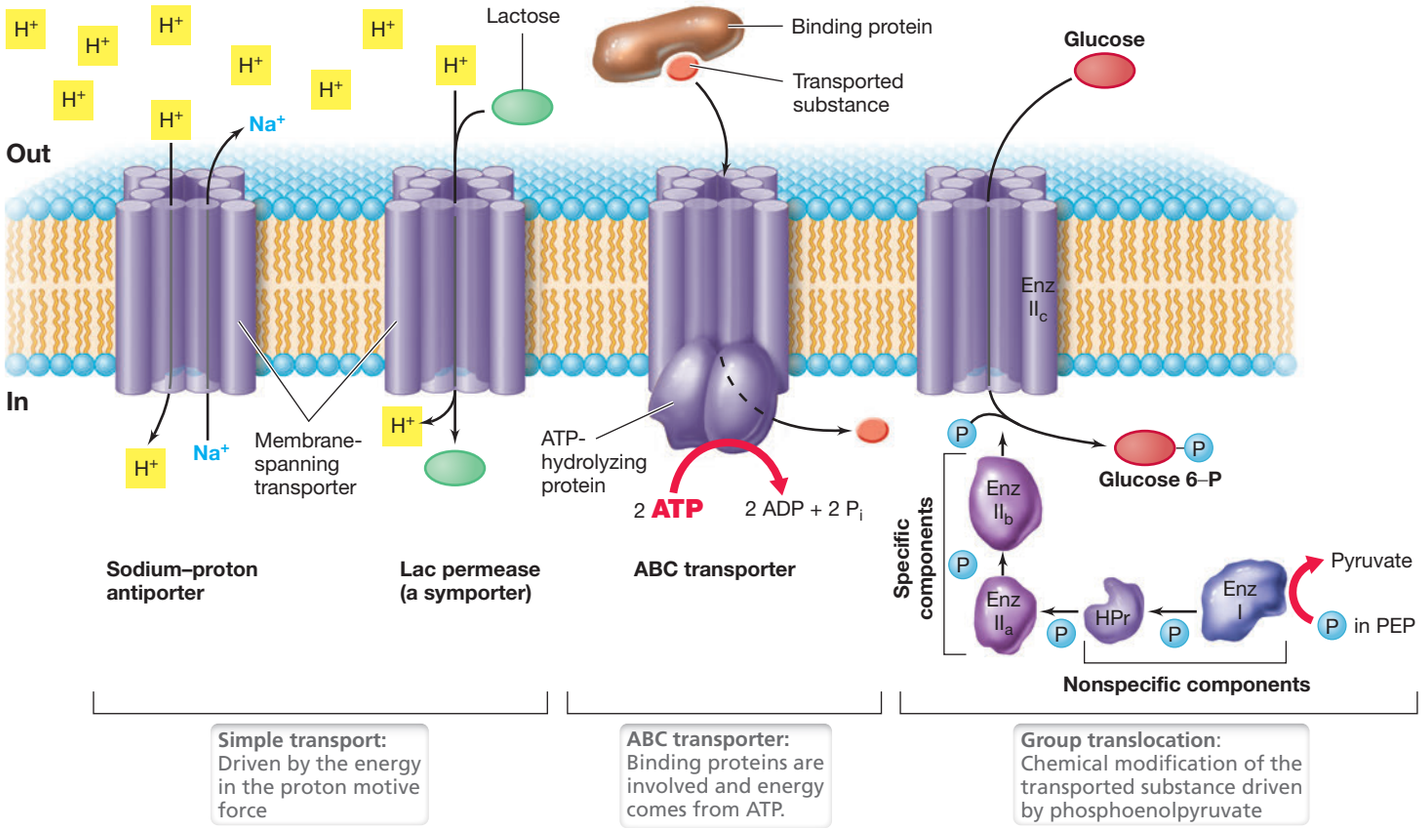


Figure 2.6 The three classes of transport systems. Transmembrane transporters are generally composed of a polypeptide that has 12 α-helices (each shown as a cylinder) that aggregate to form a channel through which solutes can cross the membrane. In simple transport the movement of a solute is coupled with the dissipation of an electrochemical gradient such as the proton motive force. ABC transporters have three components: a binding protein that has high affinity for a substrate, a transmembrane protein channel, and a cytoplasmic ATP-hydrolyzing protein, which supplies the energy required to drive substrate transport. In group translocation, the substance transported is chemically modified upon entering the cell. For example, the glucose group translocation system has five proteins: Enzyme (Enz) I, Enzymes II_a, II_b, and II_c, and HPr. A phosphate cascade occurs from phosphoenolpyruvate (PEP) to Enz II_c, and the latter protein actually transports and simultaneously phosphorylates the sugar. Proteins HPr and Enz I are nonspecific and participate in the transport of any sugar, while the three components of Enz II are specific for a particular sugar. Note how simple transporters and the ABC system transport substances without chemically modifying them, whereas group translocation results in chemical modification (in this case phosphorylation) of the transported substance.

the cell. Transport is linked to a conformational change in this transmembrane protein complex that occurs when it binds its specific solute. Like a gate swinging open, this conformational change sweeps the solute into the cell.

Simple Transporters and Group Translocation

Simple transport reactions are driven by the energy inherent in the proton motive force (Figure 2.4*c*). The two major transport events catalyzed are either *symport* reactions (where a solute and a proton are cotransported in the same direction) or *antiport* reactions (where a solute and a proton are transported in opposite directions) (Figure 2.6). A classic example of a simple transporter is the uptake of the sugar lactose by way of the *lac permease*, a well-studied symporter in *Escherichia coli*. As each lactose molecule enters the cell, the potential energy in the proton motive force is diminished slightly by the cotransport of a proton (Figures 2.4*c* and 2.6). The net result is the energy-driven accumulation of lactose in the cytoplasm against the concentration gradient. Many other solutes enter by the activity of their own simple symporters, including phosphate, sulfate, and several different organic compounds.

Group translocation differs from simple transport in two important ways: (1) the transported substance is *chemically modified* during the transport process, and (2) an energy-rich organic compound (rather than the proton motive force) drives the transport event. The best-studied group translocation systems transport the sugars glucose, mannose, and fructose in *E. coli*. During uptake, these compounds are phosphorylated by the *phosphotransferase system* (Figure 2.6). The phosphotransferase system consists of a family of five proteins that work in concert to transport any given sugar. Before the sugar is transported, the proteins in the phosphotransferase system are themselves alternately phosphorylated and dephosphorylated in a cascading fashion until Enzyme II_c phosphorylates the sugar as it enters the cytoplasm (Figure 2.6). A protein called HPr, the enzyme that phosphorylates HPr (Enzyme I), and Enzyme II_a are all cytoplasmic proteins. By contrast, Enzyme II_b is a peripheral membrane protein and Enzyme II_c is the transmembrane component.

In the phosphotransferase system, HPr and Enzyme I are *nonspecific* components and participate in the uptake of several different sugars. By contrast, distinct Enzyme II proteins exist, one set for each different sugar transported. Energy to drive the phosphotransferase system comes from phosphoenolpyruvate, an energy-rich intermediate in glycolysis (▶ Sections 3.4 and 3.6).

ABC Transporter Systems

ABC transporters are modular systems that have three components: a binding protein, a transmembrane protein channel, and an ATP-hydrolyzing protein (Figure 2.6). The ABC stands for *A*TP-*b*inding *c*assette, a structural feature of proteins that bind ATP. More than 200 different ABC transport systems are known, and these catalyze the uptake of a wide variety of organic and inorganic compounds.

Substrate-binding proteins are present outside of the cell, where they bind to a specific substrate and enable its transport into the cell. A characteristic property of binding proteins is their extremely high substrate affinity. These proteins can bind their specific substrate even when it is present at extremely low concentration; for example, less than 1 micromolar (10^{-6} M). Once its specific substrate is bound, the binding protein interacts with its respective transmembrane component to transport the substrate into the cell driven by the energy in ATP (Figure 2.6).

We move on now from our coverage of the cytoplasmic membrane to consider components of the cell envelope that confer structural strength on the cell, something the membrane cannot do.

Check Your Understanding

- Compare and contrast simple transporters, the phosphotransferase system, and ABC transporters in terms of (1) energy source, (2) chemical alterations of the solute during transport, and (3) number of proteins required.
- Which major characteristic of ABC transport systems makes them ideal for organisms living in nutrient-poor environments?

2.3 The Cell Wall

The cytoplasm of prokaryotic cells maintains a high concentration of dissolved solutes that creates significant osmotic pressure—about 2 atm (203 kPa); this is about the same as the pressure in an automobile tire. This osmotic pressure is sufficient to cause the cell membrane to burst and the cell to die—a process called *cell lysis*. To withstand this *turgor pressure*, the cell envelopes of most *Bacteria* and *Archaea* have a layer outside the cytoplasmic membrane called the *cell wall*. Besides protecting against osmotic lysis, cell walls also maintain cell shape and rigidity.

The cell envelopes of most *Bacteria* can be classified as being either *gram-positive* or *gram-negative* based on their organization and cell wall structures. The structures of gram-positive and gram-negative cell envelopes differ markedly as viewed in the electron microscope (**Figure 2.7**). The cell envelope of a gram-positive cell typically contains a cytoplasmic membrane and a thick cell wall, whereas a gram-negative cell has a cytoplasmic membrane, a thin cell wall, an outer membrane (Figure 2.7), and a periplasm, which is a compartment between the cytoplasmic and outer membranes. We will consider the outer membrane and periplasm in the next section. The names we use to describe the typical gram-positive and gram-negative cell envelopes are based on their Gram stain reactions (◀ Section 1.8). The Gram stain reaction is determined primarily by the thickness of the cell wall rather than the number of layers in the cell envelope, and so Gram stain reaction does not always correlate with cell envelope structure. However, the Gram stain reaction is sufficiently predictive of cell envelope structure in *Bacteria* that the names of the two most common bacterial cell envelopes—gram-positive and gram-negative—are based on their typical reactions to the Gram stain.

Knowledge of cell wall envelope structure and function is important not only for understanding the biology of microbial cells but also for medical reasons. Certain antibiotics, for example, the penicillins and cephalosporins, target bacterial cell wall synthesis, leaving the cell susceptible to osmotic lysis. Since human and other animal cells lack cell walls and are therefore not a target of such antibiotics, these drugs are of obvious benefit for treating bacterial infections. The major component of the bacterial cell wall, and a target of many antibiotics, is a molecule called *peptidoglycan*, and we consider this molecule in detail now.

Bacterial Cell Walls

The cell walls found in *Bacteria* contain a rigid polysaccharide called **peptidoglycan** that confers structural strength on the cell. Peptidoglycan is found in all *Bacteria* that contain a cell wall, but it is unique to *Bacteria* and is not found in *Archaea* or *Eukarya*. The sugar backbone of peptidoglycan is composed of alternating repeats of two modified glucose residues called *N-acetylglucosamine* and *N-acetylmuramic acid* joined by a β-1,4 linkage (**Figure 2.8**). Attached to the latter residue is a short peptide side chain. The amino acid composition of this peptide side chain can vary considerably between bacterial species. In *Escherichia coli* this peptide contains the amino acids L-alanine, D-alanine, D-glutamic acid, and diaminopimelic acid (DAP), though in other bacteria, L-lysine can be substituted for DAP. The presence of D stereoisomer amino acids, D-alanine and D-glutamic acid, is an unusual feature of peptidoglycan since proteins are always constructed of L-amino acids. These constituents are connected in an ordered way to form the *glycan tetrapeptide* (Figure 2.8), and long chains of this basic unit form peptidoglycan.

Strands of peptidoglycan run parallel to each other around the circumference of the cell (**Figure 2.9**). The peptide side chains of adjacent peptidoglycan strands are cross-linked together by covalent peptide bonds (Figure 2.9*a*), and in this way, the peptidoglycan forms one single enormous molecule. In gram-negative bacteria, the cross-links form primarily between the amino group of DAP on one glycan strand and the carboxyl group of the terminal D-alanine on the adjacent glycan strand (Figure 2.9*a*). The cell wall in the gram-negative cell envelope is 2–7 nm thick consisting primarily of a single layer of peptidoglycan, though it can be up to three layers thick in some places (Figure 2.9*c*).The peptidoglycan mesh so formed is flexible and porous, but strong enough to resist turgor pressure and prevent rupture of the cytoplasmic membrane and cell lysis. Additional strength against osmotic lysis in gram-negative bacteria is provided by the outer membrane, as discussed in the next section.

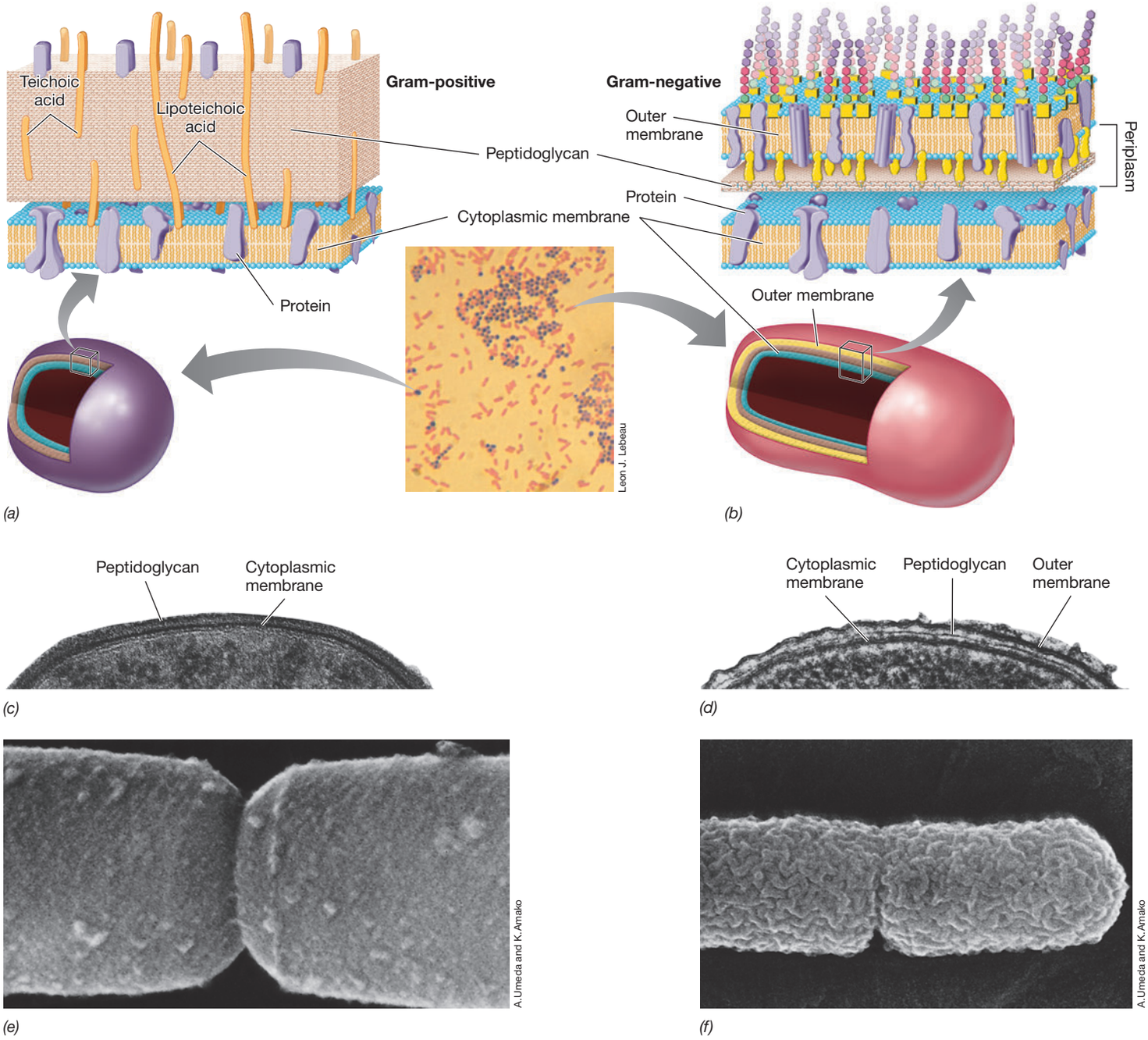


Figure 2.7 **Cell envelopes of *Bacteria*.** *(a, b)* Schematic diagrams of gram-positive and gram-negative cell envelopes; the Gram stain procedure was described in Section 1.8 and shown in Figure 1.23. The photo of Gram-stained bacteria in the center shows cells of *Staphylococcus aureus* (purple, gram-positive) and *Escherichia coli* (pink, gram-negative). *(c, d)* Transmission electron micrographs showing the cell wall of a gram-positive bacterium and a gram-negative bacterium, respectively. *(e, f)* Scanning electron micrographs of gram-positive and gram-negative bacteria, respectively. Note differences in surface texture. Each cell is about 1 μm wide.

The typical bacterial gram-positive cell envelope contains a thick peptidoglycan cell wall, which can measure 20 to 35 nm in thickness and is usually much thicker than the wall of gram-negative organisms. As much as 90% of the gram-positive cell envelope can consist of peptidoglycan. Whereas the gram-negative cell wall typically contains only a single layer of peptidoglycan, the gram-positive cell wall can be 15 or more layers thick (**Figure 2.10*a***). The peptidoglycan of the gram-positive cell wall is stabilized three-dimensionally by peptide cross-links, which form between adjacent peptidoglycan strands both horizontally and vertically. In gram-positive bacteria, peptide cross-links often contain a short peptide "interbridge," the kinds and numbers of amino acids in the interbridge varying between species. In the gram-positive bacterium *Staphylococcus aureus*, for example, the interbridge often consists of five glycines (Figure 2.9*b*).

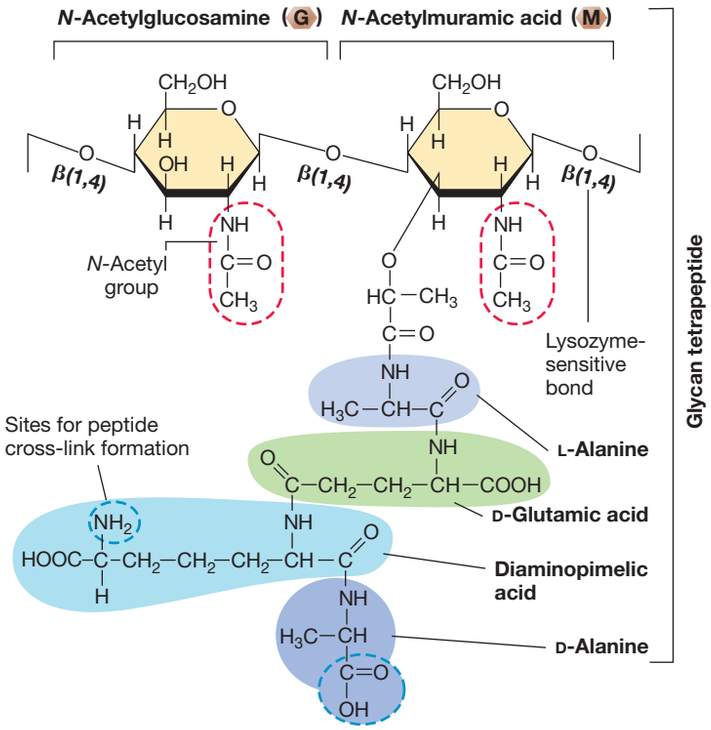


Figure 2.8 Structure of the repeating unit in peptidoglycan, the glycan tetrapeptide. The structure given is that for the peptidoglycan of *Escherichia coli* and most other gram-negative *Bacteria*. Cross-links can be formed between adjacent peptide side chains at residues having free amino and carboxyl groups (circled in blue). For example, cross-links in *E. coli* most commonly occur between the amino group of diaminopimelic acid on one peptide and the terminal carboxyl group of D-alanine on a different peptide.

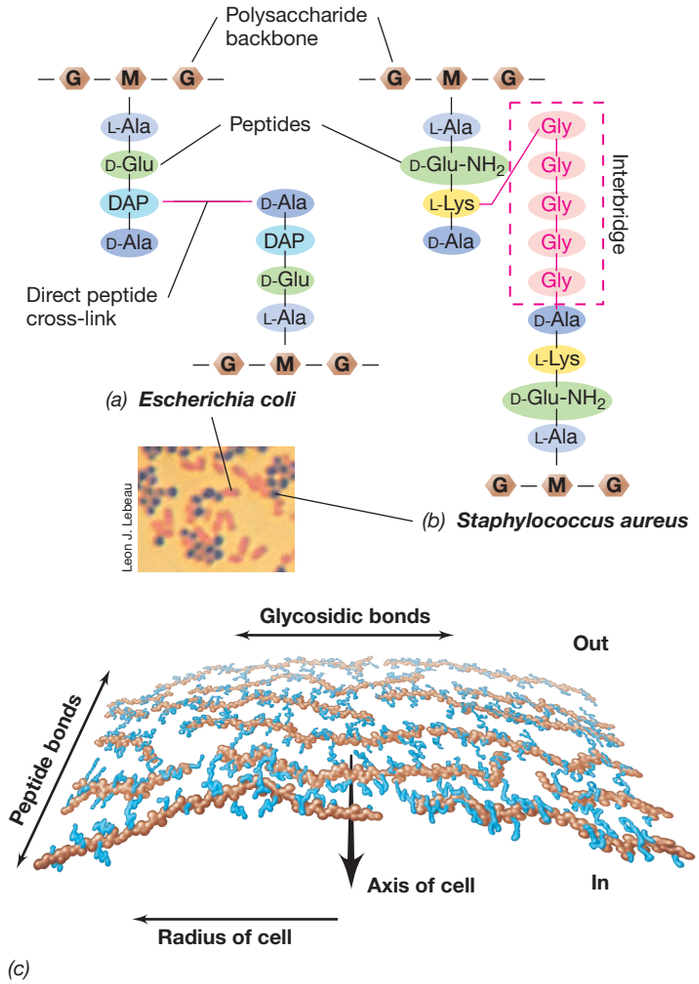


Figure 2.9 Peptidoglycan structure in the cell wall. *(a)* Gram-negative cells that have thin cell walls, such as the cell wall of *E. coli*, mostly have direct cross-links between peptide side chains. *(b)* Gram-positive cells that have thick cell walls, such as *S. aureus*, can also have peptide interbridges that extend between cross-linked peptide side chains. *(c)* Conformation of peptidoglycan in the gram-negative cell wall. G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid. Note how glycosidic bonds confer strength on peptidoglycan around the circumference of the cell whereas peptide bonds confer strength along the axis of the cell.

Mastering Microbiology
Art Activity: Figure 2.12*c* Parts of the Gram-positive cell wall

In addition to peptidoglycan, many gram-positive bacteria produce acidic molecules called **teichoic acids** embedded in their cell wall (Figure 2.10). Teichoic acids are composed of glycerol phosphate or ribitol phosphate with attached molecules of glucose or D-alanine (or both). Individual alcohol molecules are then connected through their phosphate groups to form long strands, and these are then covalently linked to peptidoglycan (Figure 2.10*b*). Some teichoic acids are covalently bonded to membrane lipids rather than to peptidoglycan, and these are called *lipoteichoic* acids.

Peptidoglycan can be destroyed by *lysozyme*, an enzyme that cleaves the glycosidic bond between *N*-acetylglucosamine and *N*-acetylmuramic acid (Figure 2.8). This weakens the peptidoglycan and can cause cell lysis. Lysozyme is present in human secretions including tears, saliva, and other bodily fluids, and functions as a major line of defense against bacterial infection. Many antibiotics, including penicillin, also target peptidoglycan. Whereas lysozyme destroys preexisting peptidoglycan, penicillin blocks the formation of peptide cross-links, which compromises the strength of the peptidoglycan, leading to cell lysis.

Archaeal Cell Walls

The cell envelopes of *Archaea* differ in fundamental ways from those of *Bacteria*. We have already learned that the cytoplasmic membranes of *Archaea*, while functionally analogous to those of *Bacteria*, differ in chemical structure (Section 2.1). Another major difference is that *Archaea* lack peptidoglycan. In addition, *Archaea* typically lack an outer membrane (see Section 2.4). One consequence of these differences is that the Gram stain reaction is not very useful for predicting the structures of archaeal cell envelopes and so we typically do not use the terms gram-positive and gram-negative to describe cells of *Archaea*. Most *Archaea* lack a polysaccharide-containing cell wall and instead have an *S-layer* (see Section 2.5), which is a rigid protein shell that functions to prevent osmotic lysis just as does the bacterial cell wall.

While some *Archaea* do have cell walls, these walls have unique chemical structures not found in *Bacteria*. For example, the cell walls of certain methane-producing *Archaea* (methanogens) contain a polysaccharide called *pseudomurein* (Figure 2.11), which is structurally remarkably similar to peptidoglycan (the term *murein* is from the Latin word for "wall" and was an old term for peptidoglycan). The backbone of pseudomurein is formed from alternating repeats

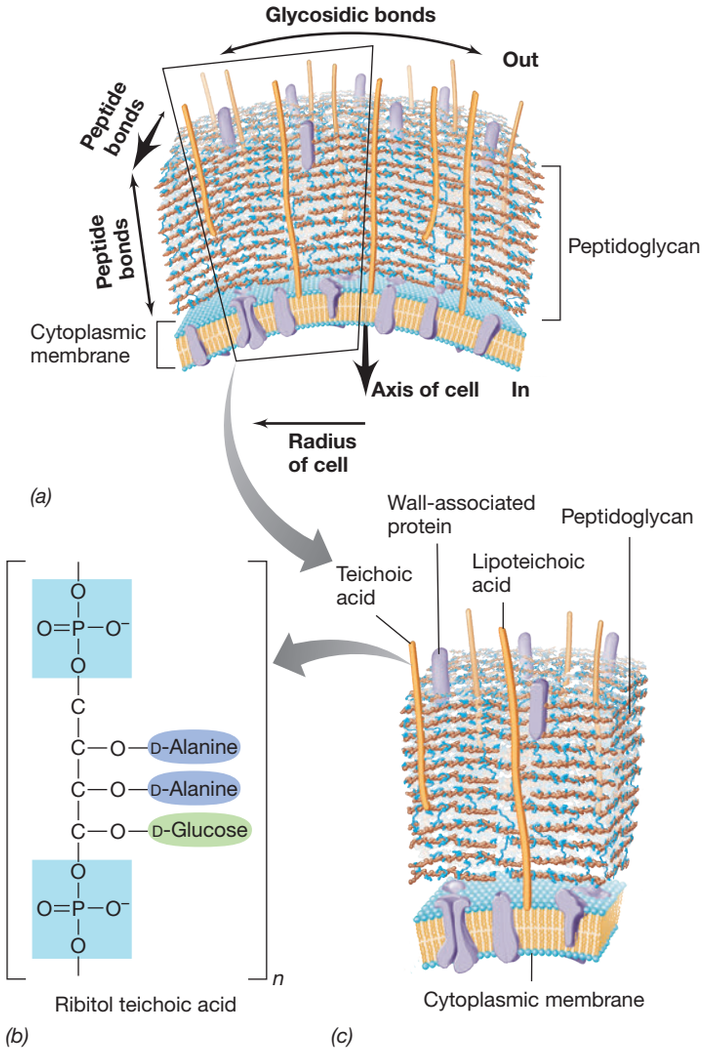


Figure 2.10 Structure of the gram-positive bacterial cell wall. *(a)* Schematic of a gram-positive cell wall showing the internal architecture of the peptidoglycan and its relationship to teichoic acids. Peptide cross-links form between peptidoglycan strands that are adjacent both horizontally and vertically, and peptidoglycan can also form covalent bonds to teichoic acids. *(b)* Structure of a ribitol teichoic acid. The teichoic acid is a polymer of the repeating ribitol unit shown here. *(c)* Summary diagram of the gram-positive bacterial cell wall. Lipoteichoic acids tether the cell wall to the cell membrane.

of *N*-acetylglucosamine (also present in peptidoglycan) and *N*-acetyltalosaminuronic acid; the latter replaces the *N*-acetylmuramic acid of peptidoglycan. Pseudomurein also differs from peptidoglycan in that the glycosidic bonds between the sugar derivatives are β-1,3 instead of β-1,4, and the amino acids are all of the L stereoisomer (compare Figures 2.9 and 2.11).

Because in many respects they are so similar, it is likely that peptidoglycan and pseudomurein are variants of a cell wall polysaccharide originally present in the common ancestor of *Bacteria* and *Archaea*. However, although they are structurally and functionally very similar, they differ sufficiently that pseudomurein is immune from destruction by both lysozyme and penicillin, molecules that destroy peptidoglycan.

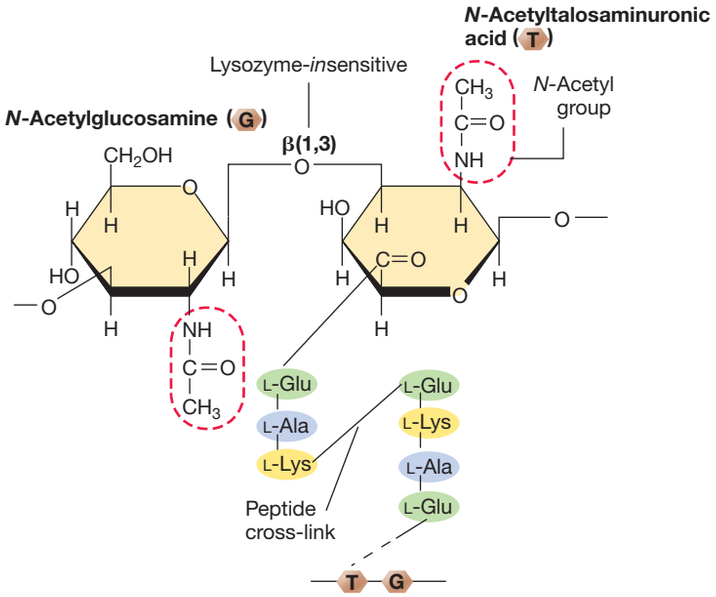


Figure 2.11 Pseudomurein. Structure of pseudomurein, the cell wall polymer of *Methanobacterium* species. Note the similarities and differences between pseudomurein and peptidoglycan (Figures 2.8 and 2.9).

Check Your Understanding

- Explain how peptidoglycan's structural arrangement resists turgor pressure.
- Describe the major differences between the cell walls of gram-negative and gram-positive bacteria.
- Explain whether you expect the enzyme lysozyme to be equally effective against *Bacteria* and *Archaea*.

2.4 LPS: The Outer Membrane

Most of the gram-negative cell envelope is composed of the **outer membrane**. The outer membrane is a second lipid bilayer found external to the cell wall (Figure 2.12), but its structure and function differs from that of the cytoplasmic membrane. The outer membrane and cytoplasmic membrane are similar in that they both contain phospholipid and protein, but a major difference is that the outer membrane also contains polysaccharide molecules covalently bound to lipids (Figure 2.12). Hence, the outer membrane is often called the **lipopolysaccharide** layer, or simply **LPS** for short.

Mastering Microbiology
Art Activity: Figure 2.14*a* Gram-negative cell wall structure

LPS molecules have several unique functions: They can facilitate surface recognition, they are important virulence factors for some bacterial pathogens, and they contribute to the mechanical strength of the cell. We will see that another major difference between the cytoplasmic and outer membranes is that the outer membrane contains *porins*, which are transmembrane proteins that allow for the nonspecific transport of solutes. Hence, we will see that the outer membrane is far more permeable than is the cytoplasmic membrane.

Structure and Activity of LPS

While the precise chemistry of LPS can vary among different species of bacteria, these molecules have several common features. As seen in Figure 2.13, LPS contains a polysaccharide that consists of two

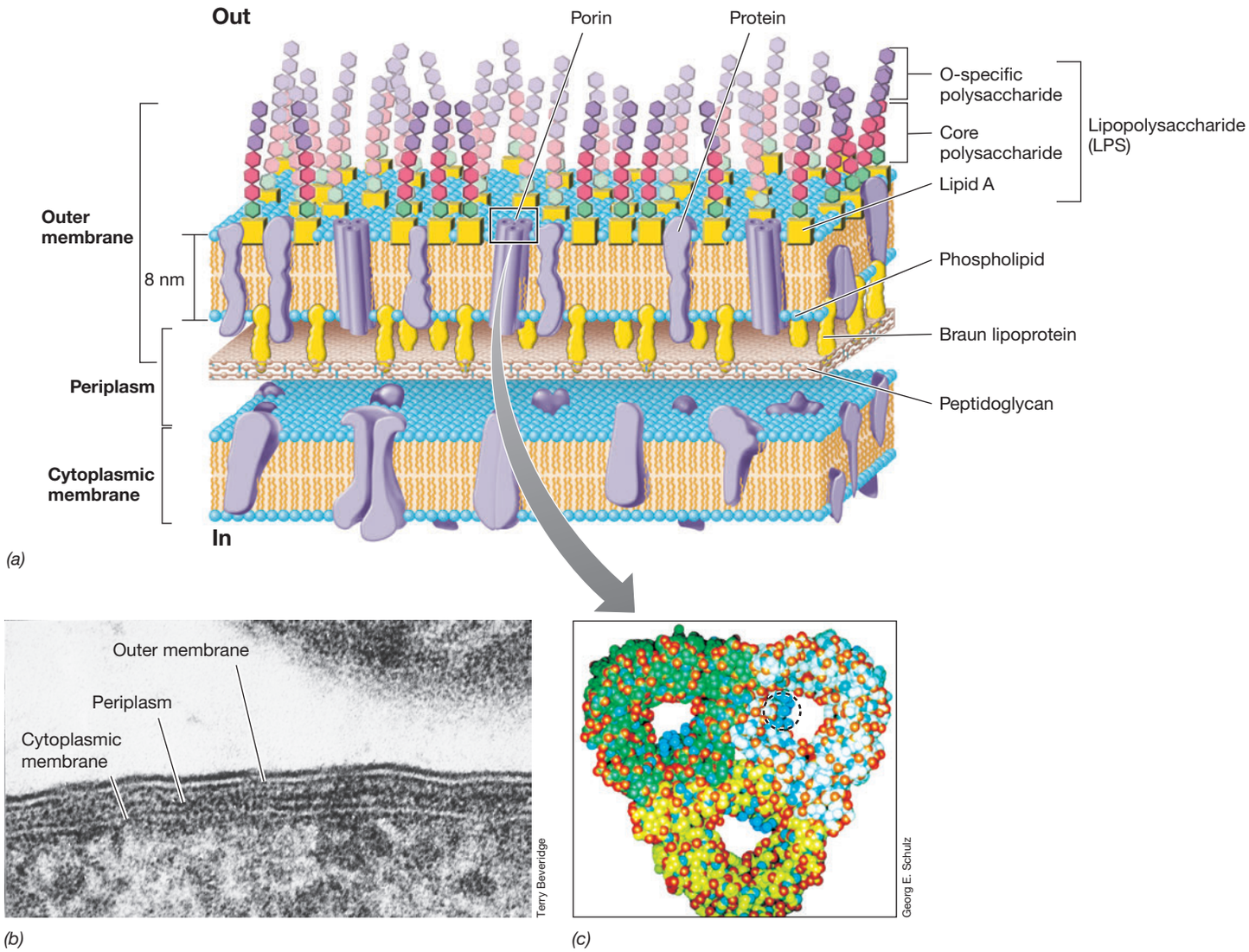


Figure 2.12 **The gram-negative bacterial cell envelope.** *(a)* Arrangement of lipopolysaccharide, lipid A, phospholipid, porins, and Braun lipoprotein in the outer membrane. See Figure 2.13 for details of the structure of LPS. *(b)* Transmission electron micrograph of a cell of *Escherichia coli* showing the cytoplasmic membrane and wall. *(c)* Molecular model of porin proteins showing their hollow pores that allow solute transport across the outer membrane. The view of the porin is perpendicular to the plane of the membrane. The black dashed circle highlights some of the hydrophilic amino acids that line the inside of the pore.

components, the *core polysaccharide* and the *O-specific polysaccharide* (see also Figure 2.12*a*). In *Salmonella* species, where LPS has been well studied, the core polysaccharide consists of ketodeoxyoctonate (KDO), various seven-carbon sugars (heptoses), the hexose sugars glucose and galactose, and *N*-acetylglucosamine. Connected to the core is the O-specific polysaccharide, which typically contains galactose, glucose, the hexoses rhamnose and mannose, and one or more dideoxyhexoses, such as abequose, colitose, paratose, or tyvelose. These sugars are connected in four- or five-membered sequences, which often are branched. When the sequences repeat, the long O-specific polysaccharide is formed. Within the outer membrane these negatively charged polysaccharides can be linked together tightly when adjacent LPS molecules mutually form ionic bonds to divalent cations (such as Ca^{2+} and Mg^{2+}). The presence of these ionic bonds confers considerable strength to the outer membrane, which rivals the gram-negative cell wall in its mechanical strength.

The lipid portion of the LPS, called *lipid A*, is not a typical glycerol lipid (see Figure 2.1*c*); instead the fatty acids are bonded through the amine groups from a disaccharide composed of glucosamine phosphate. The disaccharide is attached to the core polysaccharide through KDO (Figure 2.13). Fatty acids typically found in lipid A include caproic (C_6), lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), and stearic (C_{18}) acids. LPS replaces much of the phospholipid in the outer half of the outer membrane, and although the outer membrane is technically a lipid bilayer, its many unique components distinguish it from the cytoplasmic membrane. The outer membrane is anchored to the peptidoglycan layer by the *Braun lipoprotein*, a molecule that spans the gap between the LPS layer and the peptidoglycan layer (in the periplasm, discussed in the next subsection) (Figure 2.12*a*).

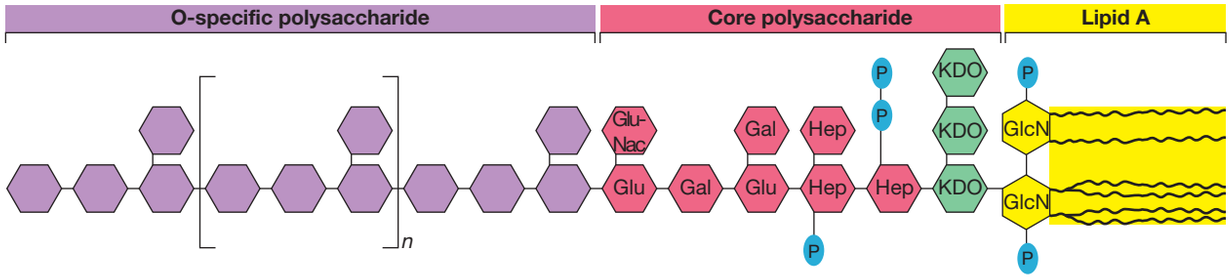


Figure 2.13 Structure of bacterial lipopolysaccharide. The chemical structures of lipid A and polysaccharides can vary among gram-negative *Bacteria*, but the major components (lipid A–KDO–core–O-specific) are typically invariant. The O-specific polysaccharide is highly variable among species. KDO, ketodeoxyoctonate; Hep, heptose; Glu, glucose; Gal, galactose; GluNac, *N*-acetylglucosamine; GlcN, glucosamine; P, phosphate. Glucosamine and the lipid A fatty acids are linked through the amine groups of GlcN. The lipid A portion of LPS can be toxic to animals and comprises the endotoxin complex. Compare the art here with that of Figure 2.12 and follow the LPS components by their color-coding.

An important biological activity of LPS is its toxicity to animals. Common gram-negative pathogens for humans include species of *Salmonella*, *Shigella*, and *Escherichia*, among many others, and some of the gastrointestinal symptoms these pathogens elicit are due to their toxic outer membrane components. Toxicity is specifically linked to the LPS layer, in particular, to lipid A. The term *endotoxin* refers to this toxic component of LPS. Some endotoxins cause violent symptoms in humans, including gas, diarrhea, and vomiting, and the endotoxins produced by *Salmonella* and enteropathogenic strains of *Escherichia coli* transmitted in contaminated foods are classic examples of this. We discuss major gram-negative enteric pathogens in Chapter 33 and endotoxin in Section 25.8.

The Periplasm and Porins

The outer membrane is impermeable to proteins and other very large molecules. In fact, a major function of the outer membrane is to prevent cellular proteins whose activities must occur outside the cytoplasm from diffusing away from the cell. These extracellular proteins reside in the **periplasm**, a space of about 15 nm located between the *outer surface* of the cytoplasmic membrane and the *inner surface* of the outer membrane (Figure 2.12*a*, *b*).

The periplasm may contain several different classes of proteins. These include hydrolytic enzymes, which function in the initial degradation of polymeric substances; binding proteins, which begin the process of transporting substrates (Section 2.2 and Figure 2.6); chemoreceptors, which are proteins that govern the chemotaxis response (Section 2.11); and proteins that construct extracellular structures (such as peptidoglycan and the outer membrane) from precursor molecules secreted through the cytoplasmic membrane. Most periplasmic proteins reach the periplasm by way of a protein-exporting system present in the cytoplasmic membrane (▶ Sections 6.12 and 6.13).

The outer membrane is relatively permeable to small molecules because of proteins called *porins* that function as channels for the entrance and exit of solutes (Figure 2.12*a*, *c*). Porins are unique to the outer membrane of *Bacteria* and should not be confused with *aquaporins*, which are a different class of proteins (aquaporins facilitate water transport across the cytoplasmic membrane). Several porins are known, including both specific and nonspecific classes. Nonspecific porins form water-filled channels through which most very small hydrophilic substances can pass. By contrast, specific porins contain a binding site for one or a group of structurally related substances. Porins are transmembrane proteins composed of three identical polypeptides; the proteins are arranged to form channels through which solutes can diffuse (Figure 2.12*c*).

While the cell envelopes of many *Bacteria* conform to either the gram-positive or the gram-negative model, we have already learned that *Archaea* have cell envelopes that diverge from both of these model structures. In the next section we will see that there is considerable diversity in cell envelope structure across the microbial world.

Check Your Understanding

- Describe and contrast the cell envelope structure of gram-negative and gram-positive bacteria.
- What is the function of porins, and where are they located in the gram-negative cell envelope?
- What component of the gram-negative cell envelope has endotoxin properties?

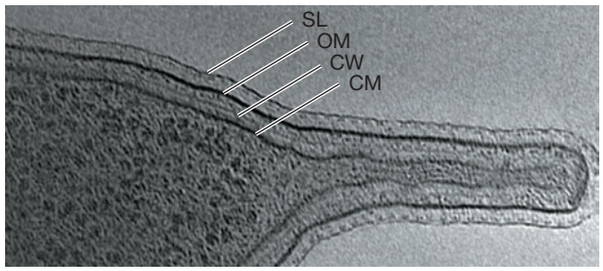
2.5 Diversity of Cell Envelope Structure

While many *Bacteria* have a gram-positive or gram-negative cell envelope organization (Figure 2.7), a variety of cell envelope structures are known. One way in which cell envelopes can vary between cells is in the presence of an *S-layer*.

S-Layers

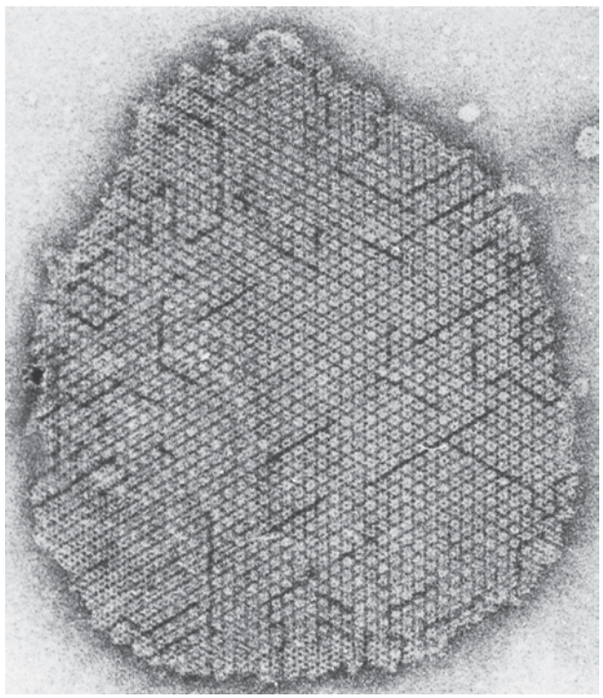
S-layers are found in many *Bacteria* and in nearly all *Archaea*. An S-layer consists of a paracrystalline monolayer of interlocking molecules of protein or glycoprotein (**Figure 2.14**). When an S-layer is present, it is always the *outermost* layer of the cell envelope (Figure 2.14*a*). The S-layer is usually composed of only one or a few subunits self-organized into repeating structures, which can have hexagonal, tetragonal, or trimeric symmetry. These repeating units form a rigid yet permeable paracrystalline lattice (Figure 2.14*b*), which can be as much as 5–20 nm thick in *Bacteria* and up to 70 nm thick in some *Archaea*.

S-layers have many important functions. In many *Archaea*, thick S-layers can take on the role of the cell wall and are responsible for providing structural strength, protecting the cell from osmotic lysis,



Tanmay A.M. Bharat

(a) **Cell envelope of *Caulobacter crescentus***



Susan F. Koval

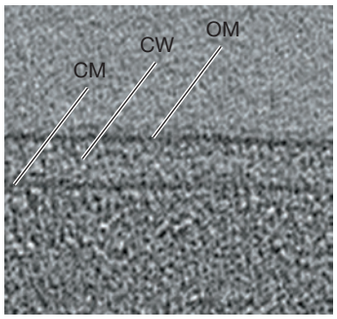
(b) **S-layer fragment from *Aquaspirillum***

Figure 2.14 S-layers. *(a)* The S-layer (SL), outer membrane (OM), peptidoglycan cell wall (CW), and cytoplasmic membrane (CM) can be clearly seen in this electron tomographic slice through a cell of *Caulobacter crescentus*, a stalked bacterium that has a gram-negative cell envelope plus an outer S-layer. *(b)* Transmission electron micrograph of a portion of an S-layer removed from the bacterium *Aquaspirillum* and flattened to show the paracrystalline nature and hexagonal symmetry common to S-layers. The stalk in *a* has an outer diameter of about 150 nm and both images are at the same scale.

and conferring cell shape. The S-layer can also create a periplasmic-like space in *Archaea*. S-layers function as molecular sieves and have pore sizes in the range of 2–10 nm in diameter. These pores are large enough to allow low-molecular-weight compounds to pass but small enough to trap large molecules—such as most proteins—in the space between the S-layer and the cytoplasmic membrane. The compartment formed between the cytoplasmic membrane and the S-layer thus functions much as the periplasm does in gram-negative bacteria, including forming a site near the cytoplasmic membrane where reactions important to cellular metabolism can occur. As the outermost layer of the cell, S-layers can also facilitate cell surface interactions, such as attachment. S-layers can also increase the ability of some bacterial pathogens to cause disease by either promoting adhesion or protecting the cell from host defenses.

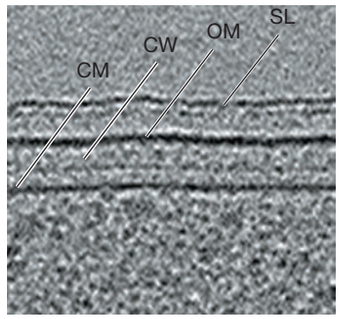
Alternative Configurations of the Cell Envelope

We have now learned about the most common components found in the cell envelope: the cytoplasmic membrane (CM), the cell wall (CW), the outer membrane (OM), and S-layers (SL). While certain configurations of these structures are common, a range of different configurations are possible (**Figure 2.15**). A common variation on cell envelope structure is to find an outer S-layer surrounding an otherwise gram-positive or gram-negative bacterium (compare Figure 2.15*a* with Figure 2.15*b*). In addition, many *Archaea* have *only* an S-layer outside of their cytoplasmic membrane, and these layers, while always constructed of some type of paracrystalline protein or



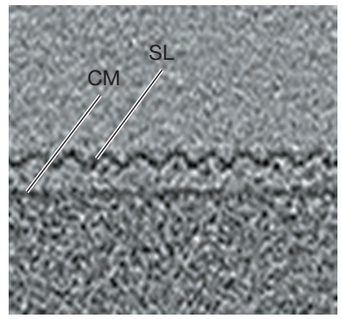
Y-W. Yang, G. Jensen Lab

(a) ***Vibrio cholerae***



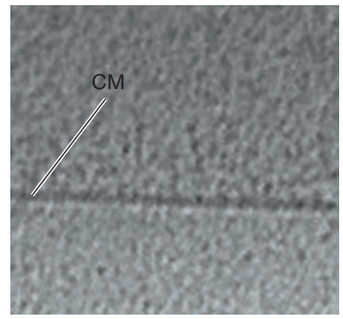
Q. Yao, G. Jensen Lab

(b) ***Caulobacter crescentus***



R. Ramdasi, G. Jensen Lab

(c) ***Nitrosopumilus maritimus***



J. Shi, G. Jensen Lab

(d) ***Mycoplasma pneumoniae***

Figure 2.15 Alternative cell envelope structures. Cell envelope structures including cytoplasmic membranes (CM), cell walls (CW), outer membranes (OM), and S-layers (SL) can be found in both bacterial and archaeal species. *(a) Vibrio cholerae* has a classic gram-negative type bacterial cell envelope. *(b) Caulobacter crescentus* is a bacterium with a gram-negative envelope and an S-layer (see also Figure 2.14*a*). *(c) Nitrosopumilus maritimus* has a typical archaeal cell envelope containing a CM and an SL. *(d) Mycoplasma pneumoniae* is a pathogenic bacterium whose cell envelope consists of only a CM. S-layers, while typically composed of a paracrystalline protein or glycoprotein layer (see also Figure 2.14*b*) can vary considerably in molecular structure (compare the S-layers in *b* and *c*). All images are transmission electron tomographs, a technique in which a special transmission electron microscope passes electrons through a specimen at different angles and then consolidates the views to form a final three-dimensional image.

glycoprotein, can vary considerably in their molecular structures (compare Figure 2.15*b* with Figure 2.15*c*). Some methanogenic *Archaea* also have cell walls made of pseudomurein, and such cell walls may or may not have an outer S-layer as well. Finally, though unusual, *Archaea* such as the heat-loving *Ignicoccus* actually have an outer membrane. This structure in *Ignicoccus* is unlike that of gram-negative bacteria in that it is composed largely of archaeal isoprenoid lipids and lacks LPS.

Although this is uncommon, a few *Bacteria* and *Archaea* lack cell walls altogether. These include in particular the mycoplasmas (Figure 2.15*d*) and other pathogenic *Bacteria* that grow within a host cell, and *Archaea* such as *Thermoplasma* and its relatives. Lacking a cell wall, these cells would be expected to contain unusually tough cytoplasmic membranes, and chemical analyses show that they do. For example, most mycoplasmas contain *sterols* in their cytoplasmic membranes; these molecules function to add strength and rigidity to the membrane as they do in the cytoplasmic membranes of eukaryotic cells. Mycoplasmas may also have little need for a cell wall because they experience little osmotic pressure when living within the cytoplasm of another cell. In addition, the loss of peptidoglycan may help mycoplasmas evade the host immune system because host defenses recognize bacterial cell wall components as one of many signals of bacterial invasion (Chapter 26).

Check Your Understanding

- What is the structure of an S-layer, and what are its functions?
- What are some alternate arrangements of cell envelope structure?

II • Cell Surface Structures and Inclusions

Many prokaryotic cells contain a cell surface layer that can have a variety of functions. Cytoplasmic inclusions may also be present and function as food reserves or bestow upon the cell a unique capacity of ecological value.

The cell envelope governs many aspects of how microbes interact with their environments and with other cells. Likewise, many other structures visible to microscopy can have profound impacts on cellular function. In particular, structures found on the cell surface and inclusions—structures present within the cell's cytoplasm—have many important functions that govern microbial interactions with the world around them.

2.6 Cell Surface Structures

Many *Bacteria* and *Archaea* secrete sticky or slimy materials on their cell surface that consist of either polysaccharide or protein. However, these are distinct from and external to the cell envelope. The terms "capsule" and "slime layer" are used to describe these layers. These outer layers can mediate attachment, they can protect the cell from attack and from environmental stresses, and they can alter the diffusive environment of the cell.

Capsules and Slime Layers

The terms *capsule* and *slime layer* are used to describe a sticky coat of polysaccharide formed outside of the cell envelope. If the polysaccharide layer is organized in a tight matrix that excludes small particles and is tightly attached to the cell, it is called a **capsule**. Capsules are readily visible by light microscopy if cells are treated with India ink, which contains particulates that stain the background but cannot penetrate the capsule; capsules can also be seen in the electron microscope (**Figure 2.16*b–d***). By contrast, if the surface

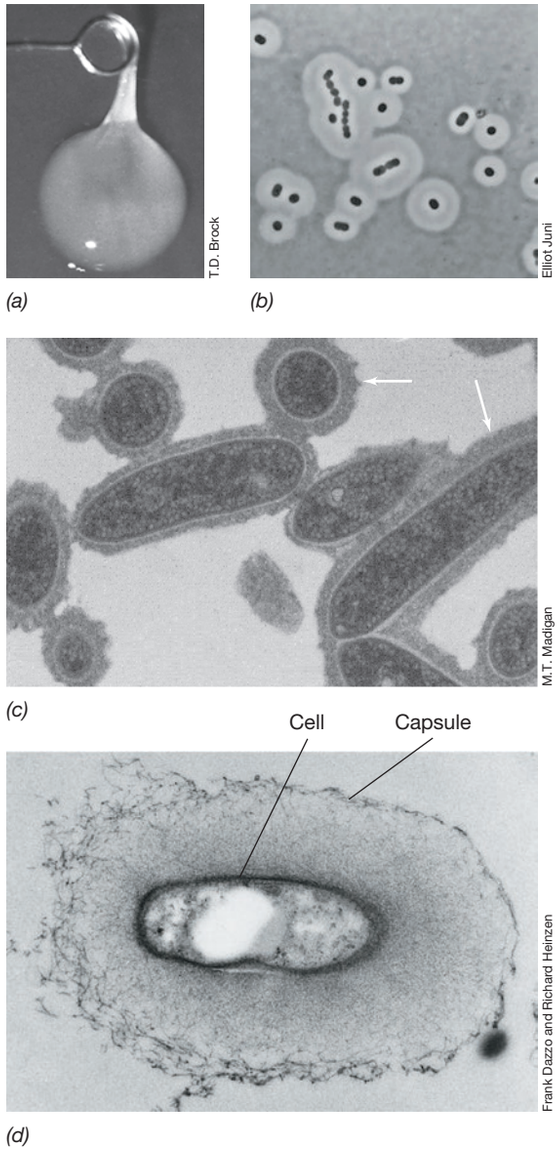


Figure 2.16 Bacterial capsules and slime formation. *(a)* A semisolid colony of the bacterium *Leuconostoc mesenteroides* (lifted up by an inoculating loop) contains a thick dextran (glucose polymer) slime layer formed by the cells. *(b)* Capsules of *Acinetobacter* species observed by phase-contrast microscopy after negative staining with India ink. India ink does not penetrate the capsule and so the capsule appears as a light area surrounding the cell, which appears black. *(c)* Transmission electron micrograph of a thin section of cells of *Rhodobacter capsulatus* with capsules (arrows) clearly evident; cells are about 0.9 μm wide. *(d)* Transmission electron micrograph of *Rhizobium leguminosarum* biovar *trifolii* stained with ruthenium red to reveal the capsule. The cell is about 0.7 μm wide.

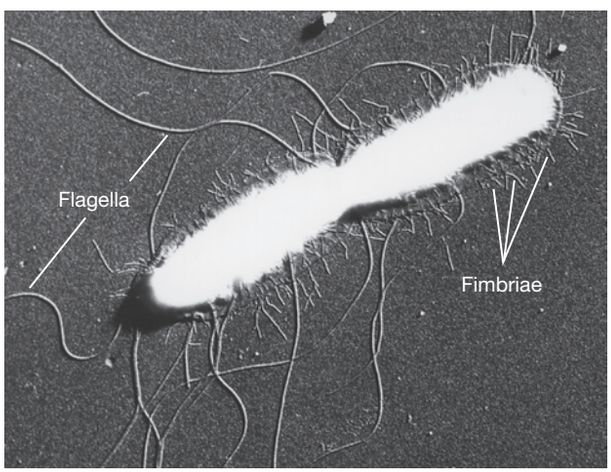
layer is easily deformed and loosely attached, it will not exclude particles and is more difficult to see microscopically. Such a loosely attached polysaccharide coat is called a *slime layer*, and it is easily detected in colonies of slime-forming species such as the lactic acid bacterium *Leuconostoc* (Figure 2.16*a*).

Outer surface layers have several functions. Surface polysaccharides assist in the attachment of microorganisms to solid surfaces. As we will see later, pathogenic microorganisms that enter the body by specific routes usually do so by first binding to specific surface components of host tissues; this binding is often facilitated by bacterial cell surface polysaccharides. When the opportunity arises, many bacteria will bind to solid surfaces, often forming a thick layer of cells called a *biofilm* (▶ Section 4.9). Extracellular polysaccharides play a key role in the development and maintenance of biofilms as well.

Besides attachment, outer surface layers have other functions. These include contributing to the infectivity of a bacterial pathogen and preventing dehydration. For example, the causative agents of the diseases anthrax and bacterial pneumonia—*Bacillus anthracis* and *Streptococcus pneumoniae*, respectively—each contain a thick capsule of either protein (*B. anthracis*) or polysaccharide (*S. pneumoniae*). Encapsulated cells of these bacteria avoid destruction by the host's immune system because the immune cells that would otherwise recognize these pathogens as foreign and destroy them are blocked from doing so by the bacterial capsule. In addition to this role in disease, bacterial outer surface layers bind water, and this helps protect the cell from desiccation in periods of dryness.

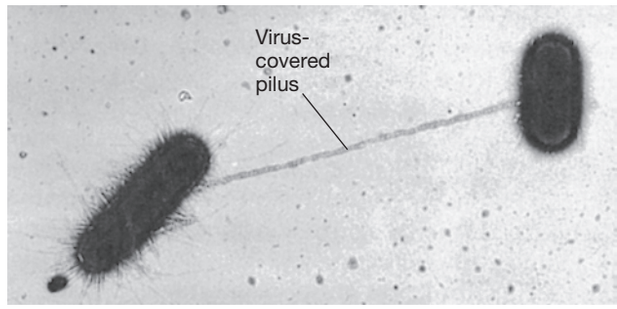
Fimbriae, Pili, and Hami

Pili are thin (2–10 nm in diameter) filamentous structures made of protein that extend from the surface of a cell and can have many functions. Short pili that mediate attachment are often called *fimbriae* (**Figure 2.17**). Pili enable bacterial cells to stick to surfaces, including animal tissues, or to form pellicles (thin sheets of cells on a liquid surface) or biofilms on solid surfaces. All gram-negative bacteria produce pili of one sort or another, and many gram-positive bacteria also contain these structures. Pili, by allowing bacteria to attach to other cells, often contribute to the virulence of pathogens.



J.P. Duguid and J.F. Wilkinson

Figure 2.17 Fimbriae. Electron micrograph of a dividing cell of *Salmonella enterica* (*typhi*), showing flagella and fimbriae. A single cell is about 0.9 μm wide.



Charles C. Brinton, Jr. and Judith Carnahan

Figure 2.18 Pili. The pilus on an *Escherichia coli* cell that is undergoing conjugation. The cells are about 0.8 μm wide. The visibility of the pilus in this electron micrograph has been improved because it is coated with viral particles that bind to the pilin protein.

Many classes of pili are known, and they can have diverse functions. As already mentioned, pili can enable bacteria to adhere to surfaces and this function can allow pathogens to target and invade specific host tissues. However, pili are diverse and they can have several other important functions as well. For example, *conjugative pili* facilitate genetic exchange by causing cell-to-cell attachment (**Figure 2.18**) during a process called *conjugation* (▶ Section 9.8). In addition, *electrically conductive pili* (also known as *nanowires*, ▶ Section 23.3) can conduct electrons toward or away from the cell and in so doing play an important role in the energy metabolism of diverse microbes (▶ Sections 14.13 and 15.13). Lastly, a type of pili called *type IV pili* not only facilitate adhesion but also support an unusual form of cell movement called *twitching motility* in certain bacterial species (see Section 2.10).

Twitching motility allows cells to move along a solid surface. In twitching motility, pili are extended away from the cell, attach to a surface, and are subsequently retracted, dragging the cell forward. ATP supplies the energy necessary for extension and retraction of the pilus. On rod-shaped cells that move by twitching, type IV pili are present only at the cell poles. Type IV pili assist in infectivity by certain pathogens, including the gram-negative bacteria *Vibrio cholerae* (cholera) and *Neisseria gonorrhoeae* (gonorrhea) and the gram-positive bacterium *Streptococcus pyogenes* (strep throat and scarlet fever). The twitching motility of these organisms assists them in locating specific sites for attachment to initiate the disease process. Type IV pili are also widespread in the *Archaea*, functioning in surface adhesion and cell aggregation events that lead to biofilm formation.

An unusual group of *Archaea*, the SM1 group, forms a unique attachment structure called a *hamus* (plural, hami) that resembles a tiny grappling hook (**Figure 2.19*a, b***). The SM1 group inhabits anoxic groundwater in Earth's deep subsurface, and hami function to affix cells to a surface to form a networked biofilm (Figure 2.19*c*). Hami structurally resemble type IV pili except for their barbed terminus, which functions to attach cells both to surfaces and to each other (Figure 2.19*c*). The biofilms formed by SM1 *Archaea* are likely an ecological strategy that allows these microbes to more efficiently trap the scarce nutrients present in their deep subsurface habitat.

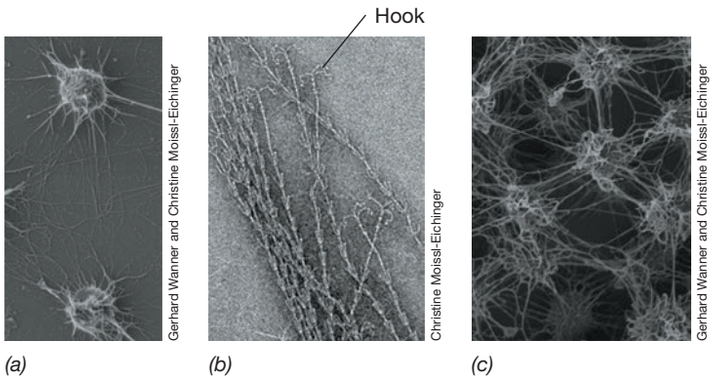


Figure 2.19 Unique attachment structures in the SM1 group of *Archaea*: Hami. *(a)* Cells of SM1 *Archaea* showing the pili-like surface structures called hami. *(b)* Transmission electron micrograph of isolated hami. A hamus "grappling hook" (labeled "Hook" in the micrograph) is about 60 nm in diameter. *(c)* A biofilm of SM1 cells showing the network of hami connecting individual cells.

Although cells of the SM1 group are not as small as the groundwater ultramicrobacterial cells described in Chapter 1 (see Explore the Microbial World, "Tiny Cells"), they are less than 1 μm in diameter and live in a similar nutrient-limiting habitat. Thus, their hami likely play an important role in preventing cells from being washed away in groundwater flowage.

In addition to the cell surface, the *cytoplasm* of a prokaryotic cell may contain enclosed and sometimes rigid structures that benefit the cell in one way or another, and we consider these now.

Check Your Understanding

- How does the thick capsule help encapsulated bacteria establish an infection in a host?
- How do fimbriae differ from pili, both structurally and functionally?
- How would inactivating the conjugative pili mechanism in bacteria help prevent antibiotic resistance genes from spreading between bacteria?

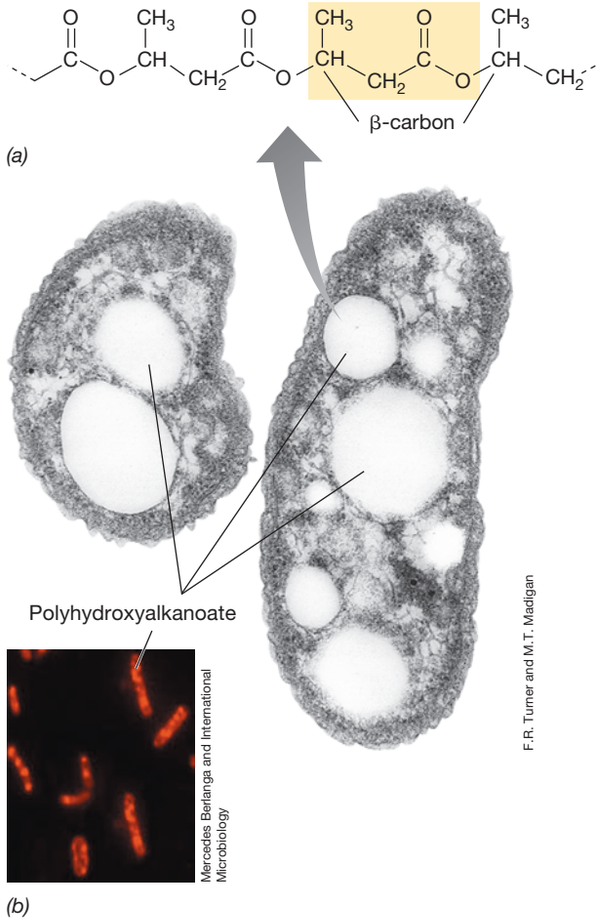


Figure 2.20 Poly-β-hydroxyalkanoates (PHAs). *(a)* Chemistry of poly-β-hydroxybutyrate, a common PHA. A monomeric unit is highlighted in color. Other PHAs are made by substituting longer-chain hydrocarbons for the $-CH_3$ group on the β-carbon. *(b)* Electron micrograph of a thin section of cells of a bacterium containing granules of PHB. Color photo: Nile red–stained cells of a PHA-containing bacterium.

2.7 Cell Inclusions

Prokaryotic cells often contain inclusions of one sort or another. Many inclusions store energy or nutrients (such as carbon or phosphorus), but some have other highly specialized functions that confer unique properties on the cells that contain them. Inclusions are often visible in cells with the light microscope and are enclosed by a single-layer (as opposed to a unit) membrane composed of proteins that partitions off the inclusion in the cytoplasm. Storing carbon or other substances in an insoluble form within the cytoplasm reduces osmotic stress and takes up less space compared with storing these substances in a soluble form.

Carbon Storage Polymers

One of the most common inclusion bodies in prokaryotic organisms is **poly-β-hydroxybutyric acid (PHB)**, a lipid that is formed from β-hydroxybutyric acid units. The monomers of PHB polymerize by ester linkage and then the polymer aggregates into granules; the granules can be seen by either light or electron microscopy (**Figure 2.20**). The monomer in the polymer is usually hydroxybutyrate (C_4) but can vary in length from as short as C_3 to as long as C_{18}. Thus, the more generic term *poly-β-hydroxyalkanoate* (PHA) is often used to describe this class of carbon- and energy-storage polymers. PHAs are synthesized by cells when there is an excess of carbon and are broken down as carbon or energy sources when conditions warrant.

Another carbon storage inclusion is *glycogen*, which is a polymer of glucose; like PHA, glycogen is a reservoir of both carbon and energy and is produced when carbon is in excess. Glycogen resembles starch, the major storage reserve of plants, but differs slightly from starch in the manner in which the glucose units are linked together.

Polyphosphate, Sulfur, and Carbonate Minerals

Many prokaryotic and eukaryotic microbes accumulate inorganic phosphate (PO_4^{3-}) in the form of *polyphosphate* granules (**Figure 2.21*a***). These granules are formed when phosphate is in excess and can be drawn upon as a source of phosphate for nucleic acid and phospholipid biosynthesis when phosphate is limiting. In

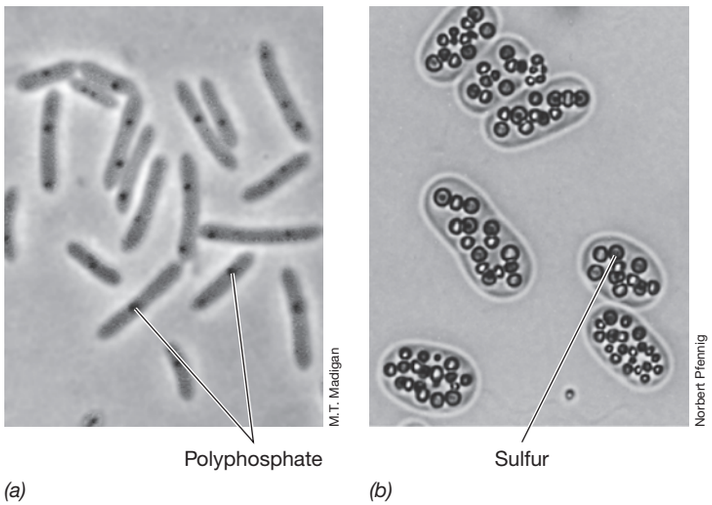


Figure 2.21 **Polyphosphate and sulfur storage products.** *(a)* Phase-contrast photomicrograph of cells of *Heliobacterium modesticaldum* showing polyphosphate as dark granules; a cell is about 1 μm wide. *(b)* Bright-field photomicrograph of cells of the purple sulfur bacterium *Isochromatium buderi*. The periplasmic inclusions are sulfur globules formed from the oxidation of hydrogen sulfide (H_2S). A cell is about 4 μm wide.

addition, in some organisms, polyphosphate can be broken down to synthesize the energy-rich compound ATP from ADP.

Many gram-negative *Bacteria* and several *Archaea* oxidize reduced sulfur compounds, such as hydrogen sulfide (H_2S); these organisms are the "sulfur bacteria," discovered by the great Russian microbiologist Sergei Winogradsky (◀ Section 1.13). The oxidation of sulfide generates electrons for use in energy metabolism (chemolithotrophy) or CO_2 fixation (autotrophy). In either case, *elemental sulfur* (S^0) from the oxidation of sulfide may accumulate in the cell in microscopically visible granules (Figure 2.21*b*). This sulfur remains as long as the source of reduced sulfur from which it was derived is still present. However, as the reduced sulfur source becomes limiting, the S^0 in the granules is oxidized to sulfate (SO_4^{2-}), and the granules slowly disappear. Interestingly, although sulfur globules appear to reside in the cytoplasm (Figure 2.21*b*), they are actually present in the periplasm (Section 2.4). In these cells the periplasm expands outward to accommodate the growing globules as H_2S is oxidized to S^0 and then contracts inward as S^0 is oxidized to SO_4^{2-}.

Filamentous cyanobacteria have long been known to form carbonate minerals on the external surface of their cells. However, some cyanobacteria also form carbonate minerals *inside* the cell, as cell inclusions. For example, the unicellular cyanobacterium *Gloeomargarita* forms intracellular granules of benstonite, a carbonate mineral that contains barium, strontium, and magnesium (**Figure 2.22**). The microbiological process of forming minerals is called *biomineralization*. It is unclear why benstonite is formed by *Gloeomargarita*, although it might function as ballast to maintain cells of this cyanobacterium in their habitat, deep in an alkaline lake. Alternatively (or in addition), the mineral could be a way to sequester carbonate (a source of CO_2) to support autotrophic growth.

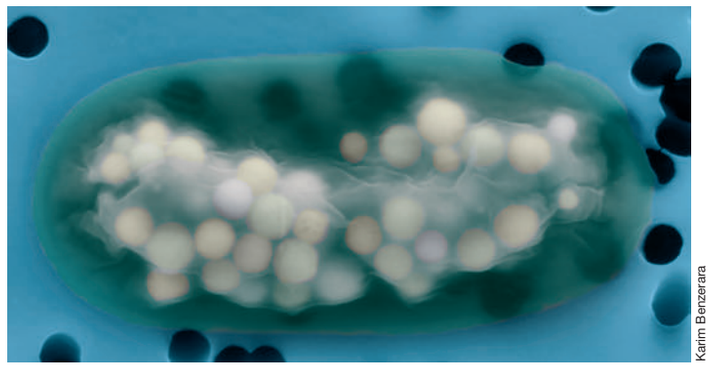


Figure 2.22 **Biomineralization by a cyanobacterium.** Electron micrograph of a cell of the cyanobacterium *Gloeomargarita* containing granules of the mineral benstonite [$(Ba,Sr)_6(Ca,Mn)_6Mg(CO_3)_{13}$]. A cell is about 2 μm wide.

Gas Vesicles

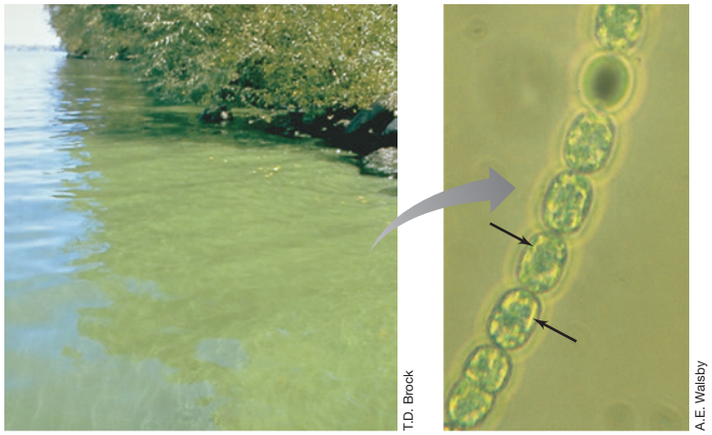
Some *Bacteria* and *Archaea* can float because they contain **gas vesicles**, structures that confer buoyancy and allow the cells to position themselves in regions of the water column that best suit their metabolisms. The most dramatic examples of gas-vesiculate microbes are those cyanobacteria that form massive accumulations called *blooms* in lakes or other bodies of water. These blooms are commonly on or near the lake surface (**Figure 2.23*a***) where sunlight is most intense and photosynthesis can occur at maximal rates.

Gas vesicles are conical-shaped structures composed of two different proteins; they are hollow yet rigid and of variable length and diameter (Figure 2.23*b*, *c*). Gas vesicles in different species vary from 300 to more than 1000 nm in length and from 45 to 120 nm in width. Gas vesicles may number from a few to hundreds per cell and are impermeable to water and solutes but permeable to gases. The composition and pressure of the gas inside a gas vesicle is that in which the organism is suspended. This could be air at 1 atm in cyanobacteria on a lake surface (Figure 2.23*a*), or a mixture of gases such as N_2, CO_2, and H_2 at greater than 1 atm in gas vesiculate species that inhabit anoxic zones deeper in the lake. The presence of gas vesicles in cells can be detected either by light microscopy, where clusters of vesicles, called *gas vacuoles*, appear as irregular bright inclusions (Figure 2.23*b*), or by transmission electron microscopy of cell thin sections (Figure 2.23*c*).

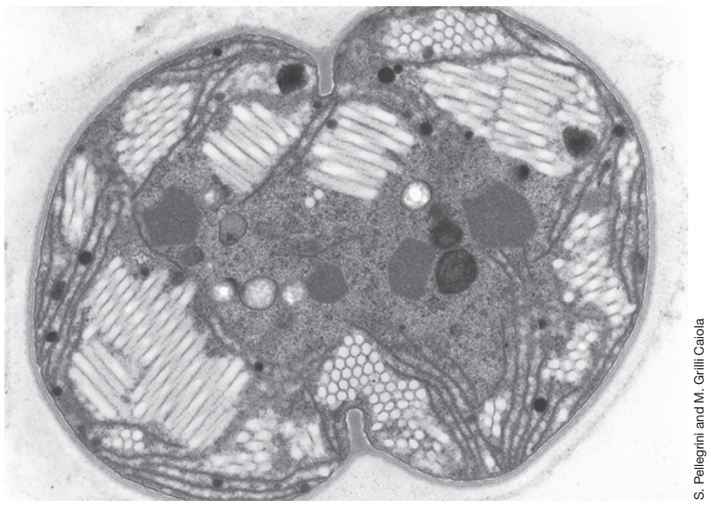
Magnetosomes

Some bacteria can orient themselves within a magnetic field because they contain **magnetosomes**. These structures are biomineralized particles of the magnetic iron oxides magnetite [$Fe(II)Fe(III)_2O_4$] or greigite [$Fe(II)Fe(III)_2S_4$] (**Figure 2.24**). Magnetosomes impart a magnetic dipole on a cell, allowing it to orient itself in a magnetic field. This allows the cell to undergo *magnetotaxis*, the process of migrating along Earth's magnetic field lines (see Section 2.12).

Magnetosome synthesis begins with insertion of magnetosome-specific proteins into the cytoplasmic membrane followed by invagination of the membrane to form a vesicle. The vesicle is then filled with iron—primarily iron in the Fe(II) oxidation state—and biomineralization proceeds through the activities of the magnetosome



(a) (b)



(c)

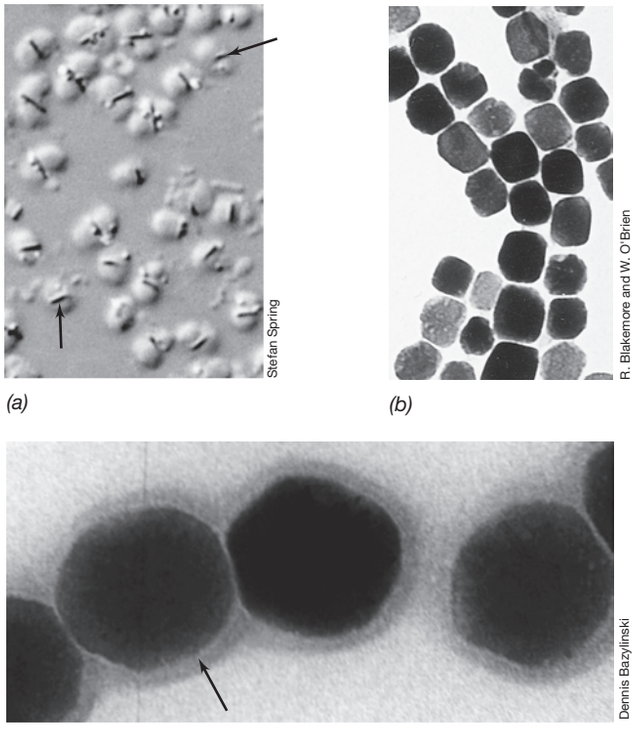
Figure 2.23 Buoyant cyanobacteria and their gas vesicles. *(a)* Flotation of a bloom of gas-vesiculate cyanobacteria in a freshwater lake. *(b)* Phase-contrast photomicrograph of *Anabaena*. Clusters of gas vesicles form phase-bright gas vacuoles (small arrows). *(c)* Transmission electron micrograph of a dividing cell of *Microcystis*. Gas vesicles are arranged in bundles, here seen in both longitudinal and cross section. A cell of *Microcystis* is about 5 μm wide.

proteins, which includes an iron oxidase enzyme that catalyzes formation of the Fe(III) needed to form the magnetic minerals. The morphology of magnetosomes varies and appears to be species-specific; several morphologies are possible, but square, rectangular, or spike-shaped magnetosomes are most common.

We now consider a special case of a cytoplasmic enclosed cell structure—the endospore.

Check Your Understanding

- Under what nutritional conditions would you expect PHAs or glycogen to be produced?
- Why would it be impossible for gram-positive bacteria to store sulfur as gram-negative sulfur-oxidizing chemolithotrophs can?
- How are magnetosomes and the *Gloeomargarita* inclusions similar and how do they differ? What are gas vesicles made of and what is inside them?



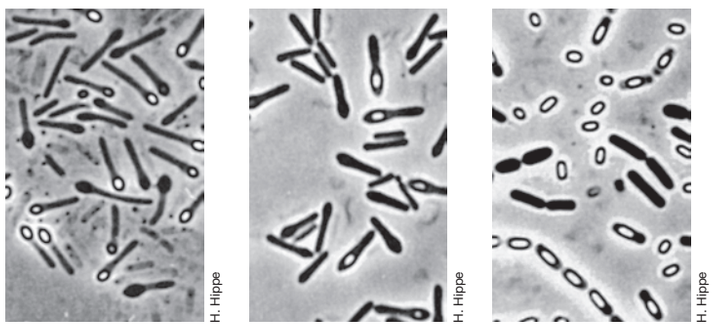
(a) (b)

(c)

Figure 2.24 Magnetotactic bacteria and magnetosomes. *(a)* Differential interference contrast micrograph of coccoid magnetotactic bacteria; note chains of magnetosomes (arrows). A cell is 2.2 μm wide. *(b)* Magnetosomes isolated from the magnetotactic bacterium *Magnetospirillum magnetotacticum*; each particle is about 50 nm wide. *(c)* Transmission electron micrograph of magnetosomes from an unnamed magnetic coccus. The arrow points to the membrane that surrounds each magnetosome. A single magnetosome is about 90 nm wide.

2.8 Endospores

Many microbes produce spore structures that allow them to survive unfavorable conditions. However, certain species of *Bacteria* produce specialized spores called **endospores** (Figure 2.25). Endospores (the prefix *endo-* means "within") are highly differentiated dormant cells that function as survival structures and can tolerate harsh



(a) Terminal endospores (b) Subterminal endospores (c) Central endospores

Figure 2.25 The bacterial endospore. Phase-contrast photomicrographs showing different intracellular locations of endospores in different species of bacteria. Endospores appear bright by phase-contrast microscopy.

environmental conditions, including extreme heat, radiation, chemical exposure, drying, and nutrient depletion. Endospores are not reproductive structures, such as the spores of fungi, but are rather the dormant stage of a bacterial life cycle: vegetative cell → endospore → vegetative cell (**Figure 2.26**).

Many microbes can form spores or spore-like structures, including the myxospores of myxobacteria (▶ Section 15.16), the arthrospores of actinomycetes (▶ Section 16.12), and various forms of cysts, but endospores are exceptional for their extreme resistance to environmental challenges. Most notably, endospores are the only type of spore that can tolerate high heat, being able to survive for hours in boiling water. And, because of their unique structure, endospores can remain dormant for hundreds and perhaps even thousands of years, only to germinate and grow when conditions become favorable. Endospores are easily dispersed by wind, water, or through the animal gut, and hence endospore-forming bacteria are widely distributed in nature.

Endospores are only produced by two groups of bacteria, the *Bacillales* and *Clostridiales*, both of which are gram-positive bacteria of the phylum *Firmicutes*. These bacteria share an ancestor and so it is likely that the ability to form endospores evolved only once, though many species within these groups have lost the ability to form endospores over time. The best-studied endospore formers are in the genera *Bacillus* and *Clostridium*. These bacteria are found widely in soil and other environments, and some are well-known pathogens of humans and other animals. In particular, endospore-forming bacteria are a major cause of food spoilage and foodborne disease. Botulism, tetanus, and several foodborne bacterial infections are caused by species of endospore-forming bacteria (Chapters 32 and 33).

Endospore Formation and Germination

The process of cellular differentiation that results in endospore formation is called *sporulation*. During endospore formation, a vegetative cell is converted through a process of cellular differentiation into a nongrowing, heat-resistant, and light-refractive structure (Figure 2.26). Sporulation is typically triggered when some nutrient becomes limiting. When the cell senses this, the developmental process that converts a vegetative cell into an endospore begins and is controlled by a complex regulatory system we will consider later (▶ Section 8.6). Our focus here is not on regulation but instead on the major steps in the process.

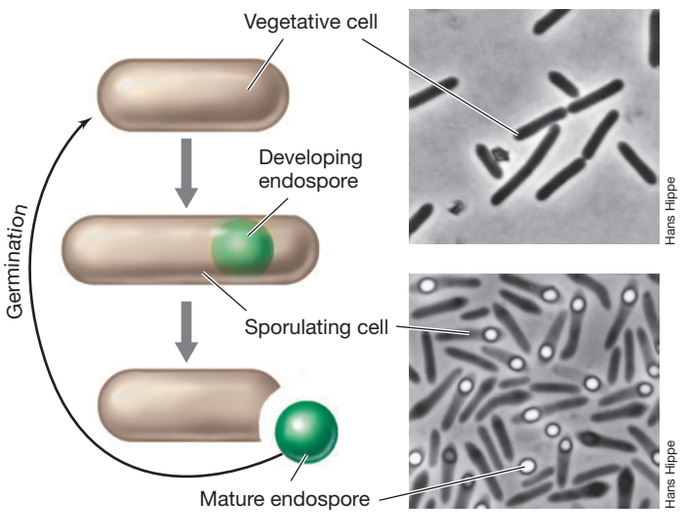


Figure 2.26 The life cycle of an endospore-forming bacterium. The phase-contrast photomicrographs are of cells of *Clostridium pascui*. A vegetative cell is about 0.8 μm wide.

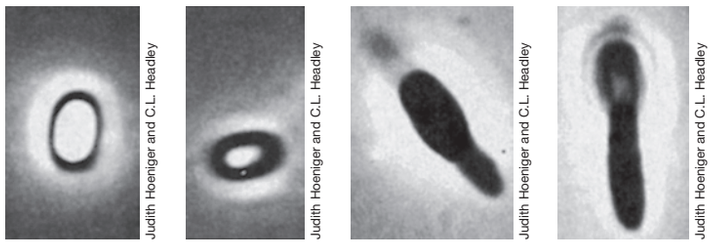


Figure 2.27 Endospore germination in *Bacillus*. Conversion of an endospore into a vegetative cell. This series of phase-contrast photomicrographs shows the sequence of events starting from *(a)* a highly refractile free endospore. *(b) Activation*: The spore becomes less refractile as the spore is hydrated. *(c) Germination*: the spore begins to develop into a vegetative cell. *(d) Outgrowth*: the vegetative cell emerges and begins to divide.

An endospore can remain dormant for years, but when conditions are favorable for growth, it can convert back to a vegetative cell rapidly through the process of *germination* (Figure 2.26). Germination is usually triggered by the availability of nutrients, such as certain amino acids or sugars. This process occurs in three steps: *activation*, *germination*, and *outgrowth* (**Figure 2.27**). The overall process occurs over a period of just a few minutes, and it is characterized by hydration of the spore, which results in loss of its heat and chemical resistance, the loss of specific spore structures and the regeneration of vegetative cell structures, and ultimately, the onset of vegetative growth by binary fission.

Endospore Structure and Features

Endospores differ in many ways from vegetative cells (**Table 2.1**). Endospores are visible by light microscopy as strongly refractile structures (Figures 2.25 and 2.26). Endospores are impermeable to most dyes, so occasionally they are seen as unstained regions within cells that have been stained with basic dyes such as methylene blue. To stain endospores, special stains and procedures must be used. In the classical endospore-staining protocol, the stain malachite green is used and is infused into the spore with steam.

The structure of the endospore as seen with the electron microscope differs distinctly from that of the vegetative cell (**Figure 2.28**). The endospore contains many layers absent from the vegetative cell. The innermost region of the endospore is called the *core*; this contains DNA and ribosomes and develops from the cytoplasm of the vegetative cell. Surrounding the core is the *inner membrane*, the *cortex*, and the *outer membrane* (Figure 2.28). The inner membrane develops from the cytoplasmic membrane of the vegetative cell, the cortex is composed of peptidoglycan, and the outer membrane is a special membrane formed during sporulation (and should not be confused with the LPS-containing outer membrane of

TABLE 2.1 Differences between endospores and vegetative cells

Characteristic	Vegetative cell	Endospore
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Respiration rate	High	Low or absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Radiation resistance	Low	High
Resistance to chemicals	Low	High
Lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble spore proteins	Absent	Present

gram-negative cells). Beyond the outer membrane is the *endospore coat* (Figure 2.28), composed of layers of spore-specific proteins, and some (but not all) endospores also have an outer proteinaceous layer called the *exosporium* (Figure 2.28).

The secret to the endospore toughness, and the reasons that endospores are so highly refractile, lies in the dehydration of the core. The endospore core contains less than one-quarter of the water found in the vegetative cell (Table 2.1). Dehydration greatly increases heat and chemical resistance and causes enzymes in the core to become inactive (but not denatured). Dehydration of the core is facilitated by the accumulation of a substance called **dipicolinic acid**, a distinctive characteristic of endospores. Endospores also contain large amounts of calcium (Ca^{2+}), most of which is complexed with dipicolinic acid. The calcium–dipicolinic acid complex forms about 10% of the dry weight of the endospore and functions to bind water, helping to dehydrate the developing endospore. In addition, the complex inserts between bases in DNA, which helps stabilize DNA against heat denaturation.

The endospore core also contains high levels of *small acid-soluble spore proteins* (SASPs). These proteins are only made during the sporulation process and have at least two functions. SASPs bind tightly to DNA in the core and protect it from potential damage from ultraviolet radiation, desiccation, and dry heat. Ultraviolet resistance is conferred when SASPs alter the physical structure of DNA, causing it to become more compact. This change in DNA structure causes it to be more resistant to mutations and other forms of potential damage caused by harsh chemicals or UV radiation (▶ Section 12.4) and also increases its resistance to thermal denaturation. In addition, SASPs function as a carbon and energy source for the outgrowth of a new vegetative cell from the endospore during germination.

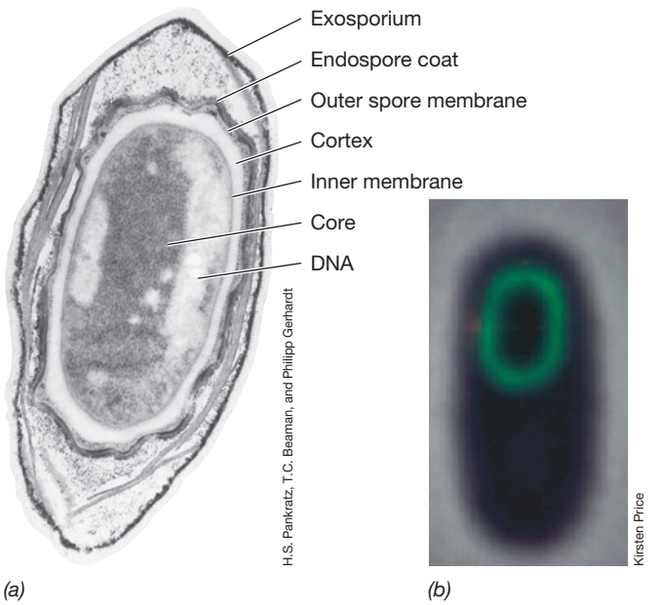


Figure 2.28 Structure of the bacterial endospore. *(a)* Transmission electron micrograph of a thin section through an endospore of *Bacillus megaterium*. Note that the composition of the endospore "outer membrane" is not the same as the outer membrane (LPS layer) of gram-negative bacteria shown in Figure 2.12. *(b)* Fluorescent photomicrograph of a cell of *Bacillus subtilis* undergoing sporulation. The green color is a fluorescent dye that specifically stains a sporulation protein in the endospore coat.

The Sporulation Cycle

Sporulation is a form of cellular differentiation (◀ Figure 1.5), and many genetically directed events occur during the conversion from vegetative growth to sporulation. The structural changes in sporulating cells of *Bacillus* are shown in **Figure 2.29**. In *Bacillus subtilis*, which has been studied in detail, the conversion of a vegetative cell into an endospore takes about 8 hours and begins with asymmetric cell division and the formation of a *forespore* (Figure 2.29). Engulfment of the forespore by the mother cell results in the formation of the outer membrane that surrounds the developing endospore; the outer membrane forms from part of the mother cell's cytoplasmic membrane. Key events in endospore formation such as asymmetric cell division, cortex formation, and SASP production take place in a defined sequence and at specific times in the sporulation cycle (Figure 2.29). Genetic studies of mutants of *Bacillus subtilis*, each blocked at one of the stages of sporulation, indicate that more than 200 sporulation-specific genes exist. These genes are turned on and off in a genetic program that governs cellular differentiation, a process we will consider in detail when we examine the genetic regulation of sporulation (▶ Section 8.6).

Prokaryotic cells are not static entities, and so we now turn our attention to how and why cell movements occur.

Check Your Understanding

- What features differentiate an endospore from a vegetative cell?
- Ingestion of honey by infants can cause botulism as it often contains *Clostridium botulinum* endospores. Why wouldn't heating honey prevent this from occurring?
- How is the outer membrane of an endospore formed, and how does this structure differ from the outer membrane in the gram-negative cell envelope?

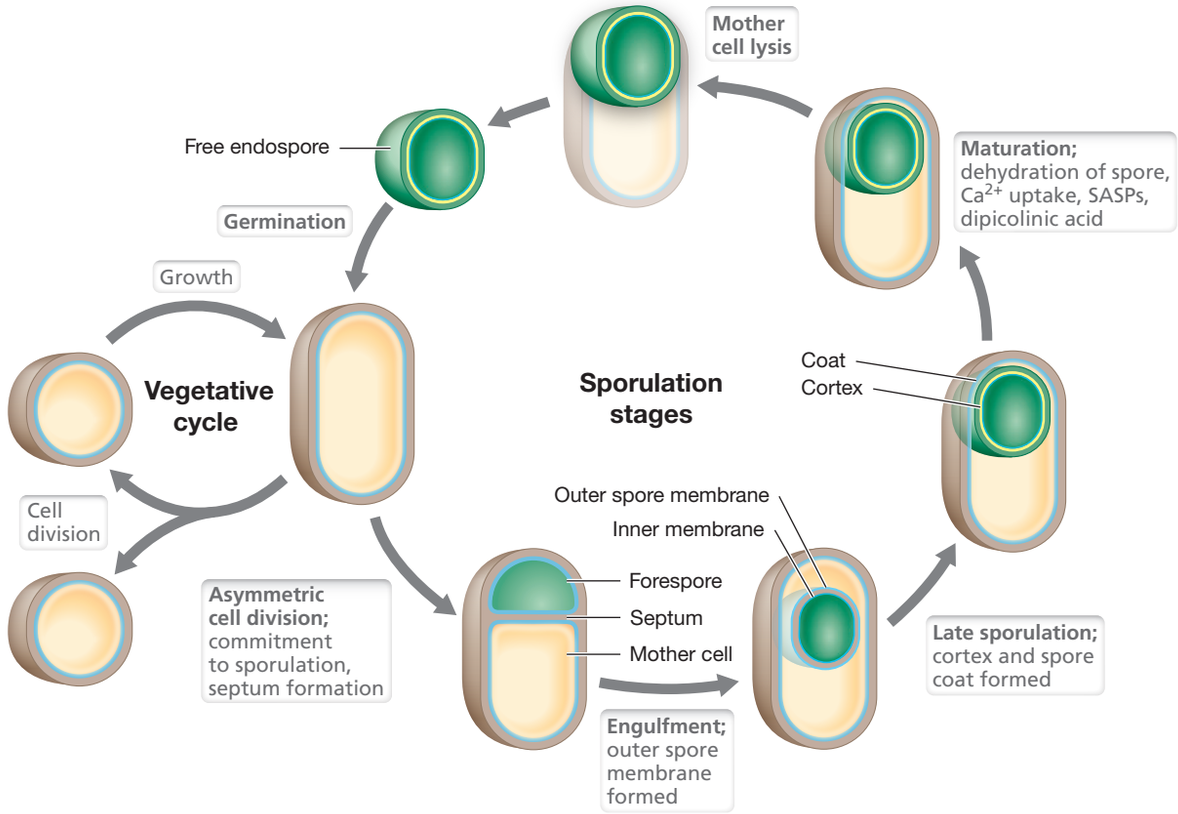


Figure 2.29 Major events in endospore formation. The steps depicted are those defined from genetic and microscopic analyses of sporulation in *Bacillus subtilis*, the model organism for studies of sporulation. SASPs, small acid-soluble proteins.

III • Cell Locomotion

Prokaryotic cells move about their environments by powering motility devices that allow them to swim or crawl, and they can move in specific directions in response to chemical or physical signals.

We finish our survey of prokaryotic cell structure and function by examining cell locomotion. Many microbial cells can move under their own power. Motility allows cells to reach different parts of their environment, and in nature, a new location may offer a cell additional resources or protection from harmful substances and ultimately spell the difference between life and death.

We examine here the two major types of prokaryotic cell movement, *swimming* and *gliding*. We then consider how motile cells are able to move in a directed fashion toward or away from particular stimuli (a phenomenon called *taxis*) and present examples of these simple behavioral responses.

2.9 Flagella, Archaella, and Swimming Motility

Mastering Microbiology Microbiology Animation: Flagella: Movement

Many *Bacteria* are motile by swimming due to a structure called the **flagellum** (plural, flagella) (Figure 2.30); an analogous structure called the **archaellum** is present in many *Archaea*. Flagella and archaella are tiny rotating machines that function to push or pull the cell through a liquid.

Flagella and Flagellation

Bacterial flagella are long, thin appendages (15–20 nm wide, depending on the species) free at one end and anchored into the cell at the other end. Flagella can be stained and observed by light microscopy (Figure 2.30) or electron microscopy (Figure 2.31).

Flagella can be anchored to a cell in different locations. In **polar flagellation**, the flagella are attached at one or both ends of a cell (Figure 2.30*b*). Occasionally, a group of many flagella (called a *tuft*) may arise at one end of the cell, a type of polar flagellation called *lophotrichous* (Figure 2.30*c*). Tufts of flagella can sometimes be seen in large unstained cells by dark-field or phase-contrast microscopy (Figure 2.32). When a tuft of flagella emerges from both poles of the cell, flagellation is called *amphitrichous*. In contrast to these more

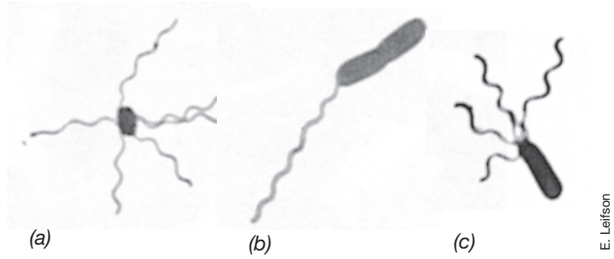


Figure 2.30 Bacterial flagella. Classic light photomicrographs taken by Einar Leifson of bacteria containing different arrangements of flagella. Cells are stained with the Leifson flagella stain. *(a)* Peritrichous. *(b)* Polar. *(c)* Lophotrichous.

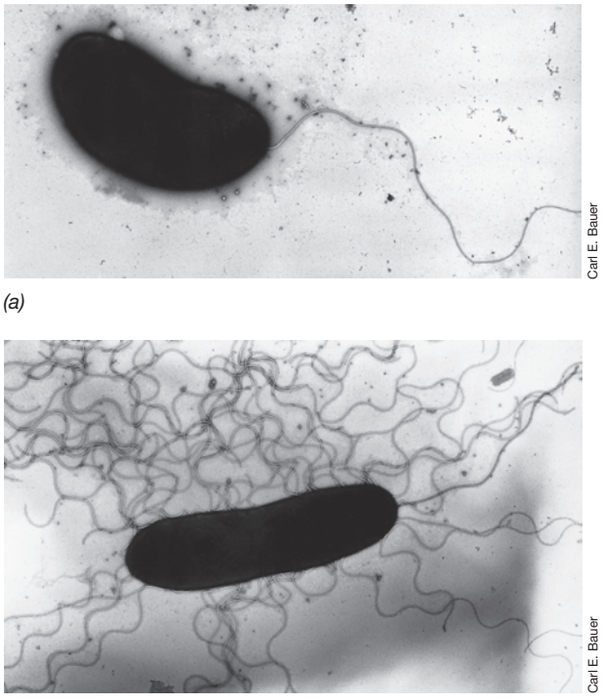


Figure 2.31 Bacterial flagella as observed by negative staining in the transmission electron microscope. *(a)* A single polar flagellum. *(b)* Peritrichous flagella. Both micrographs are of cells of the phototrophic bacterium *Rhodocista centenaria*, which are about 1.5 μm wide. Cells of *R. centenaria* are normally polarly flagellated but under certain growth conditions form peritrichous flagella. See Figure 2.41*b* for a photo of colonies of *R. centenaria* cells that move toward an increasing gradient of light (phototaxis).

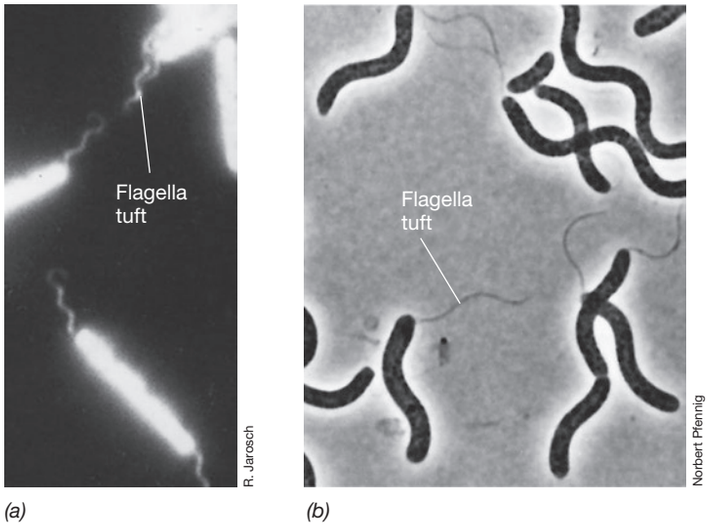


Figure 2.32 Bacterial flagellar tufts observed in living cells. *(a)* Dark-field photomicrograph of a group of large rod-shaped bacteria with flagellar tufts at each pole (a condition called *amphitrichous flagellation*). A single cell is about 2 μm wide. *(b)* Phase-contrast photomicrograph of cells of the large phototrophic purple bacterium *Rhodospirillum photometricum* that contain a tuft of lophotrichous flagella emerging from one of the poles. A cell measures about 4 × 25 μm.

specific sites of flagellation, in **peritrichous flagellation** (Figures 2.30*a* and 2.31*b*), flagella are inserted around the cell surface.

Flagella do not rotate at a constant speed but increase or decrease their rotational speed in relation to the strength of the proton motive force. Flagella can rotate at up to 1000 revolutions per second to support a swimming speed of up to 60 cell-lengths/sec. The fastest known land animal, the cheetah, can move at about 25 body-lengths/sec. Thus, a bacterium swimming at 60 cell-lengths/sec is actually moving over twice as fast—relative to its size—as the fastest animal!

The swimming motions of polarly and lophotrichously flagellated organisms differ from those of peritrichously flagellated organisms, and these can be distinguished microscopically (**Figure 2.33**). Peritrichously flagellated organisms typically move slowly in a straight line, stop and then head off in a new direction. By contrast, polarly flagellated organisms often move more rapidly and continuously, and some are able to reverse their direction. The different behavior of flagella on polar and peritrichous organisms, including differences in reversibility of the flagellum, is illustrated in Figure 2.33.

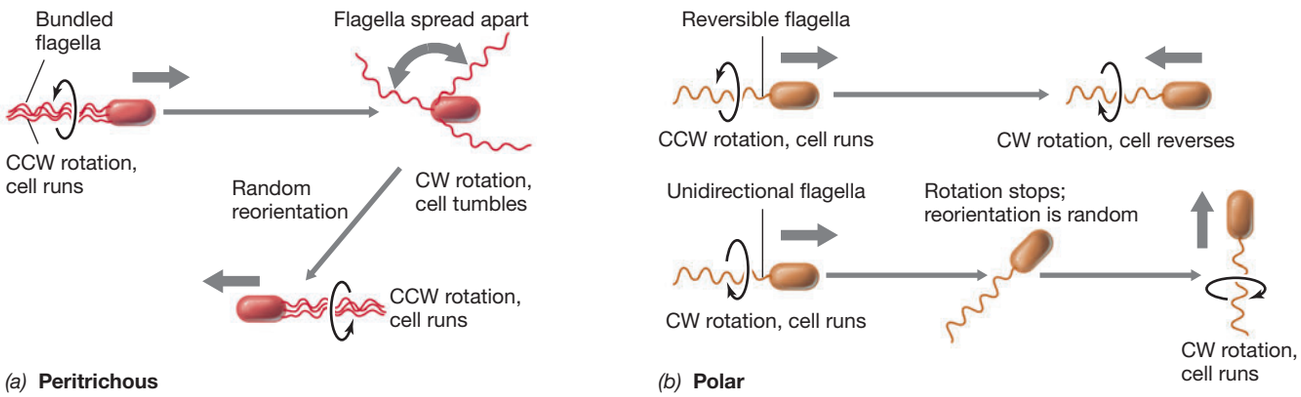


Figure 2.33 Movement in peritrichously and polarly flagellated prokaryotic cells. *(a)* Peritrichous: Forward motion is imparted by all flagella forming into a bundle and rotating counterclockwise (CCW). Clockwise (CW) rotation causes the bundle to break apart and the cell to tumble. A return to counterclockwise rotation leads the cell off in a new direction. *(b)* Polar: Cells change direction by reversing flagellar rotation (thus pulling instead of pushing the cell) or, with unidirectional flagella, by stopping periodically to reorient and then moving forward by clockwise rotation of its flagella. The gray arrow above each cell shows the direction the cell is traveling.

Flagella Structure and Activity

Bacterial flagella are rigid and helical (unlike eukaryal flagella, which are whiplike). The main part of the flagellum, called the *filament*, is composed of many copies of a protein called *flagellin*. The amino acid sequence of flagellin is highly conserved in *Bacteria*, suggesting that flagellar motility evolved early and has deep roots within this domain. In addition to the filament, a flagellum consists of several other components. A wider region at the base of the filament called the *hook* consists of a single type of protein and connects the filament to the flagellum motor in the *basal body* (**Figure 2.34**).

Mastering Microbiology
Microbiology Animation: Flagella: Structure

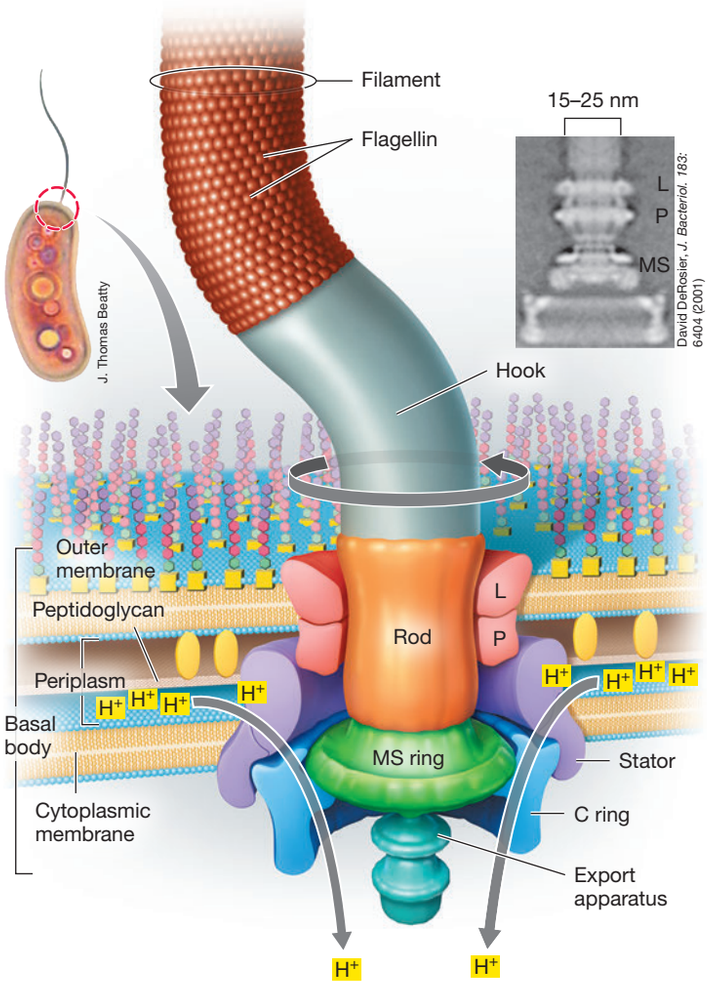


Figure 2.34 Structure and function of the flagellum in gram-negative *Bacteria*. The L ring is embedded in the LPS and the P ring in peptidoglycan. The MS ring is embedded in the cytoplasmic membrane and the C ring is found within the cytoplasm. The stator (Mot proteins) is embedded both within the cytoplasmic membrane and the peptidoglycan. Proton translocation through channels within the stator cause the MS ring to rotate, thereby driving rotation of the attached rod and flagellum. Flagellin molecules move from the export apparatus through a narrow channel in the rod and filament to reach the site of flagellar synthesis at the filament tip. The Mot proteins function as the flagellar motor. The flagellar motor rotates the filament to propel the cell through the medium. Inset photos: Top left, a cell of the purple sulfur bacterium *Chromatium* containing a tuft of polar flagella; Top right, transmission electron micrograph of a flagellar basal body from a cell of *Salmonella enterica* with the various rings labeled.

The flagellum motor is a reversible rotating machine composed of more than 25 proteins and anchored in the cytoplasmic membrane and cell wall. The motor consists of a central rod that passes through a series of rings. In gram-negative bacteria, an outer ring, called the *L ring*, is anchored in the outer membrane (Section 2.4). A second ring, called the *P ring*, is anchored in the peptidoglycan layer (Section 2.3). A third set of rings, called the *MS* and *C rings*, are located within the cytoplasmic membrane (Section 2.1) and the cytoplasm, respectively (Figure 2.34). In gram-positive bacteria, which lack an outer membrane, only the inner pair of rings is present. Surrounding the inner rings and anchored in the cytoplasmic membrane and the peptidoglycan is the *stator*, which is composed of *Mot proteins*. On the cytoplasmic side of the MS ring is the *export apparatus*, a type III secretion system (▶ Section 6.13) that facilitates synthesis of the flagellum.

The flagellum motor contains two main components: the *rotor* and the *stator*. The rotor consists of the central rod and the L, P, C, and MS rings. The stator is comprised of Mot proteins, which surround the rotor and function to generate torque. Collectively, these structures make up the flagellar **basal body** (Figure 2.34). Rotation of the flagellum occurs at the expense of the proton motive force (Section 2.1), and it is thought that rotation is caused by a type of "proton turbine" process. In this model, proton translocation through channels within the stator complex cause the MS ring to rotate, thereby driving rotation of the attached rod and flagellum. The L and P rings act like bushings within which the rod rotates. Protons flowing through the Mot proteins exert electrostatic forces on helically arranged charged residues on rotor proteins and cause the MS ring to rotate. About 1200 protons are translocated by each rotation of the flagellum. The rotational speed of the flagellum is set by the proton flow rate through the Mot proteins, which is a function of the intensity of the proton motive force. The flagellar motors of different microbes are able to generate different amounts of torque, causing significant differences in swimming speed; such adaptations are driven by adding or subtracting subunits from the stator and C ring, which presumably changes the "gear ratio" of the motor.

Flagellar Synthesis

Several genes encode the motility apparatus of *Bacteria*. In *Escherichia* and *Salmonella* species, in which motility studies have been extensive, over 50 genes are linked to motility in one way or another. These genes encode the structural proteins of the flagellum and motor apparatus, of course, but also encode proteins that export the structural proteins through the cytoplasmic membrane to the outside of the cell and proteins that regulate the synthesis of new flagella.

A flagellar filament grows not from its base, as does an animal hair, but from its tip. The MS ring is synthesized first and inserted into the cytoplasmic membrane. Then other anchoring proteins are synthesized along with the hook and the cap before the filament forms (**Figure 2.35**). Flagellin molecules synthesized in the cytoplasm are exported through the *export apparatus* (Figure 2.34) present on the cytoplasmic side of the basal body. The export apparatus shuttles flagellin molecules into a 3-nm channel that runs through the center of the basal body and the hollow flagellar filament. The flagellin

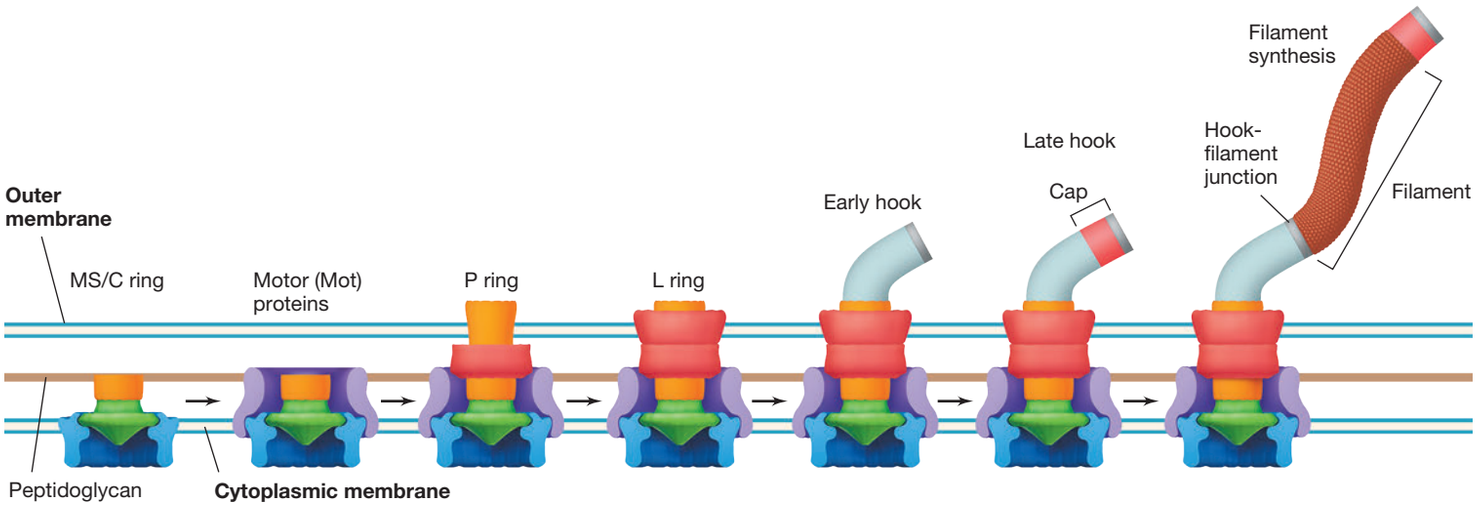


Figure 2.35 Flagella biosynthesis. Synthesis begins with assembly of MS and C rings in the cytoplasmic membrane, followed by the other rings, the hook, and the cap. Flagellin protein flows through the hook to form the filament and is guided into position by cap proteins.

subunits are ultimately passed to the end of the filament where they attach to the terminus of the growing flagellum. Cap proteins assist flagellin molecules to assemble in the proper fashion at the flagellum tip (Figure 2.35). Approximately 20,000 flagellin protein molecules are needed to make one filament. The flagellum grows more or less continuously until it reaches its final length. Broken flagella still rotate and can be repaired with new flagellin units passed through the filament channel to replace the lost ones.

Archaella

As in *Bacteria*, swimming motility is widespread among species of *Archaea* and is driven by the rotation of their flagellum analog, the *archaellum*. However, despite their similar function, archaella proteins are unrelated to those of flagella, and in evolutionary terms are more closely related to type IV pili (Section 2.6). Archaella are somewhat smaller than flagella, measuring about 10–13 nm in width (**Figure 2.36**), which is only slightly wider than type IV pili. Archaella are not hollow and are assembled from their bases much like type IV pili, whereas flagella are hollow and are assembled at their tips (Figure 2.35). Archaellar rotation, like the extension and retraction of type IV pili, is driven by ATP hydrolysis, whereas the energy for flagellar rotation is derived from the proton motive force. In addition, the archaellar motor is structurally simpler than the flagellar motor (compare Figure 2.36 with Figure 2.34), and only 7–12 genes are required to encode the major proteins that make up the archaellar motor, in contrast to the more than 25 genes that encode components of the flagellar motor.

Studies of swimming *Archaea*, such as *Halobacterium*, show that they swim at speeds only about one-tenth that of *Escherichia coli*, and in general, *Archaea* swim much more slowly than *Bacteria*. This could be due to the smaller diameter of the archaellum compared to the flagellum, which should reduce the torque the structure can generate. It could also be due to differences in the construction of the motor and how it is powered. However, some *Archaea* have found a way to overcome these limitations. For example, cells of *Methanocaldococcus* (Figure 2.36*c*) swim nearly 50 times faster than cells of *Halobacterium* and 10 times faster than cells of *Escherichia coli*. In fact, *Methanocaldococcus* swims at nearly 500 cell-lengths per second, which in a relative sense makes it one of the fastest organisms on Earth!

We now compare swimming motility with mechanistically quite different forms of cell movement but ones that still allow the cell to move about and explore its environment.

Check Your Understanding

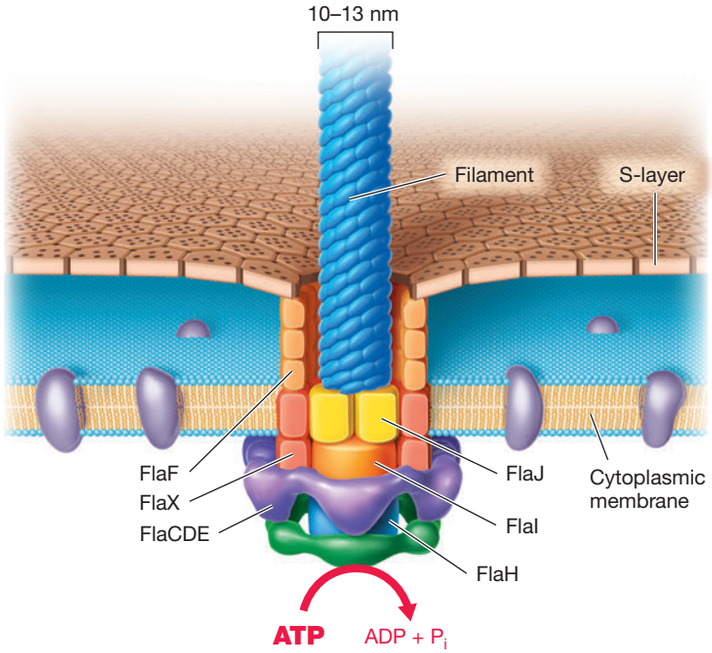
- Cells of *Salmonella* are peritrichously flagellated, those of *Pseudomonas* polarly flagellated, and those of *Spirillum* lophotrichously flagellated. Using a sketch, show how each organism would appear in a flagella stain.
- Compare flagella and archaella in terms of their structure, function, and energy source.
- The genes for flagellin are highly conserved between bacterial species. What does this imply about the importance of motility during bacterial evolution?

2.10 Surface Motility

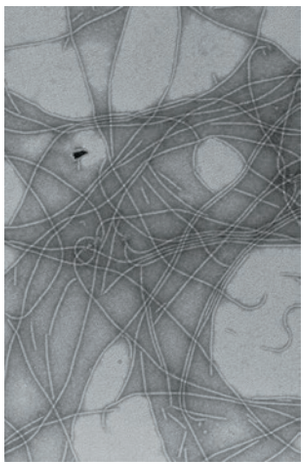
Many motile microbes are unable to swim and instead crawl over surfaces in various ways. These forms of surface motility require attachment to a surface and are independent of flagella or archaella. Surface motility results in distinctive colony morphology because cells can move out and away from the center of the colony (**Figure 2.37**). All forms of surface motility are considerably slower than swimming motility, usually less than 10 μm/sec. Finally, whereas nearly all swimming motility in *Bacteria* and *Archaea* is driven by flagella and archaella (Section 2.9), surface motility can be produced by a diversity of different systems, the most well characterized of which are *twitching motility* and *gliding motility*.

Twitching Motility

Twitching motility requires type IV pili (Section 2.6), which extend from one pole of the cell, attach to a surface, and then retract to pull

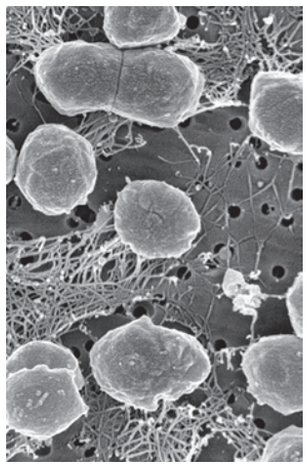


(a)



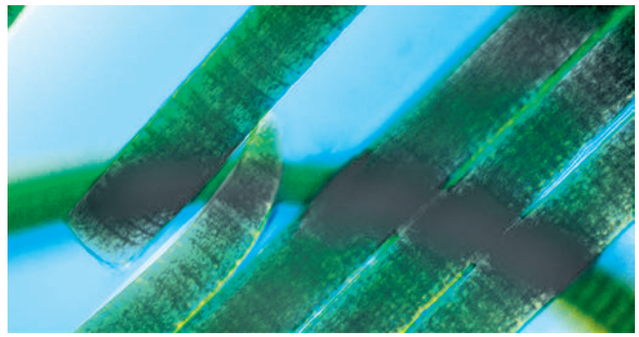
S.I. Aizawa and K.F. Jarrell

(b)



(c)

Figure 2.36 Archaella. *(a)* Depiction of an archaellum embedded in an S-layer and cytoplasmic membrane; compare with the bacterial flagellum in Figure 2.34. The archaellum and its motor differ from flagella in that the archaellum has a smaller diameter, it is not hollow, its motor has fewer proteins, and the energy for rotation comes from ATP hydrolysis and not from consumption of the proton motive force. *(b)* Negatively stained transmission electron micrograph of purified archaella. *(c)* Scanning electron micrograph of cells of *Methanocaldococcus* containing multiple archaella.

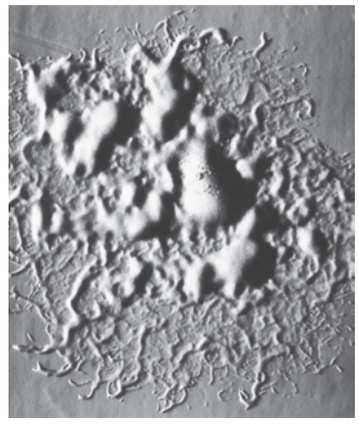


Richard W. Castenholz

(a)

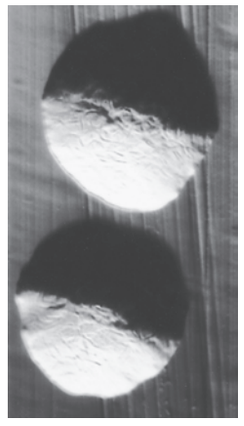
Richard W. Castenholz

(b)



Mark J. McBride

(c)



Mark J. McBride

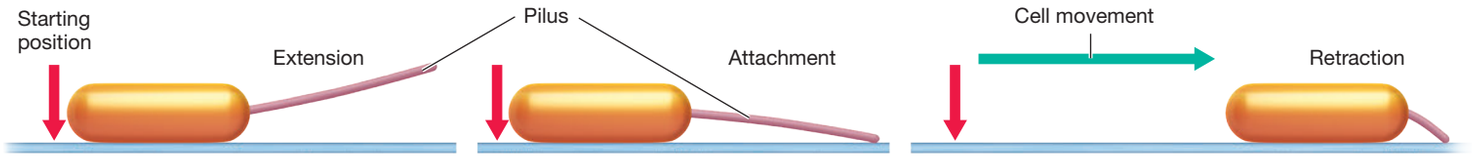
(d)

Figure 2.37 Gliding bacteria. *(a, b)* The large filamentous cyanobacterium *Oscillatoria* has cells about 35 μm wide. *(b) Oscillatoria* filaments gliding on an agar surface. *(c)* Masses of the bacterium *Flavobacterium johnsoniae* gliding away from the center of the colony (the colony is about 2.7 mm wide). *(d)* Nongliding mutant strain of *F. johnsoniae* showing typical colony morphology of nongliding bacteria (the colonies are 0.7–1 mm in diameter). See also Figure 2.41.

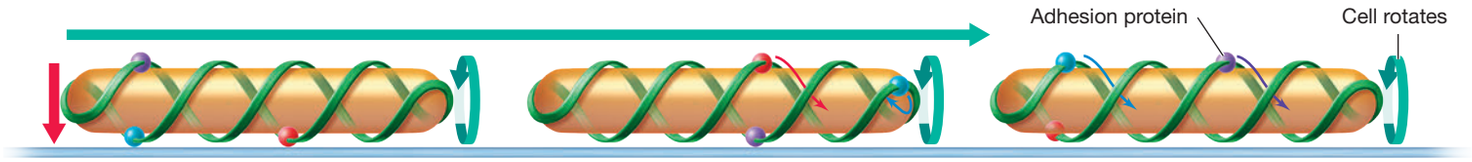
the cell forward (**Figure 2.38*a***). The energy required to catalyze the activity of type IV pili comes from ATP hydrolysis. Twitching motility has been described in many *Bacteria* and some *Archaea*. For example, species of the gram-negative bacterium *Pseudomonas* are often capable of twitching motility and this trait is important to their ability to form biofilms (▶ Section 4.9). Twitching motility has also been well described in myxobacteria, which are social predatory bacteria whose cells can work together to consume other bacteria. Myxobacteria exhibit two forms of motility: social motility, which is caused by twitching, and adventurous motility, which is caused by gliding (see below). Twitching motility allows cells to move together in groups, and this trait is facilitated by the production of both type IV pili and the secretion of extracellular polysaccharides that aid in cellular cohesion.

Gliding Motility

Gliding motility (Figure 2.38*b*) is defined as a smooth motion along the long axis of a cell without the aid of external propulsive



(a) **Twitching**



(b) **Gliding**

Figure 2.38 Surface motility. *(a)* Twitching motility employs type IV pili. These structures require ATP hydrolysis to extend (up to several micrometers) and then retract, causing the cell to move forward; movement occurs in discrete increments. *(b)* Gliding motility requires a helical intracellular protein track that interacts with gliding motors and extracellular adhesion proteins. The proton motive force drives rotation of gliding motors that translate this force to the helical track, causing adhesion proteins to move in a helical pattern; this results in continuous forward motion and clockwise rotation of the cell.

structures (such as pili or attachment organelles). Gliding motility is a continuous form of movement, very unlike swimming motility, in which cells frequently stop and then swim off in a random new direction. Gliding bacteria are typically filamentous or rod-shaped in morphology, and while gliding has been observed among diverse species of *Bacteria*, no gliding *Archaea* are known. The adventurous motility of myxobacteria is a form of gliding that has been well studied, and many species of the *Bacteroidetes* such as *Flavobacterium* also exhibit gliding motility.

Gliding has been best studied in *Myxococcus* and *Flavobacterium* (Figure 2.37*c*), and the mechanism of gliding in these two organisms is likely to be quite similar. These organisms both possess a helical intracellular track made of proteins that run in a continuous loop around the cell (Figure 2.38*b*). Associating with this track are "gliding motors," rotary motors that are driven by the proton motive force, and which are evolutionarily and operationally similar to the flagellar motor (see Figure 2.34). These organisms also have adhesion proteins that grab onto surfaces on the outside of the cell. While the exact mechanism of gliding remains unknown, it is thought that the gliding motor associates with the helical track, and rotation of the motor causes displacement of the track relative to the motor (or of the motor relative to the track). This movement is in some way transduced to the surface adhesion proteins, causing them to move in a helical direction around the surface of the cell. The net result is that the cell is propelled forward over the surface and caused to rotate around its axis as it moves forward (Figure 2.38*b*).

We now consider how these various forms of prokaryotic cell motility can move cells in specific directions, which allows them to both exploit useful nutrients and avoid harmful substances.

Check Your Understanding

- How does gliding motility differ from swimming motility in both mechanism and requirements?
- Contrast the mechanism of twitching motility with that of gliding motility.

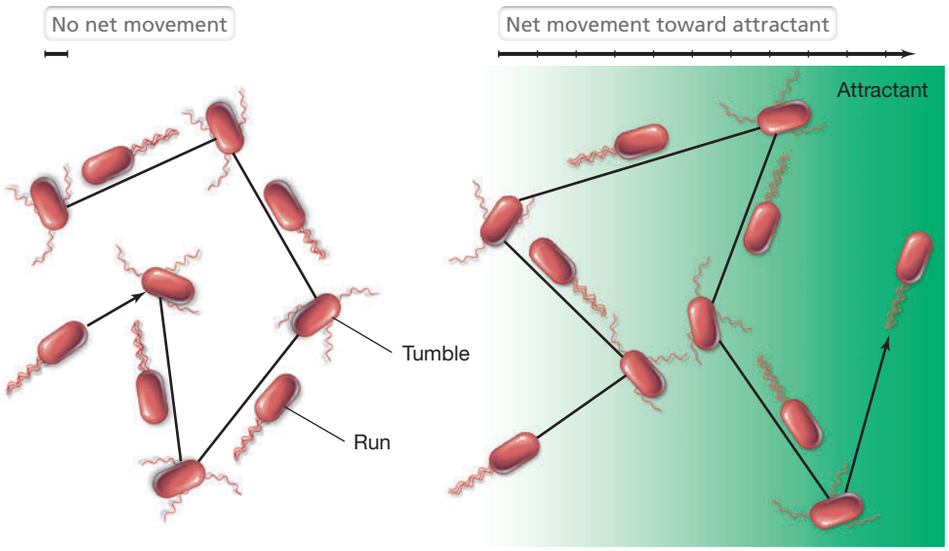
2.11 Chemotaxis

Cells of *Bacteria* and *Archaea* have evolved numerous systems that allow them to sense and respond to chemical and physical stimuli in their environments. For example, microbes are able to sense and move toward a food source. Such a directed movement is called *taxis* (plural, taxes). **Chemotaxis**, a response to chemicals, and **phototaxis**, a response to light, are two well-studied forms of taxis. The ability of a cell to move toward or away from various stimuli has ecological significance in that the directed movement may enhance a cell's access to resources or allow it to avoid harmful substances that could damage or kill it.

Chemotaxis has been well studied in *Bacteria* that use swimming motility; these organisms sense environmental stimuli and transmit signals to the flagellum causing it to alter its rotation. In addition, most *Archaea* that exhibit swimming motility are also chemotactic, and several of the proteins that control chemotaxis in *Bacteria* are similar to those found in *Archaea*. Gliding bacteria are also often chemotactic and can alter their direction in response to environmental stimuli. Phototaxis is often observed in filamentous cyanobacteria (Figure 2.37*a*, *b*) which move toward or away from a light source. In Section 7.6 we examine in detail the molecular mechanism of chemotaxis and its genetic regulation in the model bacterium *Escherichia coli*. For now, we will focus on the general principles by which chemotaxis operates.

Chemotaxis in Peritrichously Flagellated *Bacteria*

Much research on chemotaxis has been done with the peritrichously flagellated bacterium *E. coli*. In the absence of an attractant, cells move in a random fashion that includes *runs*, in which the cell is swimming forward in a smooth fashion, and *tumbles*, when the cell stops and jiggles about randomly (**Figure 2.39**). During a run, the flagellar motor rotates *counterclockwise*, causing the flagella to coil together into a bundle that propels the cell forward. By contrast, when flagella rotate *clockwise*, the bundle of flagella pushes apart, forward motion ceases, and the cells tumble randomly (Figure 2.39*a*). Following a tumble, the cell will begin a new run in a random direction. Thus, in the absence of an



(a) **No attractant present: Random movement** *(b)* **Attractant present: Directed movement**

Figure 2.39 Chemotaxis in a peritrichously flagellated bacterium. *(a)* In the absence of a chemical attractant, the cell swims randomly in runs, changing direction during tumbles. *(b)* In the presence of an attractant, runs become biased, and the cell moves up the gradient of the attractant. The attractant gradient is depicted in green, with the highest concentration where the color is most intense.

attractant, the cell moves about its environment in random fashion through a series of runs and tumbles.

To understand chemotaxis, consider what happens when *E. coli* encounters a chemical attractant, such as the sugar glucose (Figure 2.39*b*). Because of diffusion, the concentration of the attractant will increase when the cell is moving toward the attractant. During chemotaxis cells will tend to move toward the attractant over time, but cells do not move continuously nor directly toward the attractant; instead, they exhibit a behavior known as a *biased random walk*. The key to this mechanism is that cells sample the concentration of attractant not over space, but rather *over time*. As the cell is swimming in a random direction, it continues to sense the concentration of attractant over time. If the concentration goes up, runs become longer and tumbles less frequent, but if the concentration goes down, runs become shorter and tumbles more frequent. The biased random walk causes net movement toward the attractant (Figure 2.39*b*), and this mechanism is remarkably effective for bringing cells to the source of an attractant. For a repellent, the same mechanism applies, but the response is reversed, with longer runs triggered by a *decrease* in concentration of the repellent. Attractants and repellents are sensed by a series of membrane proteins called *chemoreceptors*. Chemoreceptors sense the concentration of particular chemicals and transduce this information to flagella, causing them to alter their rotation (▶ Section 7.6).

Chemotaxis in Polarly Flagellated *Bacteria*

Chemotaxis in polarly flagellated cells is similar but not identical to chemotaxis in peritrichously flagellated cells such as *E. coli*. Many polarly flagellated bacteria, such as *Pseudomonas* species, which have a single polar flagellum rather than a tuft, do not tumble when they reverse their flagellum. Instead, they swim backwards when their flagellum reverses its direction of rotation (Figure 2.33*b*). In contrast, the phototrophic bacterium *Rhodobacter* has a single polar flagellum that is not reversible; it can only start and stop its rotation. Both of these polarly flagellated bacteria rely on Brownian motion when stopped to randomly reorient the cell (Figure 2.33*b*). Then as the flagellum begins to rotate again, the cell moves off in a new direction. Despite this seemingly random activity, cells of *Rhodobacter* are strongly chemotactic to various organic compounds and to oxygen and light. Chemotactic organisms, by employing a *biased* random walk, can navigate effectively through their environments toward conditions that favor growth and away from those that could inhibit growth or otherwise cause harm.

Measuring Chemotaxis

Bacterial chemotaxis can be measured in a chemotaxis assay in which a small glass capillary tube containing an attractant is immersed into a suspension of motile bacteria. A chemical gradient extends from the tip of the capillary into the surrounding medium, with the chemical concentration decreasing with distance from the tip (**Figure 2.40**). When an attractant is present, chemotactic bacteria will move toward it, forming a swarm around the open tip (Figure 2.40*c*) with many of the bacteria swimming into the capillary tube. Of course, because of random movements some chemotactic bacteria will swim into the capillary even if it does not contain an attractant (control solution, Figure 2.40*b*). However, when an attractant is present, the number of cells within the capillary will increase significantly (Figure 2.40*e*). If the capillary contains a repellent, the number of bacteria within the capillary will be fewer than in the control (Figure 2.40*d*). This capillary method can be used to screen chemicals to see if they are attractants or repellents for a given bacterium.

Chemotaxis can also be observed microscopically. It is possible to quantify chemotactic behavior using a video camera that captures the positions of bacterial cells with time and records the motility tracks of each cell (Figure 2.40*f*). This method has been used to study chemotaxis of microbes in natural environments. In nature, nutrients are often excreted from larger microbial cells whether they are living or dead. Algae, for example, produce both organic compounds and oxygen (O_2, from photosynthesis), which can trigger chemotactic movements of bacteria toward the algal cell (Figure 2.40*f*). Chemotaxis can result in complex cellular interactions in nature including competition for resources and cooperation by recycling metabolic products.

Check Your Understanding

- Describe how bacteria use a biased random walk to move toward an attractant.
- Contrast the motility of a peritrichously flagellated bacterium such as *Escherichia coli* with that of a polarly flagellated bacterium such as a species of *Pseudomonas*.
- In a capillary tube assay, why is it necessary to use a control that lacks attractant?

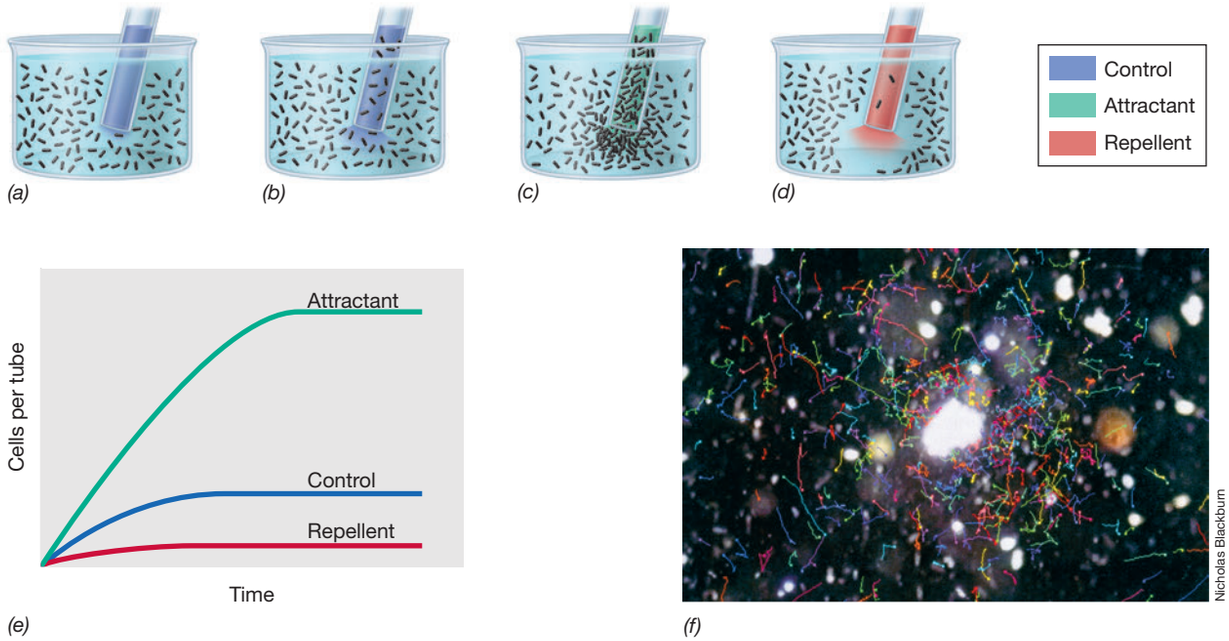


Figure 2.40 **Measuring chemotaxis using a capillary tube assay.** *(a)* Insertion of the capillary into a bacterial suspension. As the capillary is inserted, a gradient of the chemical begins to form. *(b)* A control capillary contains a salt solution that is neither an attractant nor a repellent. Cell concentration inside the capillary becomes the same as that outside. *(c)* Accumulation of bacteria in a capillary containing an attractant. *(d)* Repulsion of bacteria by a repellent. *(e)* Time course showing cell numbers in capillaries containing various chemicals. *(f)* Tracks of motile bacteria in seawater swarming around an algal cell (large white spot, center) photographed with a tracking video camera system attached to a microscope. The bacterial cells are showing positive aerotaxis by moving toward the oxygen-producing algal cell. The alga is about 60 μm in diameter.

2.12 Other Forms of Taxis

Chemotaxis is a directed movement with respect to a chemical gradient, but many other forms of sensory response can govern microbial motility. For example, *osmotaxis* is directed movement with respect to a gradient of ionic strength, *hydrotaxis* is directed movement with respect to a gradient of available water, *aerotaxis* is directed movement with respect to gradients of O_2, and *phototaxis* is directed movement with respect to a gradient in light intensity. Here we will consider phototaxis and aerotaxis, two taxes that have been particularly well studied.

Phototaxis

Many phototrophic microorganisms can move toward light, a process called *phototaxis*. Phototaxis allows a phototrophic organism to position itself most efficiently to receive light for photosynthesis. For example, if motile phototrophic purple bacteria are placed on a microscope slide that is illuminated with a spectrum of light they will move preferentially toward certain wavelengths (**Figure 2.41*a***). The wavelengths of light at which the bacteria accumulate correspond to the wavelengths of light that are absorbed by their photosynthetic pigments. These pigments include, in particular, bacteriochlorophylls and carotenoids (Chapter 14).

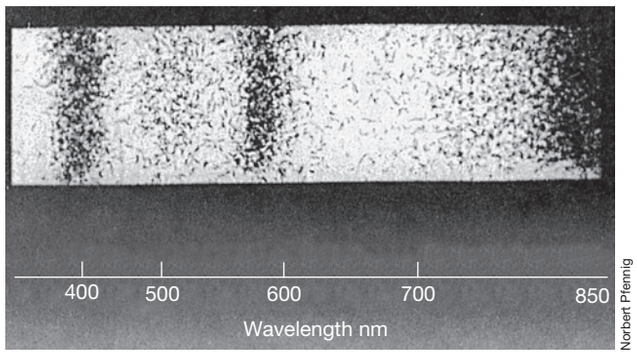
Two different light-mediated taxes are observed in phototrophic bacteria. One, called *scotophobotaxis*, is easily observed in the microscope. Scotophobotaxis occurs when a phototrophic bacterium happens to swim into darkness outside the illuminated field of view of the microscope. Entering darkness negatively affects photosynthesis, and thus cellular energy levels, and this signals the cell to tumble with greater frequency until it reenters the light by a biased random walk. Scotophobotaxis is a mechanism to prevent phototrophic cells from swimming away from a lighted zone into darkness, and this likely improves their competitive success in nature.

Phototaxis differs from scotophobotaxis in that cells move with respect to a *gradient* of light intensity, whereas scotophobotaxis is a response only to the absence of light. Phototaxis is analogous to chemotaxis except that the attractant is light instead of a chemical. In some phototactic organisms, such as the highly motile phototrophic purple bacterium *Rhodocista centenaria* (Figure 2.31), *entire colonies* of cells show phototaxis and move in unison toward the light (Figure 2.41*b*).

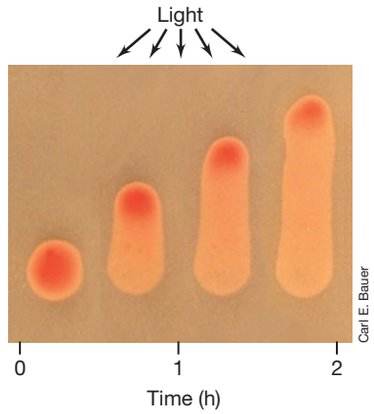
Several components of the regulatory system that control chemotaxis also control phototaxis. A *photoreceptor*, a protein that functions similarly to a chemoreceptor but which senses a gradient of light instead of chemicals, is the initial sensor in the phototaxis response. The photoreceptor then interacts with the same cytoplasmic proteins that control flagellar rotation in chemotaxis, maintaining the cell in a run if it is swimming toward an increasing intensity of light. Section 7.6 describes the activities of these proteins in more detail.

Aerotaxis and Magnetotaxis

Aerotaxis is directed motility with respect to O_2 (see Figure 2.40*f*). Aerobic organisms require O_2 and may exhibit a positive aerotactic response, swimming toward increasing concentrations of O_2. We will see in the next chapter, however, that O_2 is toxic to many



(a)



(b)

Figure 2.41 Phototaxis of phototrophic bacteria. *(a)* Scotophobic accumulation of the phototrophic purple bacterium *Thiospirillum jenense* at wavelengths of light at which its pigments absorb. A light spectrum was displayed on a microscope slide containing a dense suspension of the bacteria; after a period of time, the bacteria had accumulated selectively and the photomicrograph was taken. The wavelengths at which the bacteria accumulated are those at which the photosynthetic pigment bacteriochlorophyll *a* absorbs. *(b)* Phototaxis of an entire colony of the purple phototrophic bacterium *Rhodocista centenaria*. These strongly phototactic cells move in unison toward the light source at the top. See Figure 2.31 for electron micrographs of flagellated *R. centenaria* cells.

microbes. *Microaerophiles* are aerobic organisms that require O_2 but are killed when the concentration of O_2 is too high. Microaerophiles often use aerotaxis to position themselves at an optimum concentration of O_2 (usually between 1% and 5% O_2). Microaerophily can be a challenging lifestyle because either too much or too little O_2 can mean death, and O_2 gradients are highly dynamic because many organisms either produce or consume O_2. Hence, some microaerophiles have evolved aerotactic mechanisms that help them better navigate dynamic gradients of O_2. Indeed, a movement of only a few tens of micrometers may be necessary to place aerotactic cells in a more suitable gaseous environment.

Magnetosomes (Section 2.7) are found in specialized microaerophiles, referred to as *magnetotactic bacteria*, which are found in sediments, lakes, and ponds where concentrations of O_2 are naturally low. Because Earth is a sphere, its magnetic field lines have a significant vertical component where they intersect the surface. Magnetosomes allow magnetotactic bacteria to align themselves with these magnetic field lines. This causes the bacterial cells to point up or down so that they can swim either toward or away from O_2 at the surface. These so-called magnetotactic bacteria do not actually exhibit directed motility toward magnetic fields but instead are exhibiting aerotaxis; their magnetosomes allow them to reduce a three-dimensional biased random walk to a two-dimensional biased random walk and this vastly improves their ability to position themselves in their environment with respect to O_2.

We now segue from our consideration of the structure and function of major components of prokaryotic cells to examine structure–function issues in eukaryotic cells, many of which are microbial and coexist with prokaryotic cells in the microbial world.

Check Your Understanding

- How does scotophobotaxis differ from phototaxis?
- Explain how magnetosomes contribute to the motility of magnetotactic bacteria.

IV • Eukaryotic Microbial Cells

The cytoplasm of eukaryotic cells contains membrane-bound organelles including the nucleus, mitochondria, and chloroplasts, and several other distinct structures. During cell division, the nucleus divides by mitosis and reproductive structures are formed by the process of meiosis.

Compared with prokaryotic cells, microbial eukaryotes typically have structurally more complex and much larger cells. Microbial eukaryotes include the fungi and a vast diversity of protists as we will see in Chapter 18. Many aspects of eukaryotic cell structure set *Eukarya* apart from *Archaea* and *Bacteria*, but the defining feature of the eukaryotic cell is its nucleus.

2.13 The Nucleus and Cell Division

A double membrane–enclosed nucleus is a universal feature of the eukaryotic cell. Mitochondria are also nearly universal among eukaryotic cells, although a few unusual protists lack mitochondria. Chloroplasts are found only in phototrophic eukaryotic cells, including plants and diverse algae. Other characteristic eukaryotic structures include the Golgi complex, lysosomes, endoplasmic reticula, and microtubules and microfilaments (Figure 2.42). Some microbial eukaryotes have flagella or cilia—structures that confer motility—and a cell wall is present in many eukaryotic cells, such as the fungi and algae.

Eukaryotic cell membranes contain *sterols*. These rather rigid organic molecules, which are a subclass of steroids and absent from all but a few prokaryotic cells, lend structural strength to the eukaryotic cell, something especially important to those eukaryotes that lack a cell wall, such as many protists and animal cells.

The Nucleus

The **nucleus** contains the chromosomes of the eukaryotic cell. DNA within the nucleus is wound around basic (positively charged)

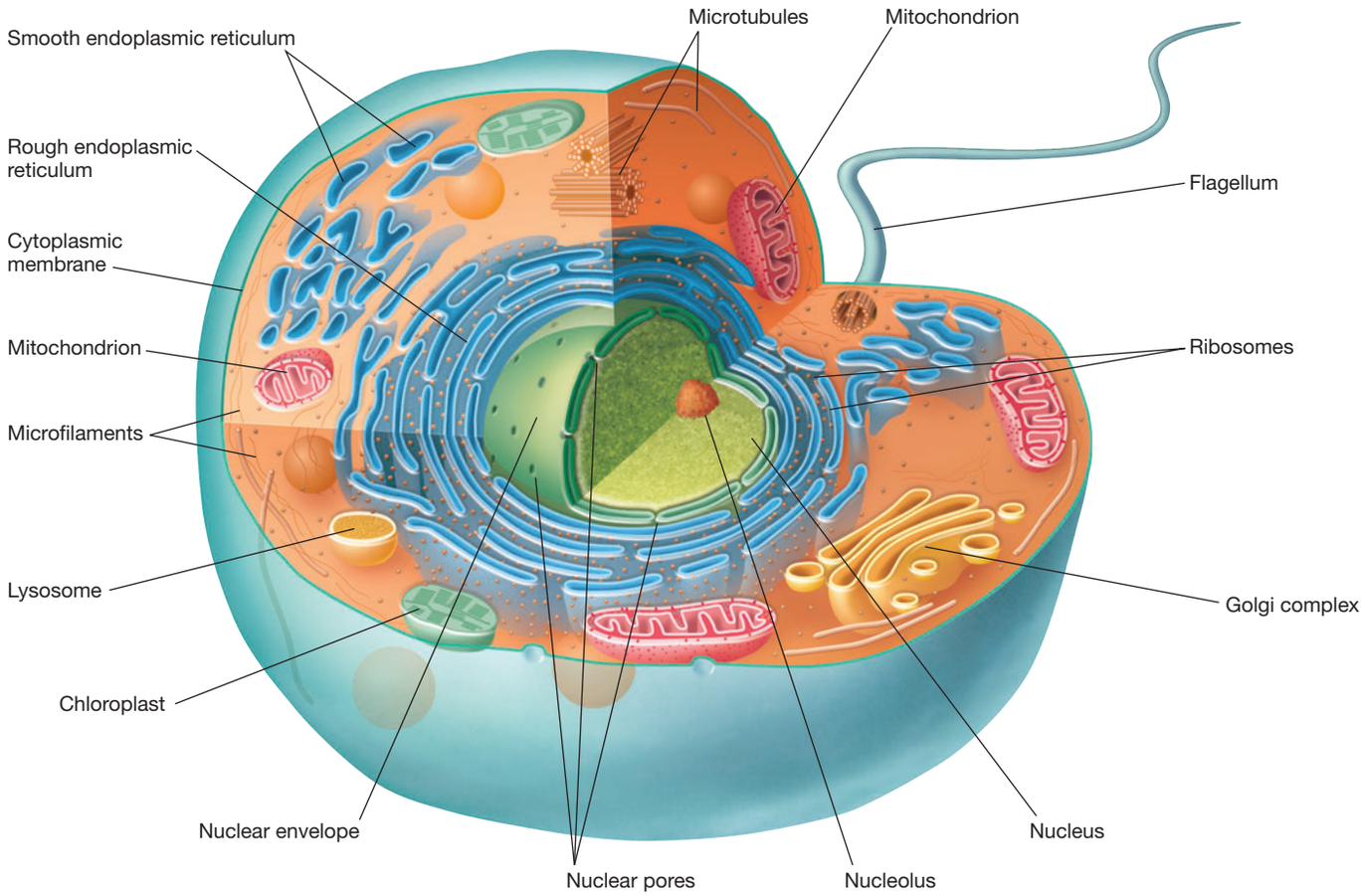


Figure 2.42 **Cutaway schematic of a cell of a microbial eukaryote.** Although all eukaryotic cells contain a nucleus, not all organelles and other structures shown are present in all microbial eukaryotes. Not shown is the cell wall, found in fungi, algae, plants, and a few protists.

proteins called **histones**, which tightly pack the negatively charged DNA to form *nucleosomes* (**Figure 2.43**); the latter are then organized into chromosomes. *Archaea* also contain histones and nucleosomes, and these are evolutionarily related to those found in *Eukarya*. Moreover, a few *Bacteria* also contain histone-like proteins that function to help organize their DNA.

Only cells of *Eukarya* contain a nucleus. The nucleus is enclosed by a *pair* of membranes, each with its own function, separated by a space. The innermost membrane is a simple sac while the outermost membrane is in many places continuous with the endoplasmic reticulum. The inner and outer nuclear membranes specialize in interactions with the nucleoplasm and the cytoplasm, respectively. The nuclear membranes contain pores (Figures 2.42 and 2.43*a*), formed from holes where the inner and outer membranes are joined. The pores allow transport proteins to import and export other proteins and nucleic acids into and out of the nucleus, a process called *nuclear transport*.

Within the nucleus is found the *nucleolus* (Figure 2.42), the site of ribosomal RNA (rRNA) synthesis. The nucleolus is rich in RNA, and ribosomal proteins synthesized in the cytoplasm are transported into the nucleolus and combine with rRNA to form the small and large subunits of eukaryotic ribosomes. These are then exported to the cytoplasm, where they associate to form the intact ribosome and function in protein synthesis.

Cell Division

Eukaryotic cell division requires a special process, called **mitosis**, in which the chromosomes are replicated, the nucleus is disassembled, the chromosomes are segregated into two sets, and a nucleus is reassembled in each daughter cell (**Figure 2.44**). Whereas many (though not all) prokaryotic cells are genetically haploid, microbial eukaryotes often alternate between haploid and diploid states. *Diploid* cells have two copies of each chromosome whereas *haploid* cells have only one. For example, the brewer's yeast *Saccharomyces cerevisiae* can exist in the haploid state (in which cells contain 16 chromosomes) as well as in the diploid state (in which cells contain 32 chromosomes). However, regardless of its genetic state, during cell division the chromosome number is first doubled and later halved to give each daughter cell its correct complement of chromosomes. During mitosis, the chromosomes condense, divide, and are separated into two sets, one for each daughter cell. Each of these distinct stages of nuclear division has a common name used widely in biology: *prophase*, *metaphase*, *anaphase*, and *telophase* (Figure 2.44*a–d*, respectively).

In contrast to mitosis, **meiosis** converts a diploid cell into several haploid cells. Meiosis consists of two successive cell divisions. In the first meiotic division, pairs of chromosomes segregate into separate cells, changing the genetic state from diploid to haploid. The second meiotic division is essentially the same as mitosis, as the two

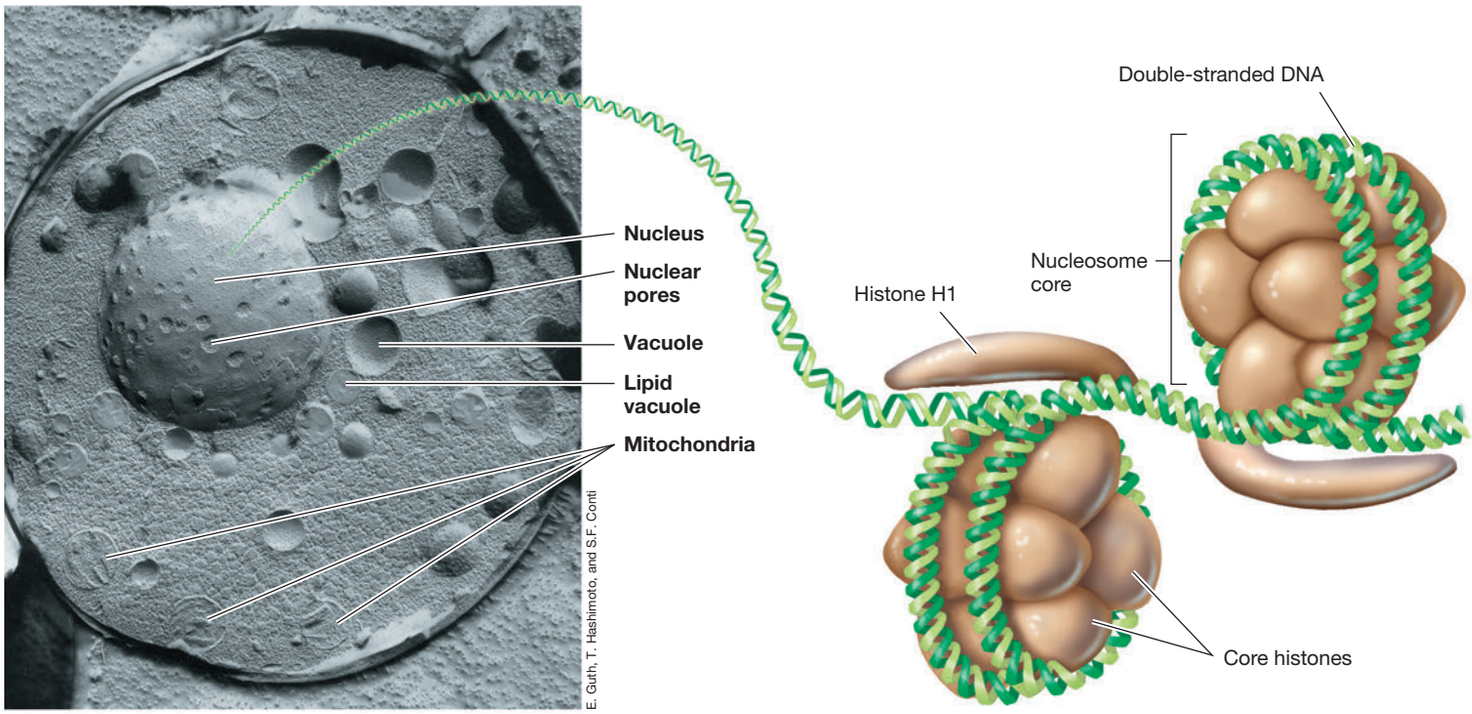


Figure 2.43 The nucleus and DNA packaging in eukaryotes. *(a)* Electron micrograph of a yeast cell prepared in such a way as to reveal a surface view of the nucleus. The cell is about 8 μm wide. *(b)* Packaging of DNA around histone proteins to form a nucleosome. Nucleosomes are arranged along the DNA strand like beads on a string and aggregate to form chromosomes during the process of mitosis (see Figure 2.44).

haploid cells divide to form a total of four haploid cells called *gametes*. This form of cell division is typically used in organisms that reproduce sexually. In animals, these gametes are the eggs and sperm; in eukaryotic microorganisms, they can be reproductive spores or other reproductive structures.

We now consider the classic organelles of the eukaryotic cell: the mitochondrion and the chloroplast.

Check Your Understanding

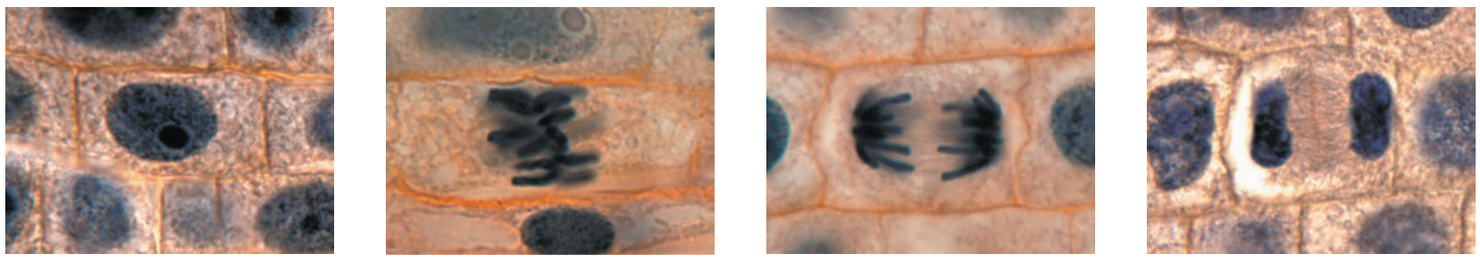
- How is DNA arranged in the chromosomes of eukaryotes?
- What are histones and what do they do?
- What are the major differences between mitosis and meiosis?

2.14 Mitochondria and Chloroplasts

Organelles that specialize in energy metabolism in eukaryotes include the mitochondrion and the chloroplast. These organelles have evolutionary roots within the *Bacteria* and provide ATP to the eukaryotic cell either from the oxidation of organic compounds or from light.

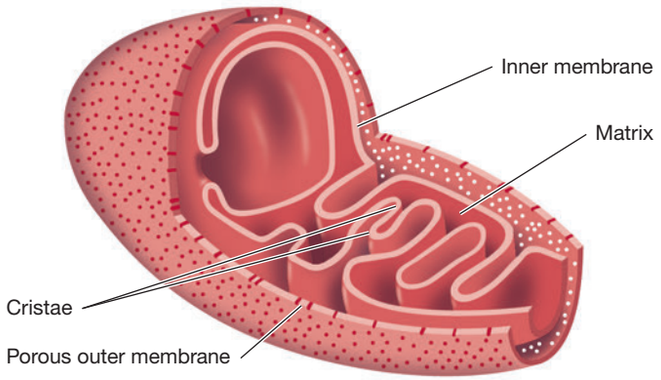
Mitochondria

In aerobic eukaryotic cells, respiration occurs in the mitochondrion. **Mitochondria** are the size of a typical bacterium, they have their own DNA and ribosomes, they can take on many shapes (Figure 2.45), and they reproduce independently of the cell. The number of

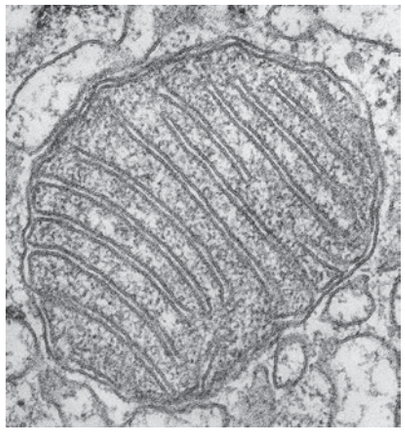


(a) **Interphase** (b) **Metaphase** (c) **Anaphase** (d) **Telophase**

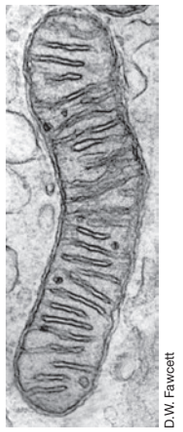
Figure 2.44 Light micrograph of eukaryotic cells undergoing mitosis. *(a)* Interphase, distinct chromosomes are not apparent. *(b)* Metaphase. Homologous chromosomes are lining up along the cell center; compare with Figure 2.47*b*. *(c)* Anaphase. Homologous chromosomes are pulling apart. *(d)* Telophase. Chromosomes have separated into the newly forming daughter cells.



(a)

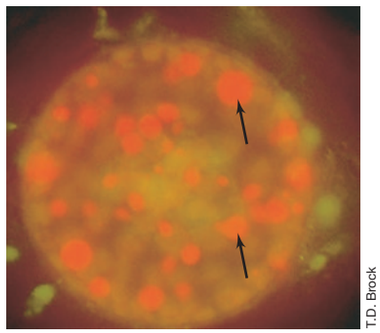


(b)

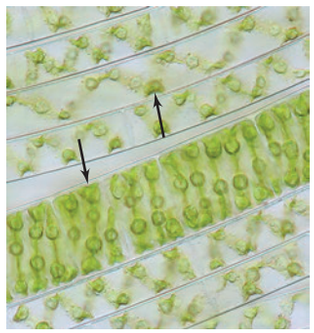


(c)

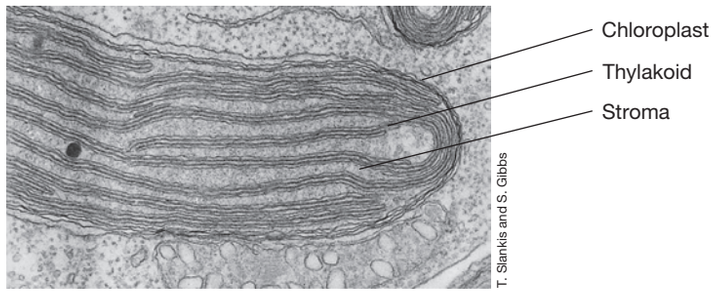
Figure 2.45 Structure of the mitochondrion. *(a)* Diagram showing the overall structure of the mitochondrion; note the inner and outer membranes. *(b, c)* Transmission electron micrographs of mitochondria from rat tissue showing the variability in morphology; note the cristae. Both mitochondria are about 0.7 μm wide.



(a)



(b)



(c)

Figure 2.46 Chloroplasts of a diatom and a green alga cell. *(a)* Fluorescence photomicrograph of a diatom shows chlorophyll fluorescence; arrows, chloroplasts. The cell is about 40 μm wide. *(b)* Phase-contrast photomicrograph of the filamentous green alga *Spirogyra* showing the characteristic spiral-shaped chloroplasts (arrows) of this phototroph. A cell is about 20 μm wide. *(c)* Transmission electron micrograph showing a chloroplast of a diatom; note the thylakoids.

mitochondria per cell varies depending on the cell type, its size, and its physiological state. A yeast cell may have only a few mitochondria per cell, whereas an animal cell may have over a thousand. The mitochondrion is enclosed by a double membrane system. Like the nuclear membrane, the outermost mitochondrial membrane is somewhat permeable and contains pores that allow the passage of small molecules. The innermost membrane is much less permeable, and its structure more closely resembles that of the cytoplasmic membrane of *Bacteria*.

Mitochondria also contain folded internal membranes called **cristae**. These membranes, formed by invagination of the inner membrane, contain the enzymes needed for respiration and ATP production. Cristae also contain transport proteins that regulate the passage of key molecules such as ATP into and out of the *matrix*, the innermost compartment of the mitochondrion (Figure 2.45*a*). The matrix contains enzymes for the oxidation of organic compounds, in particular, enzymes of the citric acid cycle, the major pathway for the combustion of organic compounds to CO_2 (▶ Section 3.6).

Chloroplasts

Chloroplasts are the chlorophyll-containing organelles found in plants and algae and are the site of photosynthesis. Chloroplasts are about the size of unicellular cyanobacteria and are readily visible with the light microscope (**Figure 2.46**). Like mitochondria, the number of chloroplasts per cell varies among species, and chloroplasts contain their own DNA and ribosomes.

Also like mitochondria, chloroplasts are enclosed by a double membrane composed of a permeable outer membrane and a less-permeable inner membrane. The innermost membrane surrounds the **stroma**, analogous to the matrix of the mitochondrion (Figure 2.46*c*). The stroma contains large levels of the enzyme *ribulose bisphosphate carboxylase* (RuBisCO), the key enzyme of the *Calvin cycle*, which is the series of biosynthetic reactions by which phototrophs convert CO_2 to organic compounds (▶ Section 3.12). The permeability of the outermost chloroplast membrane allows glucose and ATP produced during photosynthesis to diffuse into the cell cytoplasm where they are consumed in biosynthesis.

Chlorophyll and all other components needed for ATP synthesis in chloroplasts are located in a series of flattened membrane discs called **thylakoids** (Figure 2.46*c*). Like the cytoplasmic membrane, the thylakoid membrane is highly impermeable, and its major function is to form a proton motive force (Figure 2.4*c*) that results in ATP synthesis.

The Endosymbiotic Origin of Organelles

On the basis of their relative autonomy, size, and morphological resemblance to bacteria, it was hypothesized over 100 years ago that mitochondria and chloroplasts were descendants of respiratory

and phototrophic bacterial cells, respectively. According to this hypothesis, when these symbiotic bacteria associated with nonphototrophic hosts, the hosts gained new forms of energy metabolism while the bacterial partners received a stable and supportive growth environment inside the host cell. Then, over time, these originally free-living symbionts became an intimate part of the eukaryotic cell. The idea that symbiotic bacteria are ancestors to the mitochondrion and chloroplast is called the **endosymbiotic theory** and is well accepted in biology today (▶ Sections 13.4 and 18.1). Several lines of evidence support the endosymbiotic theory. These include, most notably, the fact that mitochondria and chloroplasts contain their own genomes and ribosomes whose structures are similar to the genomes and ribosomes of *Bacteria* but not to those of *Eukarya*. These organellar genomes are also circular, typical of bacterial chromosomes (▶ Section 10.4).

Eukarya are a distinct domain of life, a domain that evolved after *Bacteria* and *Archaea*. Nevertheless, eukaryotic cells share many genes with both *Bacteria* and *Archaea*. In this respect, *Eukarya* are genetic chimeras that contain genes from two domains of life. The domain *Eukarya* is hypothesized to have originated from a symbiotic fusion between an archaeal host cell and a mitochondrial endosymbiont (derived from *Bacteria*). Sometime later, a second major symbiotic event occurred, in which a eukaryotic host cell acquired a chloroplast endosymbiont (derived from cyanobacteria), becoming the ancestor of all phototrophic eukaryotes (including plants and algae).We consider these important concepts in detail in Chapter 13.

Check Your Understanding

- What key reactions occur in the mitochondrion and in the chloroplast, and what key products are made in each? Why is it important to keep these reactions behind a double membrane?
- What is the endosymbiotic theory, and what evidence is there to support it?

2.15 Other Eukaryotic Cell Structures

Several other complex structures are present within the cytoplasm of microbial eukaryotes in addition to mitochondria and chloroplasts (Figure 2.42). These include the endoplasmic reticulum, the Golgi complex, lysosomes, and a dynamic cytoskeleton. Unlike the nucleus, mitochondria, and chloroplasts, these structures lack both DNA and a double membrane and are not of endosymbiotic origin. In addition, these structures control the transport of proteins and nutrients within the cell and govern cell shape and movement. Cell walls are also present in certain microbial eukaryotes (such as fungi and algae), though there is great diversity in cell wall structure among eukaryotic cells.

Cytoskeleton

The eukaryotic cytoplasm is crisscrossed by a series of dynamic protein filaments, which are used to transport substances, to position cell structures, and to control cell movement. This internal support network consists of *microtubules, microfilaments*, and *intermediate filaments*; together, these structures form the cell **cytoskeleton** (Figure 2.42). Some cytoskeleton-like elements are also important for determining the shape and molecular organization of prokaryotic cells, but the eukaryotic cytoskeleton is notable for its complexity and dynamism.

Microtubules are hollow tubes about 25 nm in diameter and are composed of the proteins *α-tubulin* and *β-tubulin*. Microtubules (**Figure 2.47*a***) have many functions including maintaining cell shape and facilitating cell motility, moving chromosomes during mitosis (Figures 2.44 and 2.47*b*), and in the movement of organelles within the cell. **Microfilaments** (Figure 2.47*c*) are smaller than microtubules,

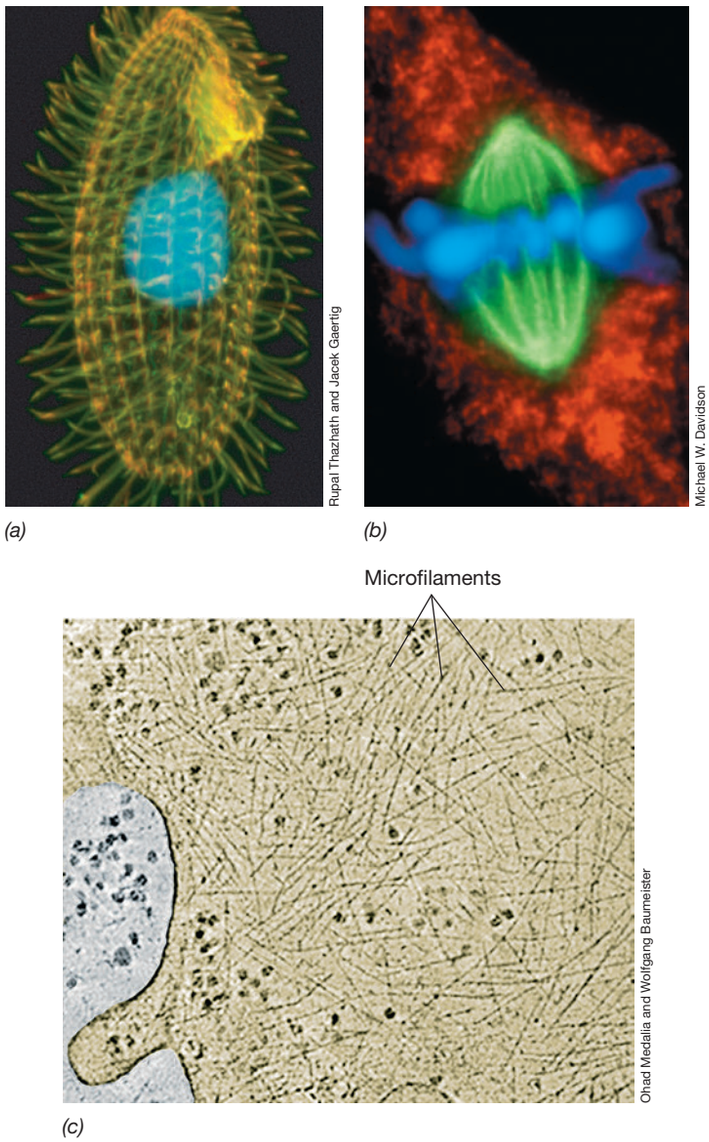


Figure 2.47 Tubulin and microfilaments. *(a)* Fluorescence photomicrograph of a cell of the ciliated protozoan *Tetrahymena thermophila* with red- and green-labeled antitubulin antibodies (the two dyes combine to give yellow) and with DAPI, which stains DNA (blue, nucleus). A cell is about 10 μm wide. *(b)* A mouse cell showing the role of tubulin (green) in separating chromosomes (blue) during metaphase of mitosis (cytoplasmic proteins stain red). Compare with Figure 2.44*c*. *(c)* Electron microscopic image of the cellular slime mold *Dictyostelium discoideum* showing the network of actin microfilaments that along with microtubules functions as the cell cytoskeleton. Microfilaments are about 7 nm in diameter.

about 7 nm in diameter, and are polymers of two intertwined strands of the protein *actin*. Microfilaments function in maintaining or changing cell shape, in cell motility by cells that move by amoeboid movement, and during cell division. **Intermediate filaments** are fibrous keratin proteins that are arranged into fibers 8–12 nm in diameter and function in maintaining cell shape and positioning organelles in the cell.

Endoplasmic Reticulum, the Golgi Complex, and Lysosomes

The endoplasmic reticulum (ER) is a network of membranes continuous with the nuclear membrane. Two types of endoplasmic reticulum exist: *rough* ER, which contains attached ribosomes, and *smooth* ER, which does not (Figure 2.42). Smooth ER participates in the synthesis of lipids and in some aspects of carbohydrate metabolism. Rough ER, through the activity of its ribosomes, is a major producer of glycoproteins and also produces new membrane material that is transported throughout the cell to enlarge the various membrane systems before cell division.

The Golgi complex is a stack of membrane-bound sacs. In the Golgi complex, products of the ER are chemically modified and sorted into those destined for secretion versus those that will function in other membranous structures in the cell. Many of the modifications made in the Golgi complex are glycosylations (addition of sugar residues) that convert the proteins into glycoproteins that can then be targeted to specific locations in the cell.

Lysosomes (Figure 2.42) are membrane-enclosed compartments that contain digestive enzymes that hydrolyze proteins, fats, and polysaccharides. The lysosome fuses with food that enters the cell in vacuoles and then releases digestive enzymes that break down the foods for biosynthesis and energy generation. Lysosomes also function in degrading damaged cellular components and recycling these materials for new biosyntheses. The lysosome thus allows the cell's lytic activities to be partitioned away from the cytoplasm proper. Following the degradation of macromolecules in the lysosome, the resulting nutrients pass from the lysosome into the cytoplasm for use by cytoplasmic enzymes.

Flagella and Cilia

Flagella and cilia are present on the surface of many eukaryotic microbes and function as motility structures, allowing cells to move by swimming. As we learned when we considered motility in prokaryotic cells (Sections 2.9–2.12), motility has survival value, as the ability to move allows motile organisms to move about their habitat and exploit new resources. *Cilia* are essentially short flagella that beat in synchrony to propel the cell—usually quite rapidly—through the medium. *Flagella*, by contrast, are long appendages present singly or in groups that propel the cell along—typically more slowly than

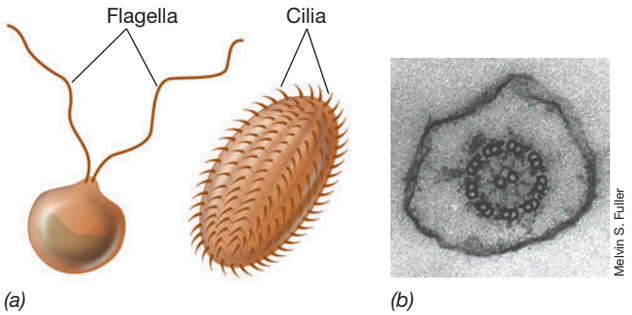


Figure 2.48 Motility organelles in eukaryotic cells: Flagella and cilia. *(a)* Flagella can be present as single or multiple filaments. Cilia are structurally similar to flagella but much shorter. Eukaryotic flagella move in a whiplike motion. *(b)* Cross section through a flagellum of the fungus *Blastocladiella* showing the outer sheath, the outer nine pairs of microtubules, and the central pair of microtubules.

by cilia—through a whiplike motion (**Figure 2.48*a***). In cross section, eukaryotic cilia and flagella appear similar. Each contains a bundle of nine pairs of microtubules surrounding a central pair of microtubules (Figure 2.48*b*). A protein called *dynein* is attached to the microtubules and uses ATP to drive motility. Movement of flagella and cilia is similar. In both cases, movement is the result of the coordinated sliding of microtubules against one another in a direction toward or away from the base of the cell. This movement confers the whiplike motion on the flagellum or cilium that results in cell propulsion.

Eukaryotic flagella differ structurally and functionally from the flagella of *Bacteria* and the archaella of *Archaea*, and these three structures should not be confused (contrast Figure 2.48 with Figure 2.34). Eukaryotic flagella are much larger than flagella or archaella, are enclosed by the cytoplasmic membrane and contain a cytoskeleton, and do not rotate but rather whip back and forth through the activity of their cytoskeleton proteins.

Armed with the major principles of microbial cell structure and function, we move on to Chapter 3 where the metabolic events that provide the energy necessary to build cellular components will unfold as a prelude to our consideration of the building process itself.

Check Your Understanding

- Why are the activities in the lysosome partitioned from the rest of the cytoplasm?
- Describe the structural elements that comprise the cytoskeleton.
- In terms of structure and function, how do the flagella of eukaryotic cells differ from those of prokaryotic cells?

Go to **Mastering Microbiology** for videos, animations, practice tests, and more.

CHAPTER REVIEW

I • The Cell Envelope

2.1 The cytoplasmic membrane is a highly selective permeability barrier constructed of lipids and proteins that form a bilayer, with a hydrophobic interior and hydrophilic exterior. In contrast to *Bacteria* and *Eukarya*, where fatty acids are ester-linked to glycerol, *Archaea* contain ether-linked lipids, and some form monolayer instead of bilayer membranes. The major functions of the cytoplasmic membrane are permeability, transport, and energy conservation, and nutrient accumulation requires energy.

Q Does the cytoplasmic membrane of prokaryotic cells provide shape and rigid support to the cell? Contrast the typical structure of the cytoplasmic membranes of *Bacteria* and *Archaea*.

2.2 The active transport of nutrients into the cell is an energy-requiring process driven by ATP (or some other energy-rich compound) or by the proton motive force. Three transporters are employed by prokaryotic cells: simple, group translocation, and ABC systems. Each mechanism accumulates solutes against the concentration gradient.

Q Cells of *Escherichia coli* transport lactose via lac permease, glucose via the phosphotransferase system, and maltose via an ABC-type transporter. For each of these sugars describe: (1) the components of the transport system and (2) the source of energy that drives the transport event.

2.3 Peptidoglycan is a polysaccharide found only in *Bacteria* that consists of an alternating repeat of *N*-acetylglucosamine and *N*-acetylmuramic acid, the latter cross-linked by tetrapeptides in adjacent strands. The enzyme lysozyme and the antibiotic penicillin both destroy peptidoglycan, leading to cell lysis.

Q Why is the rigid layer of the bacterial cell wall called peptidoglycan? What are the structural reasons for the rigidity that is conferred on the cell wall by the peptidoglycan structure?

2.4 Gram-negative *Bacteria* have an outer membrane consisting of LPS, protein, and lipoprotein. Porins allow for permeability across the outer membrane. The gap between the outer and cytoplasmic membranes is called the periplasm and contains proteins that function in transport, sensing chemicals, and other important cell functions.

Q Is the outer membrane more selective or less selective than the cytoplasmic membrane? Describe the periplasm and its possible functions.

2.5 Cell envelopes can exhibit a range of different structures. One common variation is the presence of an outer S-layer composed of protein or glycoprotein. S-layers function as the cell wall for many *Archaea* and they are also found in many *Bacteria*.

Q What functions can S-layers provide to those cells that make them?

II • Cell Surface Structures and Inclusions

2.6 Many prokaryotic cells have capsules, slime layers, or pili. These structures have several functions, including attachment, genetic exchange, and twitching motility. Hami, present on the surface of certain *Archaea*, function as miniature grappling hooks to attach cells to a surface or to one another.

Q What function(s) do polysaccharide layers outside the cell wall have in prokaryotic cells?

2.7 Prokaryotic cells can contain inclusions of sulfur, polyphosphate, carbon polymers, various minerals formed by biomineralization, or atmospheric gases. These substances function as nutrient storage materials, control magnetotaxis (in the case of magnetosomes), or confer buoyancy (in the case of gas vesicles).

Q What are some of the roles of poly-β-hydroxyalkanoates (PHAs)?

2.8 The endospore is a highly resistant and differentiated structure produced by certain gram-positive *Bacteria*. Endospores are highly dehydrated and contain calcium dipicolinate and small acid-soluble spore proteins, both of which are absent from vegetative cells. Endospores can remain dormant indefinitely but can germinate quickly when conditions warrant.

Q Is an endospore still the same bacterial cell? What are the different types of endospores as to their location in the cell? Describe the structure of endospores.

III • Cell Locomotion

2.9 Swimming motility in prokaryotic cells is due to flagella (*Bacteria*) or archaella (*Archaea*). Both structures are composed of several proteins, are anchored in the cell wall and cytoplasmic membrane, and cause cells to swim by their rotation. However, flagella and archaella differ in both structure and mechanism of operation.

Q Compare and contrast the differences in structure and mechanism between the flagella of *Bacteria* and the archaella of *Archaea*.

2.10 Some microbes are capable of surface motility in which they move along solid surfaces by one of several mechanisms including twitching or gliding.

Q Contrast the mechanisms of gliding motility in *Flavobacterium* with twitching motility in *Pseudomonas* and swimming motility in *Escherichia coli*.

2.11 Swimming bacteria respond to chemical and physical gradients in their environment by controlling the lengths of runs and frequency of tumbles, resulting in a biased random walk. Tumbles are controlled by the direction of rotation of the flagellum, which in turn is controlled by a network of sensory and response proteins.

Q Chemotaxis causes motile bacteria to either move towards attractants or move away from repellents. Movement is determined by coordinated runs and tumbles, leading to biased random walks in response to the concentration of the chemotactic chemical. How would simple diffusion of an attractant over time affect the biased random walk?

2.12 Phototaxis describes directed motility in response to light intensity, while scotophobotaxis describes directed motility away from the dark. Aerotaxis describes directed motility in response to O_2 gradients.

Q Magnetotactic bacteria do not exhibit directed motility in response to magnetic fields and hence their name is a misnomer. Describe the role of magnetosomes and aerotaxis in the directed movements of magnetotactic bacteria.

IV • Eukaryotic Microbial Cells

2.13 Microbial eukaryotes contain several organelles including the nucleus, mitochondria, and chloroplasts. The nucleus contains the cell's DNA wrapped around histone proteins. Cells of microbial eukaryotes divide following the process of mitosis and may also undergo meiosis if a haploid/diploid life cycle occurs.

Q Eukaryotic cell division's aim is to create two identical, functional daughter cells. Hence, the DNA in the parent cell is replicated so that both daughter cells receive a full set of chromosomes. Do other organelles such as mitochondria, endoplasmic reticulum, etc. in the cytoplasm and cell membrane need to be replicated too? Explain.

2.14 Mitochondria and chloroplasts are energy-generating organelles. According to the endosymbiotic theory, these organelles were once free-living *Bacteria* that later established symbiotic relationships within ancestral cells of *Eukarya*.

Q Describe the evidence that supports the idea that the major organelles of eukaryotes were once *Bacteria*.

2.15 Endoplasmic reticula are membranous structures in eukaryotes that either contain attached ribosomes (rough ER) or do not (smooth ER). Flagella and cilia are means of motility and in eukaryotic cells move by a whiplike mechanism instead of by rotation. Lysosomes specialize in degrading proteins and other macromolecules no longer needed by the cell. Microtubules, microfilaments, and intermediate filaments function as internal cell structures that combine to form the cell cytoskeleton.

Q Compare and contrast the structure and function of eukaryotic flagella with those of prokaryotic cells.

APPLICATION QUESTIONS

1. Assume you are given two cultures, one of a species of gram-negative *Bacteria* and one of a species of *Archaea*. Discuss at least four different ways you could analyze these two cultures and tell which culture was which.
2. Gram-negative and gram-positive bacteria have different susceptibility to antibiotics because of differences in cell envelope structure. Assume that you are given an antibiotic that is effective against gram-positive cells but ineffective against gram-negative cells because it cannot cross the outer membrane. How might you chemically modify this antibiotic to increase its efficacy against gram-negative organisms?

CHAPTER GLOSSARY

ABC transport system a membrane transport system consisting of three proteins, one of which hydrolyzes ATP; the system transports specific nutrients into the cell

Archaellum a long, thin cellular appendage present in some *Archaea* that rotates and is responsible for swimming motility

Basal body the "motor" portion of the bacterial flagellum, embedded in the cytoplasmic membrane and cell wall

Capsule a polysaccharide or protein outermost layer, usually rather slimy, present on some bacteria

Cell envelope the system of layered structures that surround the cytoplasm and define the outer boundary of the cell

Chemotaxis directed movement of an organism toward (positive chemotaxis) or away from (negative chemotaxis) a chemical gradient

Chloroplast the photosynthetic organelle of phototrophic eukaryotes

Cristae the internal membranes of a mitochondrion

Cytoplasmic membrane a semipermeable barrier that separates the cell interior (cytoplasm) from the environment

Cytoskeleton the cellular scaffolding typical of eukaryotic cells, made of microtubules, microfilaments, and intermediate filaments

Dipicolinic acid a substance unique to endospores which, when complexed with Ca^{2+}, confers heat resistance on these structures

Endospore a highly heat-resistant, thick-walled, differentiated structure produced by certain gram-positive *Bacteria*

Endosymbiotic theory the idea that mitochondria and chloroplasts originated from *Bacteria*

Flagellum a long, thin cellular appendage that rotates in *Bacteria* or has a whiplike motion in *Eukarya* and is responsible for swimming motility

Gas vesicles gas-filled cytoplasmic structures bounded by a single layered protein membrane that confer buoyancy on cells

Gliding motility a form of surface motility characterized by smooth continuous movement

Group translocation an energy-dependent transport system in which the substance transported is chemically modified during the process of being transported by a series of proteins

Histones highly basic proteins that compact and wind DNA in the nucleus of eukaryotic cells

Intermediate filament a filamentous polymer of fibrous keratin proteins, supercoiled into thicker fibers, that functions in maintaining cell shape and the positioning of certain organelles in the eukaryotic cell

Lipopolysaccharide (LPS) a combination of lipid with polysaccharide and protein that forms the major portion of the outer membrane in gram-negative *Bacteria*

Lysosome an organelle containing digestive enzymes for hydrolysis of proteins, fats, and polysaccharides

Magnetosome a particle of magnetite (Fe_3O_4) or greigite (Fe_3S_4) enclosed by a single membrane in the cytoplasm of magnetotactic *Bacteria*

Meiosis the nuclear division that halves the diploid number of chromosomes to the haploid

Microfilament a filamentous polymer of the protein actin that helps maintain the shape of a eukaryotic cell

Microtubule a filamentous polymer of the proteins α-tubulin and β-tubulin that functions in eukaryotic cell shape and motility

Mitochondrion the respiratory organelle of eukaryotic organisms

Mitosis nuclear division in eukaryotic cells in which chromosomes are replicated and partitioned into two daughter cells during cell division

Nucleus a membrane-enclosed structure in eukaryotic cells that contains the cell's DNA genome

Outer membrane a phospholipid- and polysaccharide-containing unit membrane that lies external to the peptidoglycan layer in cells of gram-negative *Bacteria* and is a component of the cell envelope in these organisms

Peptidoglycan a polysaccharide composed of alternating repeats of *N*-acetylglucosamine and *N*-acetylmuramic acid arranged in adjacent layers cross-linked by short peptides; a component of the cell envelope of virtually all *Bacteria*

Periplasm a region between the outer surface of the cytoplasmic membrane and the inner surface of the lipopolysaccharide layer of gram-negative *Bacteria*

Peritrichous flagellation having flagella located in many places around the surface of the cell

Phototaxis movement of an organism toward light

Pili thin, filamentous structures that extend from the surface of a cell and, depending on type, facilitate cell attachment, genetic exchange, or twitching motility

Polar flagellation having flagella emanating from one or both poles of the cell

Poly-β-hydroxybutyric acid (PHB) a common storage material of prokaryotic cells consisting of a polymer of β-hydroxybutyrate or another β-alkanoic acid or mixtures of β-alkanoic acids

S-layer an outermost cell surface layer composed of protein or glycoprotein present on some *Bacteria* and many *Archaea*

Simple transport system a transporter that consists of only a membrane-spanning protein and is typically driven by energy from the proton motive force

Stroma the lumen of the chloroplast, surrounded by the inner membrane

Teichoic acid a phosphorylated polyalcohol found in the cell wall of some gram-positive *Bacteria*

Thylakoids membrane stacks containing the photosynthetic pigments in cyanobacteria or in the chloroplast of eukaryotic phototrophs

Twitching motility a form of surface motility caused by extension and retraction of type IV pili

Microbial Metabolism 3

MICROBIOLOGY**NOW**

Life Begins with Metabolism

Metabolism is the foundation upon which life is formed. Microorganisms have evolved tremendous metabolic diversity, but all microbes in their vast diversity have a common set of requirements. Life requires liquid water, a source of energy to do work, a source of electrons to perform biochemical reactions, and nutrients required to build macromolecules. Once we understand these requirements, we can begin to make predictions about how life behaves in any environment, whether a caustic hypersaline pond, inside a rock from the cold deserts of Antarctica, in a hydrothermal vent chimney from the ocean's depths, or even on another world. In short, the search for life begins with an understanding of metabolism.

Water is common in our solar system. There is water on Mars and on Earth's moon, on asteroids and comets, and there is evidence for water on several other planets and moons. Liquid water, however, is harder to find. One place that has liquid water is the moon Enceladus, which orbits Saturn. Enceladus has an ice-covered ocean with cracks in its surface through which erupt geysers that spew materials into space (see photo). The Cassini spacecraft photographed and flew through these plumes, detecting silicate minerals that form only in the presence of liquid water.

The gravity from Saturn squeezes Enceladus, resulting in volcanic activity and liquid water beneath its icy crust. These plumes also contain diverse organic and inorganic compounds and the gases H_2, CO_2, and methane (CH_4). Taken as a whole, these molecules are sufficient to provide free energy, a source of electrons, and nutrients required to support life. Indeed, microbes could likely survive at the hydrothermal vents thought to exist on Enceladus. We now know that all of the fundamental requirements for life are present on other worlds, and the search for extraterrestrial life is a search for microbial life.

Source: Waite, J.H., et al. 2017. Cassini finds molecular hydrogen in the Enceladus plume: Evidence for hydrothermal processes. *Science* 356: 155.