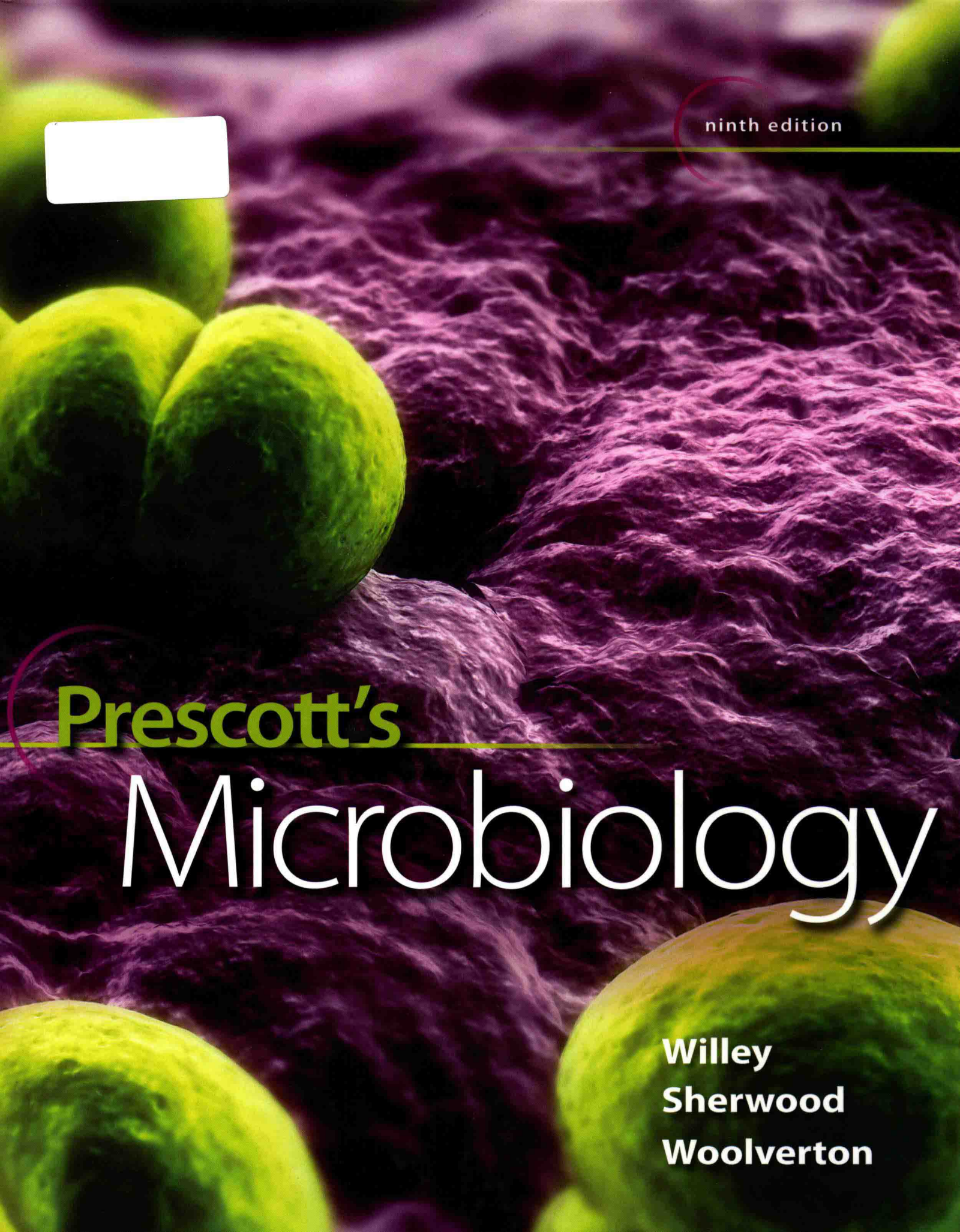
ninth edition
Prescott's
Microbiology
Willey
Sherwood
Woolverton

ninth edition

Prescott's Microbiology

Joanne M. Willey
HOFSTRA UNIVERSITY

Linda M. Sherwood
MONTANA STATE UNIVERSITY

Christopher J. Woolverton
KENT STATE UNIVERSITY

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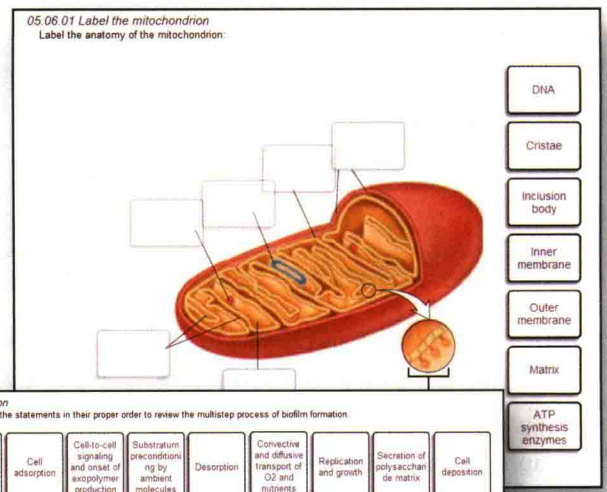

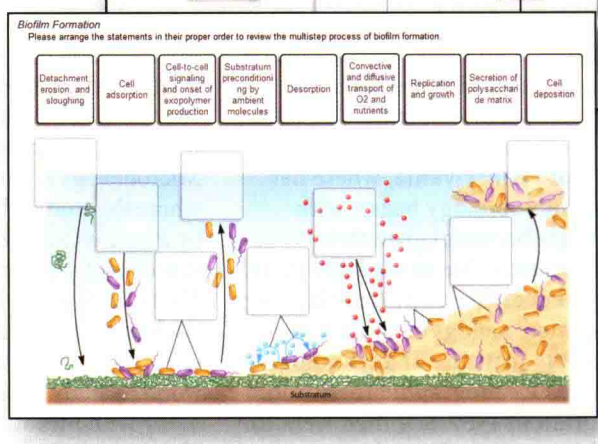

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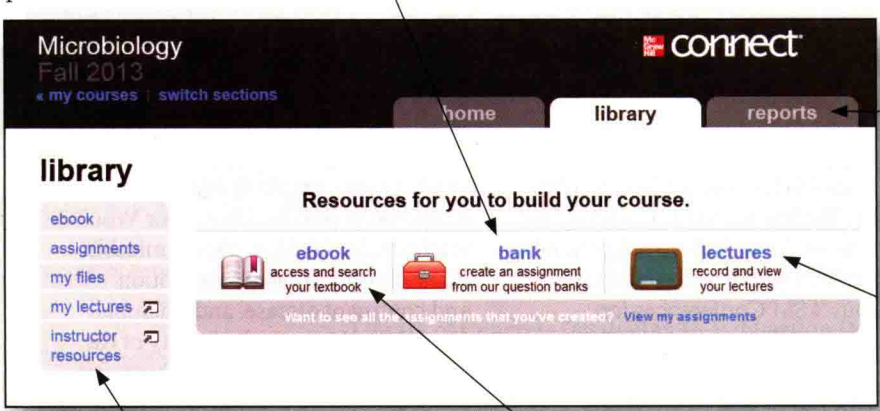

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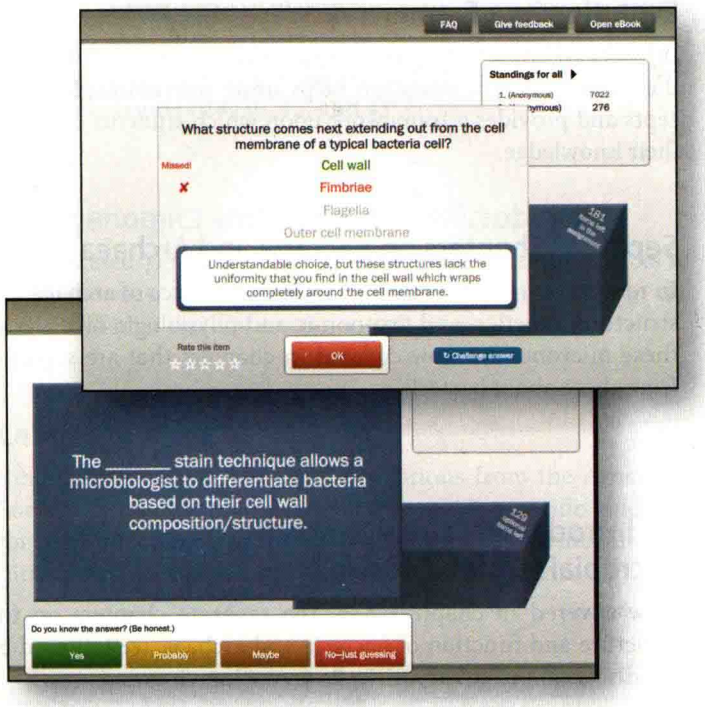

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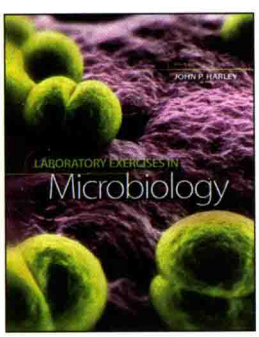

Laboratory Exercises in Microbiology, Ninth Edition

John P. Harley has revised this laboratory manual to accompany the ninth edition of *Prescott's Microbiology.* The class-tested exercises are modular to allow instructors to easily incorporate them into their course. This balanced introduction to each area of microbiology now also has accompanying Connect content for additional homework and assessment opportunities. In addition, all artwork from the lab manual is now available through the Instructor Resources in Connect for incorporation into lectures.

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A Modern Approach to Microbiology

Evolution as a Framework

Introduced immediately in chapter 1 and used as an overarching theme throughout, evolution helps unite microbiological concepts and provides a framework upon which students can build their knowledge.

Separate Chapters on Bacteria and Archaea

In recognition of the importance and prevalence of archaea, the structure, genetics, and taxonomic and physiologic diversity of these microbes are now covered in chapters that are separate from those about bacteria.

An Introduction to the Entire Microbial World

Now covered in chapters 3–6, the separate chapters on the structure and function of bacteria and archaea are followed by the discussion of eukaryotic cells preceding viruses.

Brief Contents

Secondary Lymphoid Organs and Tissues

The **spleen** is the most highly organized secondary lymphoid organ. It is a large organ located in the abdominal cavity that functions to filter the blood and trap blood-borne particles to be assessed for foreignness by phagocytes (figure 33.14). Macrophages and dendritic cells are present in abundance, and once trapped by splenic macrophages or dendritic cells, a pathogen is phagocytosed, killed, and digested. The resulting antigens are presented to lymphocytes, activating a specific immune response.

Lymph nodes lie at the junctions of lymphatic vessels, where macrophages and dendritic cells trap particles that enter the lymphatic system (figure 33.14c). If a particle is found to be foreign, it is then phagocytosed and degraded, and the resulting antigens are presented to lymphocytes.

Lymphoid tissues are found throughout the body as highly organized or loosely associated cellular complexes (figure 33.14). Some lymphoid cells are closely associated with specific tissues such as skin (skin-associated lymphoid tissue, or SALT) and mucous membranes (mucosal-associated lymphoid tissue, or MALT). SALT and MALT are good examples of highly organized lymphoid tissues that feature macrophages surrounded by specific areas of B and T lymphocytes and sometimes dendritic cells. Loosely associated lymphoid tissue is best represented by the bronchial-associated lymphoid tissue (BALT), because it lacks cellular partitioning. The primary role of these lymphoid tissues is to efficiently organize leukocytes to increase interaction between the innate and the adaptive arms of the immune response. Thus, the lymphoid tissues serve as the interface between the innate resistance mechanisms and adaptive immunity of a host. We now discuss these tissues in more detail.

Despite the skin's defenses, at times pathogenic microorganisms gain access to the tissue under the skin surface. Here they encounter a specialized set of cells called the **skin-associated lymphoid tissue (SALT)** (figure 33.15). The major function of SALT is to confine microbial invaders to the area immediately underlying the epidermis and to prevent them from gaining access to the bloodstream. One type of SALT cell is the **Langerhans cell,** a dendritic cell that phagocytoses microorganisms that penetrate the skin. Once the Langerhans cell has internalized a foreign particle or microorganism, it migrates from the epidermis to nearby lymph nodes, where it presents antigen to activate nearby lymphocytes, inducing a specific immune response to that antigen. This dendritic cell–lymphocyte interaction illustrates another bridge between innate resistance and adaptive immunity.

The epidermis also contains another type of SALT cell called the **intraepidermal lymphocyte** (figure 33.15), a specialized T cell having potent cytolytic and immunoregulatory responses to antigen. These cells are strategically located in the skin so that they can intercept any antigens that breach the first line of defense. Most of these specialized SALT cells have limited receptor diversity and have likely evolved to recognize common skin pathogen patterns.

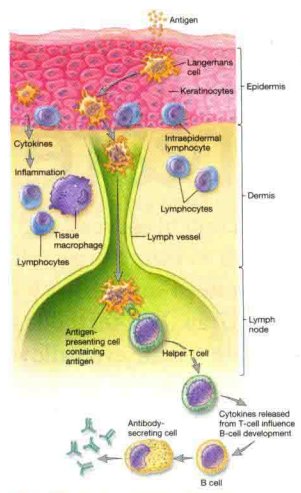


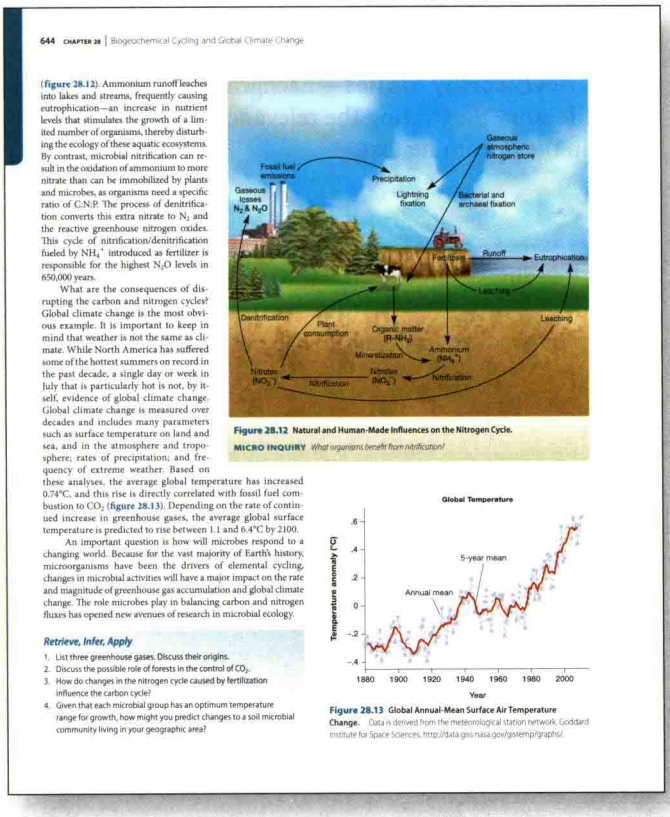
Figure 33.15 Skin-Associated Lymphoid Tissue (SALT). Keratinocytes make up 90% of the epidermis. They are capable of secreting cytokines that cause an inflammatory response to invading pathogens. Langerhans cells internalize antigen and move to a lymph node, where they differentiate into dendritic cells that present antigen to helper T cells. The intraepidermal lymphocytes may function as T cells that can activate B cells to induce an antibody response.

The specialized lymphoid tissue in mucous membranes is called ***mucosal-associated lymphoid tissue*** **(MALT).** There are several types of MALT. The system most studied is the ***gut-associated lymphoid tissue*** **(GALT).** GALT includes the tonsils, adenoids, diffuse lymphoid areas along the gut, and specialized regions in the intestine called Peyer's patches. Less well-organized MALT also occurs in the respiratory system and

Molecular Microbiology and Immunology

The ninth edition includes updates on genetics, biotechnology, genomics, and immunology. The discussion of eukaryotic and archaeal genetics has been expanded and makes up a separate chapter to reflect the relatedness of genetic information flow. A streamlined discussion of immunity with enhanced detail between innate and adaptive linkages helps students grasp the complexity and specificity of immune responses.

A Modern Approach to Microbiology



644 CHAPTER 28 | Biogeochemical Cycling and Global Climate Change

(figure 28.12). Ammonium runoff leaches into lakes and streams, frequently causing eutrophication—an increase in nutrient levels that stimulates the growth of a limited number of organisms, thereby disturbing the ecology of these aquatic ecosystems. By contrast, microbial nitrification can result in the oxidation of ammonium to more nitrate than can be immobilized by plants and microbes, as organisms need a specific ratio of C:N:P. The process of denitrification converts this extra nitrate to N_2 and the reactive greenhouse nitrogen oxides. This cycle of nitrification/denitrification fueled by NH_4^+ introduced as fertilizer is responsible for the highest N_2O levels in 650,000 years.

What are the consequences of disrupting the carbon and nitrogen cycles? Global climate change is the most obvious example. It is important to keep in mind that weather is not the same as climate. While North America has suffered some of the hottest summers on record in the past decade, a single day or week in July that is particularly hot is not, by itself, evidence of global climate change. Global climate change is measured over decades and includes many parameters such as surface temperature on land and sea, and in the atmosphere and troposphere; rates of precipitation; and frequency of extreme weather. Based on these analyses, the average global temperature has increased 0.74°C, and this rise is directly correlated with fossil fuel combustion to CO_2 (figure 28.13). Depending on the rate of continued increase in greenhouse gases, the average global surface temperature is predicted to rise between 1.1 and 6.4°C by 2100.

An important question is how will microbes respond to a changing world. Because for the vast majority of Earth's history, microorganisms have been the drivers of elemental cycling, changes in microbial activities will have a major impact on the rate and magnitude of greenhouse gas accumulation and global climate change. The role microbes play in balancing carbon and nitrogen fluxes has opened new avenues of research in microbial ecology.

Retrieve, Infer, Apply

1. List three greenhouse gases. Discuss their origins.
2. Discuss the possible role of forests in the control of CO_2.
3. How do changes in the nitrogen cycle caused by fertilization influence the carbon cycle?
4. Given that each microbial group has an optimum temperature range for growth, how might you predict changes to a soil microbial community living in your geographic area?

Figure 28.12 Natural and Human-Made Influences on the Nitrogen Cycle.
MICRO INQUIRY *What organisms benefit from nitrification?*

Figure 28.13 Global Annual-Mean Surface Air Temperature Change. Data is derived from the meteorological station network. Goddard Institute for Space Sciences, http://data.giss.nasa.gov/gistemp/graphs/.

21st-Century Microbiology

Prescott's Microbiology leads the way with updated text devoted to global climate change, biofuels, and microbial fuel cells. For more, see chapters 28, 30, 42, and 43.

Metagenomics and the Human Microbiome

The updated genomics chapter covers the technical aspects of metagenomics, and the human microbiome is discussed in the context of microbial interactions in chapters 18 and 32.

Laboratory Safety

Reflecting forthcoming recommendations from the American Society for Microbiology, chapter 37 provides specific guidance for laboratory best practices to help instructors provide safe conditions during the teaching of laboratory exercises.

Special Interest Essays

Organized into four themes—Microbial Diversity & Ecology, Techniques & Applications, Historical Highlights, and Disease—these focused and interesting essays provide additional insight to relevant topics.

Microbial Diversity & Ecol

3.1 Gram Positive and Gram Negative or Monoderms and Diderms?

The importance of the Gram stain in the history of microbiology cannot be overstated. The Gram stain reaction was for many years one of the critical pieces of information used by bacterial taxonomists to construct taxa, and it is still useful in identifying bacteria in clinical settings. The initial studies done to differentiate bacteria that stained Gram positive from those that stain Gram negative were done using model organisms such as *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative). At the time, it was thought that all other bacteria would have similar cell wall structures. However, as the cell walls of more bacteria have been characterized, it has become apparent that it may be misleading to refer to bacteria as Gram positive or Gram negative. In other words, the long-held models of Gram-positive and Gram-negative cell walls do not hold true for all bacteria. Recently Iain Sutcliffe has proposed that microbiologists stop referring to bacteria as either Gram positive or Gram negative. He suggests that instead we should more precisely describe bacterial cell envelope architectures by focusing on the observation that some bacteria have envelopes with a single membrane—the plasma membrane as seen in typical Gram-positive bacteria—while others have envelopes with two membranes—the plasma membrane and an outer membrane as seen in typical Gram-negative bacteria. He proposed calling the former monoderms and the latter diderms.

But why make this change? Sutcliffe begins by pointing out that some bacteria staining Gram positive are actually diderms and some staining Gram negative are actually monoderms. By referring to Gram-positive-staining diderms as Gram-positive bacteria, it is too easy to mislead scientists and many a budding microbiologist into thinking t bacterium has a typical Gram-positive envelope. He a gues that by relating cell envelope architecture to the p enies of various bacterial taxa, we may gain insight i evolution of these architectures. He notes that the ph *micutes* and *Actinobacteria* are composed almost com of monoderm bacteria, whereas almost all other ba phyla consist of diderms.

There are interesting exceptions to the relation phylogeny and cell envelope structure. For instance, m of the genus *Mycobacterium* (e.g., *M. tuberculosis*) to the predominantly monoderm phylum *Actinob* Mycobacteria have cell walls that consist of peptido and an outer membrane. The outer membrane is con of mycolic acids rather than the phospholipids an polysaccharides (LPSs) found in the typical Gram-n cells' outer membrane. ▶▶| *Suborder* Corynebacte *(section 24.1)*

Members of the genus *Deinococcus* are another i ing exception. These bacteria stain Gram positive but derms. Their cell envelopes consist of the plasma mem what appears to be a typical Gram-negative cell wall, outer S-layer. Their outer membrane is distinctive because it lacks LPS. Deinococci are not unique in this respect, however. It is now known that there are several taxa with outer membranes that substitute other molecules for LPS.

Source: Sutcliffe, I. C. 2010. A phylum level perspective on bacterial cell envelope architecture. Trends Microbiol. *18(10):464–70.*

Disease

26.1 White-Nose Syndrome Is Decimating North American Bat Populations

Bats evoke all kinds of images. Some people immediately think of vampire bats and are repulsed. Others think of the large fruit bats often called flying foxes. If you have spent a summer evening outdoors on the east coast of North America, mosquitoes and the small bats that eat them may come to mind. A new scene can now be added to these: bats with white fungal hyphae growing around their muzzles (**box figure**). This is the hallmark of white-nose syndrome (WNS), and if its rate of infection continues unchecked, it is projected to eliminate the most common bat species in eastern North America (*Myotis lucifugus*) by 2026.

WNS was first spotted in 2006 among bats hibernating in a cave near Albany, NY. Scientists quickly became alarmed for two reasons. First, it spreads rapidly—it's known to occur in at least six bat species and is now found from the mid-Atlantic United States, northward into Canada (Ontario, Quebec, and New Brunswick), and as far west as Oklahoma. Second, it is deadly. A population of bats declines from 30 to 99% in any given infected hibernacula (the place where bats hibernate, which unfortunately rhymes with Dracula).

WNS is caused by the ascomycete *Geomyces destructans*. It colonizes a bat's wings, muzzle, and ears where it first erodes the epidermis and then invades the underlying skin and connective tissue. Despite the name WNS, the primary site of infection (and the anatomical site harmed most) is the wing. Wings provide a large surface area for colonization, and once infected, the thin layer of skin is easily damaged, leading to adverse physiological changes during hibernation. These in turn result in premature awakening, loss of essential fat reserves, and strange behavior.

Where did this pathogen come from and why does it infect bats? The best hypothesis regarding its origin is that humans inadvertently brought it from Europe, where it causes mild infection in at least one hibernating bat species. This makes *G. destructans* an apparent case of pathogen pollution—the human introduction of invasive pathogens of wildlife and domestic animal populations that threaten biodiversity and ecosystem function.

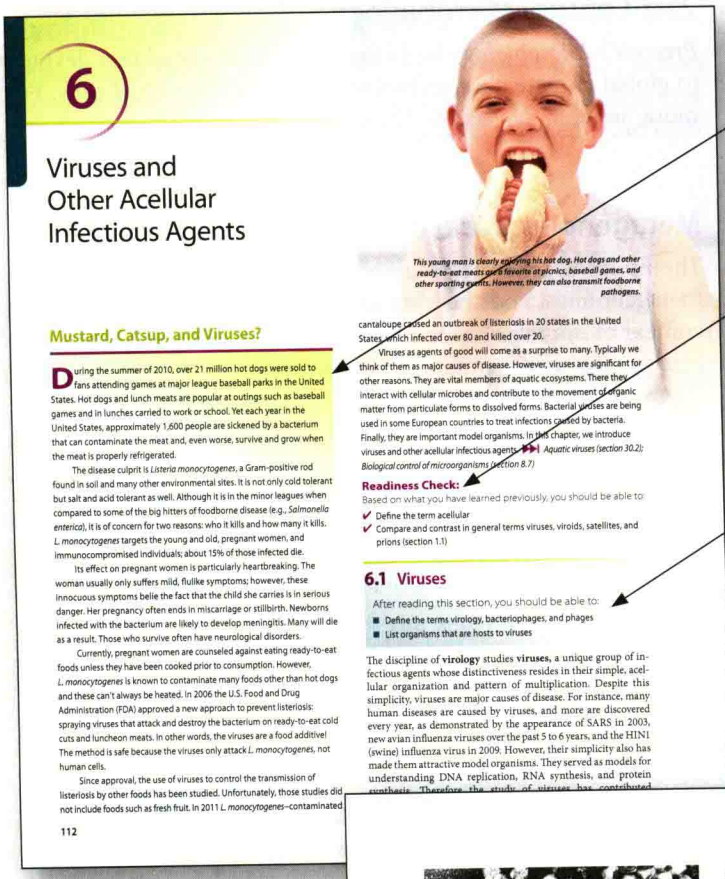
The capacity of *G. destructans* to sweep through bat populations results from a "perfect storm" of host- and pathogen-associated factors. *G. destructans* is psychrophilic, with a growth optimum around 12°C; it does not grow above 20°C. All infected bat species hibernate in cold and humid environments such as caves and mines. Because their metabolic rate is drastically reduced during hibernation, their body temperature reaches that of their surroundings, between 2 and 7°C. Thus WNS is only seen in hibernating bats or those that have just emerged from hibernation. When metabolically active, the bat's body temperature is too warm to support pathogen growth.

While it is too late to save the estimated 6 million bats that have already succumbed to WNS, microbiologists, conservationists, and government agencies are trying to limit the continued decline in bat populations. Caves have been closed to human traffic, and protocols for decontamination after visiting hibernacula have been developed to limit the spread from cave to cave. Although we cannot cure sick bats, it is our responsibility to stop the continued spread of this pathogen.

Geomyces destructans causes WNS. A little brown bat (*Myotis lucifugus*) with the white fungal hyphae (arrow) for which WNS is named.

Read more: Frick, W. F. et al., 2010. An emerging disease causes regional population collapse of a common North American bat species. Science 329:679–682.

Student-Friendly Organization



6

Viruses and Other Acellular Infectious Agents

This young man is clearly enjoying his hot dog. Hot dogs and other ready-to-eat meats are favorite at picnics, baseball games, and other sporting events. However, they can also transmit foodborne pathogens.

Mustard, Catsup, and Viruses?

During the summer of 2010, over 21 million hot dogs were sold to fans attending games at major league baseball parks in the United States. Hot dogs and lunch meats are popular at outings such as baseball games and in lunches carried to work or school. Yet each year in the United States, approximately 1,600 people are sickened by a bacterium that can contaminate the meat and, even worse, survive and grow when the meat is properly refrigerated.

The disease culprit is *Listeria monocytogenes*, a Gram-positive rod found in soil and many other environmental sites. It is not only cold tolerant but salt and acid tolerant as well. Although it is in the minor leagues when compared to some of the big hitters of foodborne disease (e.g., *Salmonella enterica*), it is of concern for two reasons: who it kills and how many it kills. *L. monocytogenes* targets the young and old, pregnant women, and immunocompromised individuals; about 15% of those infected die.

Its effect on pregnant women is particularly heartbreaking. The woman usually only suffers mild, flulike symptoms; however, these innocuous symptoms belie the fact that the child she carries is in serious danger. Her pregnancy often ends in miscarriage or stillbirth. Newborns infected with the bacterium are likely to develop meningitis. Many will die as a result. Those who survive often have neurological disorders.

Currently, pregnant women are counseled against eating ready-to-eat foods unless they have been cooked prior to consumption. However, *L. monocytogenes* is known to contaminate many foods other than hot dogs and these can't always be heated. In 2006 the U.S. Food and Drug Administration (FDA) approved a new approach to prevent listeriosis: spraying viruses that attack and destroy the bacterium on ready-to-eat cold cuts and luncheon meats. In other words, the viruses are a food additive! The method is safe because the viruses only attack *L. monocytogenes*, not human cells.

Since approval, the use of viruses to control the transmission of listeriosis by other foods has been studied. Unfortunately, those studies did not include foods such as fresh fruit. In 2011 *L. monocytogenes*–contaminated cantaloupe caused an outbreak of listeriosis in 20 states in the United States, which infected over 80 and killed over 20.

Viruses as agents of good will come as a surprise to many. Typically we think of them as major causes of disease. However, viruses are significant for other reasons. They are vital members of aquatic ecosystems. There they interact with cellular microbes and contribute to the movement of organic matter from particulate forms to dissolved forms. Bacterial viruses are being used in some European countries to treat infections caused by bacteria. Finally, they are important model organisms. In this chapter, we introduce viruses and other acellular infectious agents. ⏮⏭ *Aquatic viruses (section 30.2); Biological control of microorganisms (section 8.7)*

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✔ Define the term acellular
- ✔ Compare and contrast in general terms viruses, viroids, satellites, and prions (section 1.1)

6.1 Viruses

After reading this section, you should be able to:

- Define the terms virology, bacteriophages, and phages
- List organisms that are hosts to viruses

The discipline of **virology** studies **viruses,** a unique group of infectious agents whose distinctiveness resides in their simple, acellular organization and pattern of multiplication. Despite this simplicity, viruses are major causes of disease. For instance, many human diseases are caused by viruses, and more are discovered every year, as demonstrated by the appearance of SARS in 2003, new avian influenza viruses over the past 5 to 6 years, and the H1N1 (swine) influenza virus in 2009. However, their simplicity also has made them attractive model organisms. They served as models for understanding DNA replication, RNA synthesis, and protein synthesis. Therefore the study of viruses has contributed

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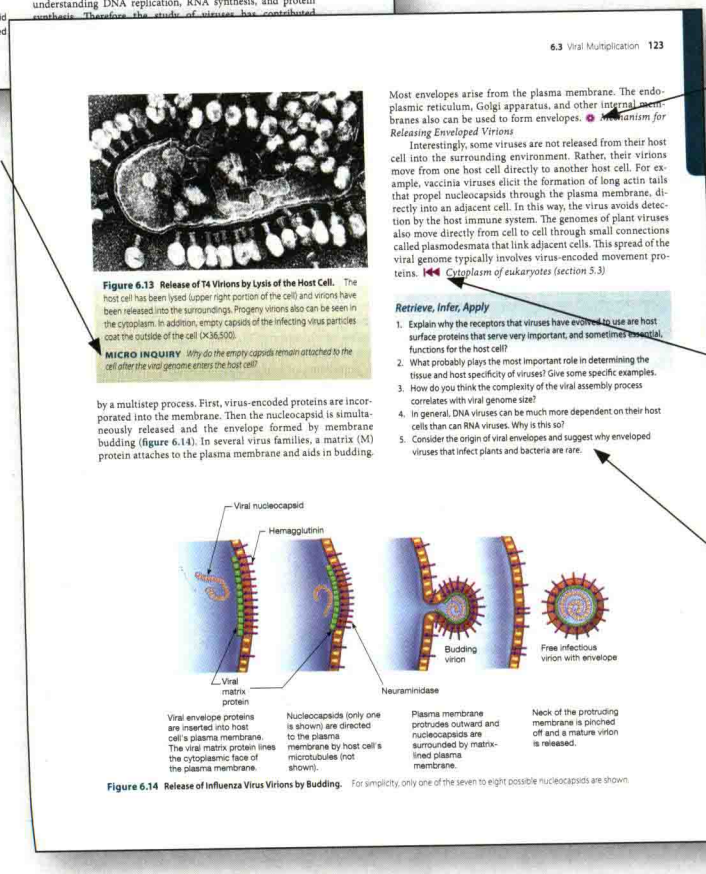


6.3 Viral Multiplication 123

Figure 6.13 Release of T4 Virions by Lysis of the Host Cell. The host cell has been lysed (upper right portion of the cell) and virions have been released into the surroundings. Progeny virions also can be seen in the cytoplasm. In addition, empty capsids of the infecting virus particles coat the outside of the cell (×36,500).

MICRO INQUIRY *Why do the empty capsids remain attached to the cell after the viral genome enters the host cell?*

by a multistep process. First, virus-encoded proteins are incorporated into the membrane. Then the nucleocapsid is simultaneously released and the envelope formed by membrane budding (**figure 6.14**). In several virus families, a matrix (M) protein attaches to the plasma membrane and aids in budding.

Most envelopes arise from the plasma membrane. The endoplasmic reticulum, Golgi apparatus, and other internal membranes also can be used to form envelopes. ● *Mechanism for Releasing Enveloped Virions*

Interestingly, some viruses are not released from their host cell into the surrounding environment. Rather, their virions move from one host cell directly to another host cell. For example, vaccinia viruses elicit the formation of long actin tails that propel nucleocapsids through the plasma membrane, directly into an adjacent cell. In this way, the virus avoids detection by the host immune system. The genomes of plant viruses also move directly from cell to cell through small connections called plasmodesmata that link adjacent cells. This spread of the viral genome typically involves virus-encoded movement proteins. ⏮ *Cytoplasm of eukaryotes (section 5.3)*

Retrieve, Infer, Apply

1. Explain why the receptors that viruses have evolved to use are host surface proteins that serve very important, and sometimes essential, functions for the host cell?
2. What probably plays the most important role in determining the tissue and host specificity of viruses? Give some specific examples.
3. How do you think the complexity of the viral assembly process correlates with viral genome size?
4. In general, DNA viruses can be much more dependent on their host cells than can RNA viruses. Why is this so?
5. Consider the origin of viral envelopes and suggest why enveloped viruses that infect plants and bacteria are rare.

Figure 6.14 Release of Influenza Virus Virions by Budding. For simplicity, only one of the seven to eight possible nucleocapsids are shown.

New! Newsworthy Stories—Each chapter begins with a real-life story illustrating the relevance of the content covered in the upcoming text.

New! Readiness Check—The introduction to each chapter includes a skills checklist that defines the prior knowledge a student needs to understand the material that follows.

New! Learning Outcomes—Every section in each chapter begins with a list of content-based activities students should be able to perform after reading.

Micro Inquiry—Select figures throughout every chapter contain probing questions, adding another assessment opportunity for the student.

Animation Icon—This symbol indicates material presented in the text is also accompanied by an animation on the text website at www.mhhe.com/willey9.

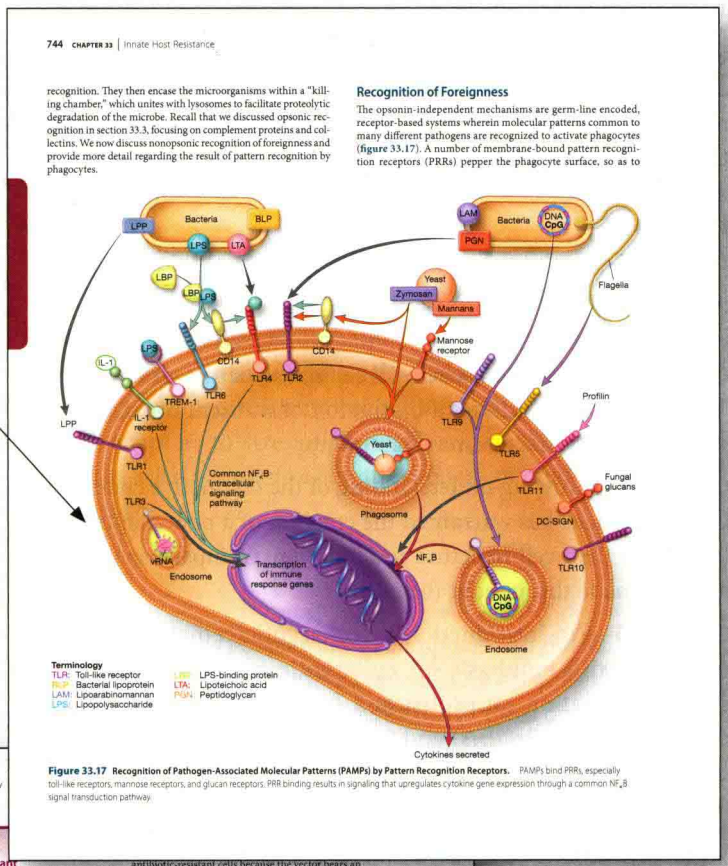
Cross-Referenced Notes—In-text references refer students to other parts of the book to review.

Retrieve, Infer, Apply—Questions within the narrative of each chapter assist students in mastering section concepts before moving on to other topics.

Student-Friendly Organization

Vivid Instructional Art Program—Three-dimensional renditions and bright, attractive colors enhance learning.

More Annotated Figures—All key metabolic pathways and molecular processes are now annotated, so that each step is clearly illustrated and explained.



744 CHAPTER 33 | Innate Host Resistance

recognition. They then encase the microorganisms within a "killing chamber," which unites with lysosomes to facilitate proteolytic degradation of the microbe. Recall that we discussed opsonic recognition in section 33.3, focusing on complement proteins and collectins. We now discuss nonopsonic recognition of foreignness and provide more detail regarding the result of pattern recognition by phagocytes.

Recognition of Foreignness

The opsonin-independent mechanisms are germ-line encoded, receptor-based systems wherein molecular patterns common to many different pathogens are recognized to activate phagocytes (**figure** 33.17). A number of membrane-bound pattern recognition receptors (PRRs) pepper the phagocyte surface, so as to

Figure 33.17 **Recognition of Pathogen-Associated Molecular Patterns (PAMPs) by Pattern Recognition Receptors.** PAMPs bind PRRs, especially toll-like receptors, mannose receptors, and glucan receptors. PRR binding results in signaling that upregulates cytokine gene expression through a common NF$_\kappa$B signal transduction pathway.

Key Concepts—At the end of each chapter and organized by numbered headings, this feature distills the content to its essential components with completely cross-referenced figures and tables.

Compare, Hypothesize, Invent—Includes questions taken from current literature; designed to stimulate analytical problem-solving skills.

422 CHAPTER 17 | Recombinant DNA Technology

Key Concepts

17.1 Key Developments in Recombinant DNA Technology

- Genetic engineering became possible after the discovery of restriction enzymes and reverse transcriptase, and the development of essential methods in nucleic acid chemistry such as the Southern blotting technique.
- Restriction enzymes are important because they cut DNA at specific sequences, thereby releasing fragments of DNA that can be cloned or otherwise manipulated (**figure 17.3** and **table 17.2**).
- Gel electrophoresis is used to separate molecules according to charge and size.
- DNA fragments are separated on agarose and acrylamide gels. Because DNA is acidic, it migrates from the negative to the positive end of a gel (**figure 17.6**).

17.2 Polymerase Chain Reaction

- The polymerase chain reaction (PCR) allows small amounts of specific DNA sequences to be increased in concentration thousands of times (**figure 17.8**).
- PCR has numerous applications. It often is used to obtain genes for cloning and in diagnostic and forensic science.

17.3 Cloning Vectors and Creating Recombinant DNA

- There are four types of cloning vectors: plasmids, viruses, cosmids, and artificial chromosomes. Cloning vectors generally have at least three components: an origin of replication, a selectable marker, and a multicloning site or polylinker (**table 17.3; figures 17.10** and **17.12**).
- The most common approach to cloning is to digest both vector and DNA to be inserted with the same restriction enzyme or enzymes so that compatible sticky ends are generated. The vector and DNA to be cloned are then incubated in the presence of DNA ligase, which catalyzes the formation of phosphodiester bonds once the DNA fragment inserts into the vector.
- Once the recombinant plasmid has been introduced into host cells, cells carrying vector must be selected. This is often accomplished by allowing the growth of only antibiotic-resistant cells because the vector bears an antibiotic-resistance gene. Cells that took up vector with inserted DNA must then be distinguished from those that contain only vector. Often a blue-versus-white colony phenotype is used; this is based on the presence or absence, respectively, of a functional *lacZ* gene (**figure 17.11**).

17.4 Construction of Genomic Libraries

- It is sometimes necessary to find a gene without the knowledge of the gene's DNA sequence. A genomic library is constructed by cleaving an organism's genome into many fragments, each of which is cloned into a vector to make a unique recombinant plasmid.
- Genomic libraries are often screened for the gene of interest by either phenotypic rescue (genetic complementation) or DNA hybridization with an oligonucleotide probe (**figure 17.13**).

17.5 Introducing Recombinant DNA into Host Cells

- The bacterium *E. coli* and the yeast *S. cerevisiae* are the most common host species.
- DNA can be introduced into microbes by transformation or electroporation.

17.6 Expressing Foreign Genes in Host Cells

- An expression vector has the necessary features to express any recombinant gene it carries.
- If a eukaryotic gene is to be expressed in a bacterium, cDNA is used because it lacks introns; a bacterial leader must also be fused to the 5′ end of the gene.
- Purification of recombinant proteins is often accomplished by fusing the coding sequence of a protein to six histidine residue codons found on some expression vectors. When introduced and expressed in bacteria, the His-tagged protein can be selectively purified (**figure 17.14**).
- Green fluorescent protein can be used to study the regulation of gene expression (transcriptional fusions) and protein localization (translational fusions) (**figure 17.15**).

Compare, Hypothesize, Invent

1. You are performing a PCR to amplify a gene encoding a tRNA from a bacterium that has only recently been grown in pure culture. You are expecting a product of 954 bp. However, you generate three different products; only one is the expected size. List at least two possible explanations (excluding experimental error).
2. You have cloned a structural gene required for riboflavin synthesis in *E. coli*. You find that an *E. coli* riboflavin auxotroph carrying the cloned gene on a vector makes less riboflavin than does the wild-type strain. Why might this be the case?

List of Content Changes

Each chapter has been thoroughly reviewed and many have undergone significant revision. All now feature pedagogical elements, including a Readiness Check for the chapter and Learning Outcomes for each section therein.

Part I

Chapter 1—Evolution is the driving force of all biological systems; this is made clear by introducing essential concepts of microbial evolution first.

Chapter 3—Coverage of bacterial cellular structure and function. The chapter now includes a discussion of nutrient uptake in the section on bacterial plasma membranes.

Chapter 4—Growing understanding of the distinctive characteristics of archaea has warranted the creation of a new chapter that focuses on their cell structure and function. Comparisons to bacteria are made throughout the chapter.

Chapter 5—An introduction to eukaryotic cell structure and function, with emphasis on eukaryotic microbes. More detailed information on protist and fungal cells is presented in chapters 25 (*The Protists*) and 26 (*The Fungi*), which also focus on the diversity of these microbes. Comparisons between bacteria, archaea, and eukaryotes are included throughout the chapter.

Chapter 6—This chapter, entitled *Viruses and Other Acellular Infectious Agents*, surveys the essential morphological, physiological, and genetic elements of viruses as well as viroids, satellites, and prions. This chapter completes our four-chapter introduction of microbial life.

Part II

Chapter 7—Reorganized to initially focus on the growth of microbes outside the laboratory (including growth in oligotrophic environments) and the environmental factors that influence microbial reproduction. Topics related to laboratory culture of microbes follow.

Chapter 8—Reorganized to reflect emphasis on interruption of normal growth and reproduction functions to control microorganisms.

Chapter 9—Content focuses on the mechanism of action of each antimicrobial agent and stresses usage to limit drug resistance.

Part III

Chapter 10—This introduction to metabolism includes a new section that outlines the nature of biochemical pathways and introduces the concept of metabolic flux through the interconnected biochemical pathways used by cells.

Chapter 11—The chapter now begins with an introduction to metabolic diversity and nutritional types.

Chapter 12—Updated coverage of CO_2-fixation pathways.

Part IV

Chapter 13—Now focuses on bacterial genetic information flow with improved coverage of bacterial promoters, sigma factors, termination of DNA replication, transcription cycle, and protein folding and secretion.

Chapter 14—Now focuses on the regulation of bacterial cellular processes. The coverage of regulation of complex cellular behaviors has been significantly updated and expanded, including new material on cyclic dimeric GMP.

Chapter 15—A new chapter that considers eukaryal and archaeal genome replication and expression together. In both cases, the discussion has been updated and expanded, and reflects the similarity of information flow as carried out by members of *Archaea* and *Eukarya*.

Chapter 16—Covers mutation, repair, and recombination in the context of processes that introduce genetic variation into populations. This is now related to the evolution of antibiotic-resistant bacteria.

Chapter 17—The use of recombinant DNA approaches to construct a synthetic genome is highlighted.

Chapter 18—New principles and applications of genomic techniques, including massively parallel genome sequencing and single cell genome sequencing, are now reviewed. The growing importance of metagenomics to environmental microbiology and its use in exploring the human microbiome are introduced here.

Part V

Chapter 19—Microbial evolution, introduced in chapter 1, is expanded with a complete discussion of the endosymbiotic theory, and the concept and definition of a microbial species.

Chapter 20—Expanded coverage of archaeal physiology includes new figures presenting archaeal-specific anabolic and catabolic pathways. The evolutionary advantage of each pathway is discussed in the context of archaeal ecology.

Chapter 21—Now includes mycoplasmas, in keeping with *Bergey's Manual;* new figures illustrating the life cycle of *Chlamydia* are included.

List of Content Changes

Chapter 22—Expanded coverage of proteobacterial physiology with content on C1 metabolism, including several figures.

Chapter 24—Increased coverage of streptomycetes, with new graphics illustrating their life cycle and their importance in antibiotic production.

Chapter 27—Updated discussion of virus taxonomy and phylogeny, including increased coverage of archaeal viruses and the CRISPR/CAS system.

Part VI

Chapter 28—The description of each nutrient cycle is accompanied by a new "student-friendly" figure that distinguishes between reductive and oxidative reactions. Expanded coverage of the interaction between nutrient cycles is also newly illustrated.

Chapter 29—This chapter continues to emphasize culture-based techniques as the "gold standard" and reviews some new, innovative approaches. The chapter also discusses a variety of culture-independent techniques used to assess populations and communities.

Chapter 30—Updated and expanded discussion of freshwater microbiology is complemented by discussion of carbon cycling in the open ocean and its implications for global climate change.

Chapter 31—New and updated coverage of mycorrhizae, with an emphasis on host-microbe communication and evolutionary similarities to rhizobia.

Chapter 32—Microbial relationships are presented along with human-microbe interactions, helping to convey the concept that the human body is an ecosystem. New and increased coverage of the human microbiome.

Part VII

Chapter 33—Reorganized and updated, this chapter on innate host resistance provides in-depth coverage of physical and chemical components of the nonspecific host response followed by an overview of cells, tissues, and organs of the immune system. This includes a step-by-step discussion of how microorganisms and damaged tissues are identified by the host using pattern recognition to remove them. Discussions of phagocytosis and inflammation are updated and reflect molecular mechanisms. The groundwork is laid for a full appreciation of the connections between the adaptive and innate arms of the immune system.

Chapter 34—Reorganized and updated to enhance linkages between innate and adaptive immune activities. Discussions integrate cell biology, physiology, and genetics concepts to present the immune system as a unified response having various components. Implications of dysfunctional immune actions are also discussed.

Chapter 35—This chapter has been re-titled *Pathogenicity and Infection,* reflecting its emphasis on microbial strategies for survival that can lead to human disease. The essential elements required for a pathogen to establish infection are introduced and virulence mechanisms highlighted. It follows the immunology chapters to stress that the host-parasite relationship is dynamic, with adaptations and responses offered by both host and parasite.

Part VIII

Chapter 36—This chapter has been updated to reflect the workflow and practice of a modern clinical laboratory. Emphasis is on modern diagnostic testing to identify infectious disease.

Chapter 37—Expanded focus on the important role of laboratory safety, especially in the teaching laboratory. Discussion emphasizes modern epidemiology as an investigative science and its role in preventative medicine. Disease prevention strategies are highlighted.

Chapter 38—Updated and expanded coverage includes viral pathogenesis and common viral infections.

Chapter 39—Expanded coverage of bacterial organisms and their common methods leading to human disease.

Chapter 40—Refocused to reflect disease transmission routes as well as expanded coverage of fungal and protozoal diseases.

Part IX

Chapter 41—Expanded discussion of probiotics in the context of the human microbiome.

Chapter 42—This chapter has been reorganized to illustrate the importance of industrial microbiology by presenting common microbial products—including biofuels—first. This is followed by an updated discussion of strain development, including in vivo and in vitro directed evolution.

Chapter 43—Updated discussion of water purification, wastewater treatment, and bioremediation. This includes the development and use of microbial fuel cells.

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