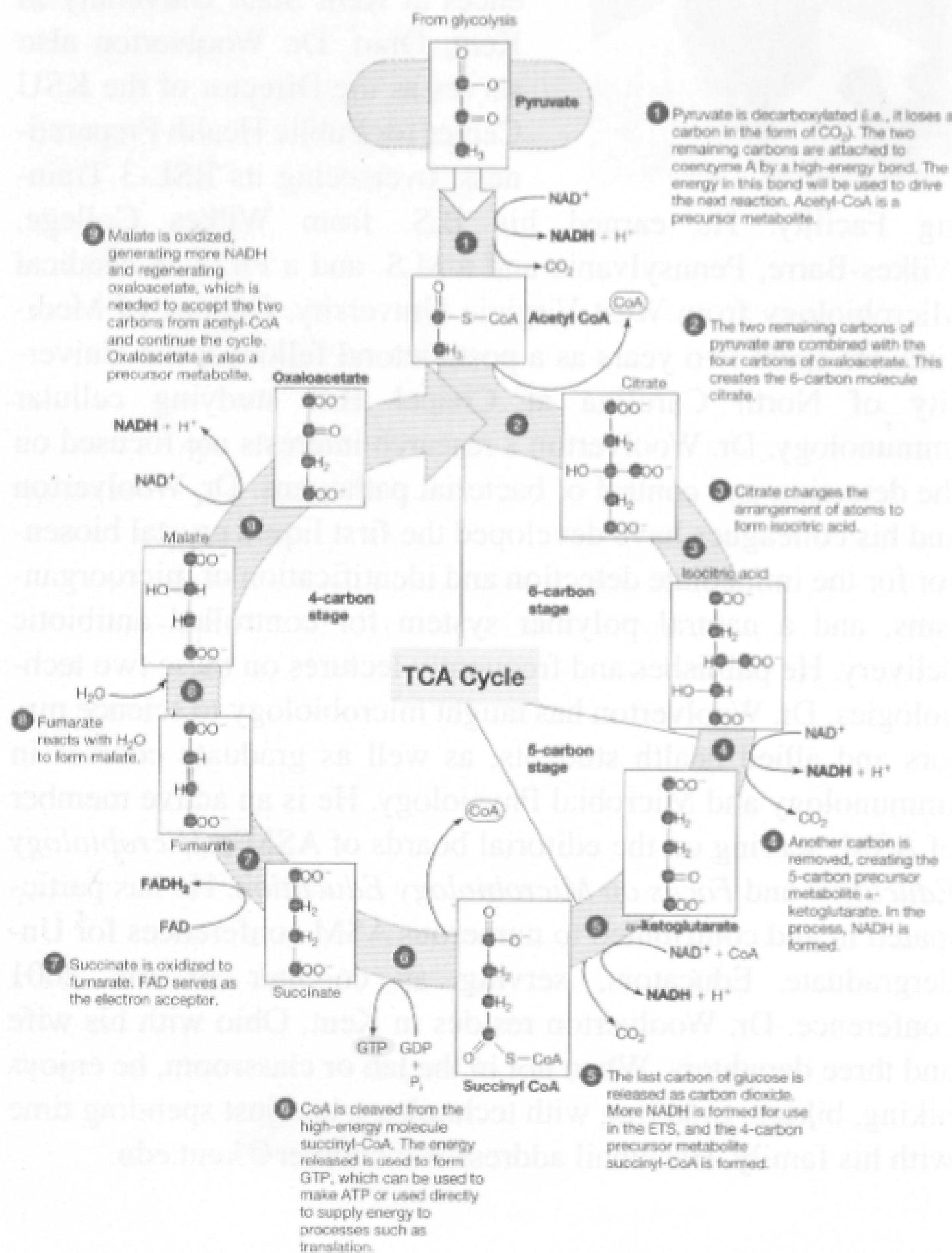
Preface

Prescott, Harley, and Klein's Microbiology has acquired the reputation of covering the broad discipline of microbiology at a depth not found in any other textbook. The seventh edition introduces a new author team. As new authors, we were faced with the daunting task of making a superior textbook even better. We bring over 40 years of combined research and teaching experience. Our keen interest in teaching has been fostered by our involvement in workshops and conferences designed to explore, implement, and assess various pedagogical approaches. Thus one of our goals for this edition was to make the book more accessible to students. To accomplish this we focused on three specific areas: readability, artwork, and the integration of several key themes throughout the text.

Our Strengths

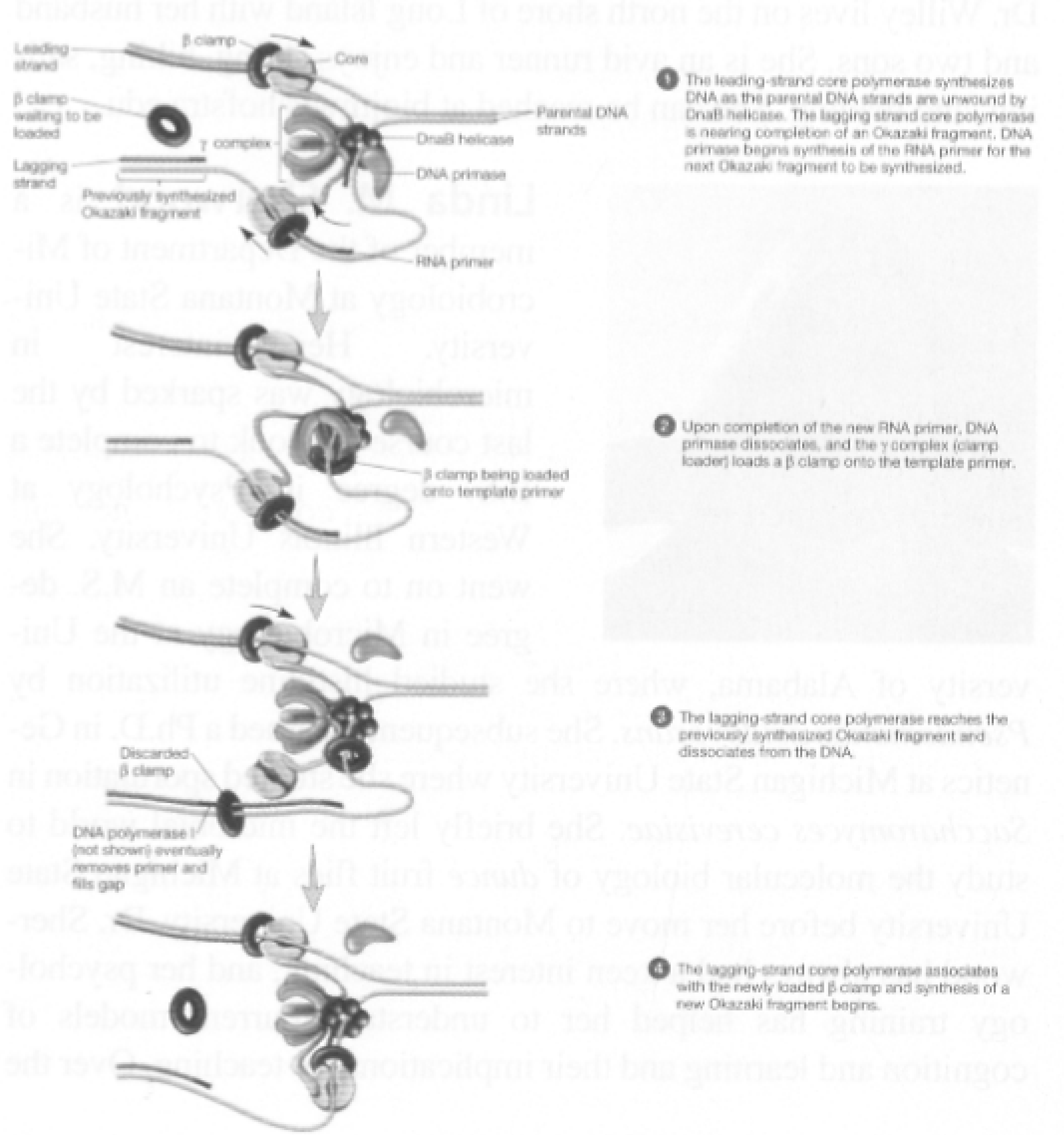
Readability

We have retained the relatively simple and direct writing style used in previous editions of *Prescott, Harley, and Klein's Microbiology*. However, for the seventh edition, we have added style elements designed to further engage students. For example, we have introduced the use of the first person to describe the flow of information (e.g., see chapter openers) and we pose questions within the text, prompting students to reflect on the matter at hand. Each chapter is divided into numbered section headings and organized in an outline format. Some chapters have been significantly reorganized to present the material in a more logical format (e.g., chapters 12, 28, and 39). As in previous editions, key terminology is boldfaced and clearly defined. In addition, some words are now highlighted in red font: these include names of scientists with whom the students should be acquainted, as well as names of techniques and microbes. Every term in the extensive glossary, which includes over 200 new and revised entries, includes a page reference.



Artwork

To engage today's students, a textbook must do more than offer text and images that just adequately describe the topic at hand. Our goal is to make the students *want* to read the text because they find the material interesting and appealing. The seventh edition brings a new art program that features three-dimensional renditions and bright, attractive colors. However, not only have existing figures been updated, over 200 new figures have been added. The updated art program also includes new pedagogical features such as concept maps (*see figures 8.1, 12.1, and 31.1*) and annotation of key pathways and processes (*see figures 9.9 and 11.17*).



Thematic Integration

With the advent of genomics and the increased reach of cell biology, the divisions among microbiology subdisciplines have become blurred; for instance, the microbial ecologist must also be well-versed in microbial physiology, evolution, and the principles and practices of molecular biology. In addition, the microbiologist must be acquainted with all major groups of microorganisms: viruses, bacteria, archaea, protists, and fungi. Students new to microbiology are asked to assimilate vocabulary, facts, and most importantly, concepts, from a seemingly vast array of subjects. The challenge to the professor of microbiology is to integrate essential concepts throughout the presentation of material while conveying the beauty of microbes and excitement of this dynamic field.

While previous editions of *Microbiology* excelled in incorporating genetics and metabolism throughout the text, in this edition we have attempted to bring the diversity of the microbial world into each chapter. Of course this was most easily done in those chapters devoted to microbial evolution, diversity, and ecology (chapters 19 to 30), but we challenged ourselves to bring microbial diversity into chapters that are traditionally *E. coli*-based. So, although the chapters on genetics (chapters 11 to 13) principally review processes as they are revealed in *E. coli,* we also explore other systems as well, such as the regulation of sporulation in *Bacillus subtilis* and quorum sensing in *V. fischerii* (*see figures 12.19 through 12.21*).

We also thought it was important to weave the thread of evolution throughout the text. We start in the first chapter with a discussion of the universal tree of life (*see figure 1.1*), with various renditions of "the big tree" appearing in later chapters. Importantly, we remind students that structures and processes *evolved* to their current state; that natural selection is always at work (e.g., the title and the tone of chapter 13—now called *Microbial Genetics: Mechanisms of Genetic Variations*—have been changed). Finally, the seventh edition of *Microbiology* explores theories regarding the origin of life at a depth not seen in other microbiology texts (chapter 19).

Indeed, depth of coverage has been one of the mainstays of *Prescott, Harley, and Klein's Microbiology.* The text was founded on two fundamental principles: (1) students need an introduction to the whole of microbiology before concentrating on specialized areas, and (2) this introduction should provide the level of understanding required for students to grasp the conceptual underpinning of facts. We remain committed to this approach. Thus the seventh edition continues to provide a balanced and thorough introduction to all major areas of microbiology. This book is suitable for courses with orientations ranging from basic microbiology to medical and applied microbiology. Students preparing for careers in medicine, dentistry, nursing, and allied health professions will find the text as useful as will those aiming for careers in research, teaching, and industry. While two courses each of biology and chemistry are assumed, we provide a strong overview of the relevant chemistry in appendix I.

Changes to the Seventh Edition

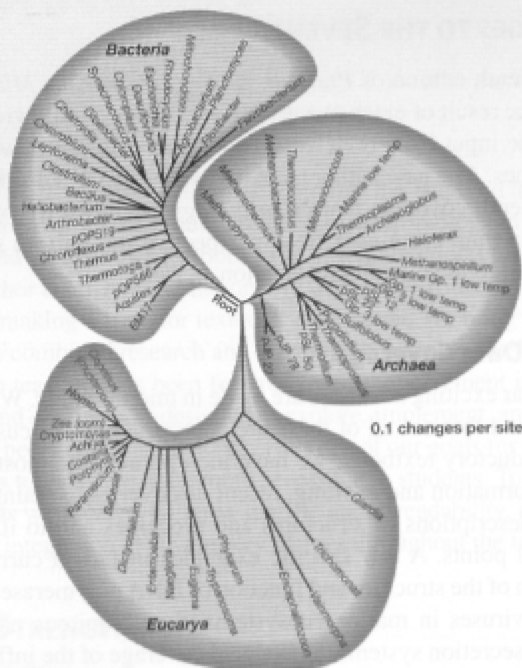
The seventh edition of *Prescott, Harley, and Klein's Microbiology* is the result of extensive review and analysis of previous editions, the input from reviewers, and casual discussions with our colleagues. As a new author team, we were committed to keeping the in-depth coverage that *Microbiology* is known for, while at the same time bringing a fresh perspective not only to specific topics but to the overall presentation as well.

Up-to-Date Coverage

Each year exciting advances are made in microbiology. While we understand that not all of these are appropriate for discussion in an introductory textbook, we have incorporated the most up-to-date information and exciting, recent discoveries to maintain accurate descriptions of structures and processes and to illustrate essential points. A few specific examples include a current description of the structure and function of DNA polymerase III, the role of viruses in marine ecosystems, the ubiquitous nature of type III secretion systems, an updated coverage of the inflammatory response, and the current understanding of HIV origins and avian influenza epidemiology.

Increased Emphasis on Microbial Evolution and Diversity

Microbial evolution, diversity, and ecology are no longer subdisciplines to be ignored by those interested in microbial genetics, physiology, or pathogenesis. For example, within the last 10 years, polymicrobial diseases, intercellular communication, and biofilms have been recognized as important microbial processes that closely tie evolution to genetics, ecology to physiology, and ecology to pathogenesis. The seventh edition strives to integrate these themes throughout the text. We begin chapter 1 with a discussion of the universal tree of life and whenever possible, bring diverse microbial species into discussions so that students can begin to appreciate the tremendous variation in the microbial world. Chapter 19 now covers microbial evolution in greater depth than other texts. It has been retitled *Microbial Evolution, Taxonomy, and Diversity* and the content significantly revised so that microbial evolution is presented as a key component of microbiology. We also introduce and frequently remind students of the enormity of microbial diversity. Like previous editions, the seventh edition features specific chapters that review the members of the microbial world. The chapters that are specifically devoted to ecology (chapters 27 through 29) have undergone significant revisions. We continue to use the classification scheme set forth in the second edition of *Bergey's Manual of Systematic Bacteriology;* in addition, we have introduced the Baltimore System of virus classification and the International Society of Protistologists' new classification scheme for eucaryotes in chapters 18 and 25, respectively.



Writing for Student Understanding

Our goal as a new author team was to retain the straightforward writing style of previous editions while at the same time making the text more readable for the average college student. We have thus added style elements designed to help the reader understand the larger context of the topic at hand. For example, the opening text in several chapters is accompanied by a concept map, enabling the student to visualize the relationships among component topics found within a chapter. Parts of the text are now written in first person; we want students to appreciate that we, as authors, understand that learning is a process that needs to be guided.

Significantly Enhanced Art Program

Today's student must be visually engaged. The artwork in each chapter of the seventh edition has been revised and updated to include realistic, three-dimensional images designed to spark student interest and curiosity. This new program uses bright and appealing colors that give the text an attractive look. We have taken the opportunity to both update and annotate a number of images so that students can picture a complex process step-by-step. New pedagogical features such as concept maps and annotation of key pathways have been added. The three-dimensional renderings help the student appreciate the beauty and elegance of the cell, while at the same time making the material more comprehendible.

Questions for Review and Reflection

Our belief that concepts are just as important as facts, if not more, is also reflected in the questions for review and reflection that appear throughout each chapter. Those who have used previous editions of *Microbiology* may notice that in addition to questions that quiz the retention of key facts, new questions designed to be more thought provoking have been added.

Content Changes By Part

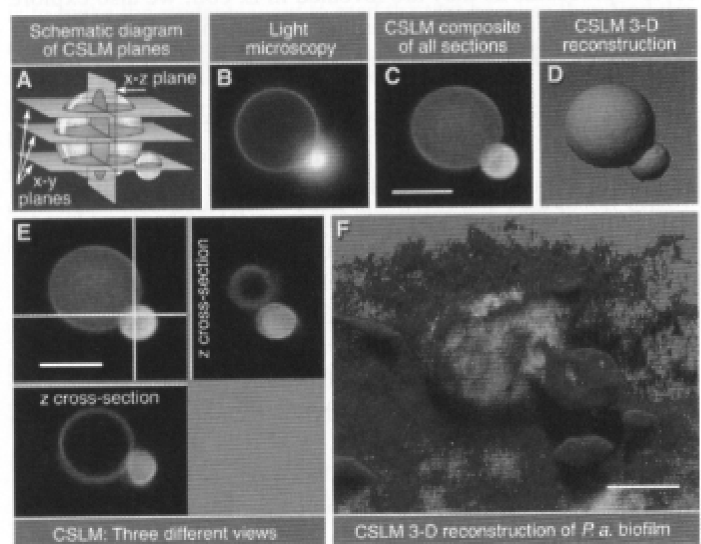
Each chapter has been thoroughly reviewed and almost all have undergone significant revision. In some chapters, there are changes in both organization and content (e.g., chapters 11 to 13), while many other chapters retain the same order of presentation but the content has been updated. A summary of important new material by parts includes:

Part I

Chapter 1—Expanded introduction to the three domains of life and the microbes found in each domain.

Chapter 3—Increased coverage of the difference between archaeal and bacterial cellular structure.

Chapter 4—Reorganized and updated discussion of the biosynthetic-secretory pathway and endocytosis.



Part II

Chapter 6—Updated discussion of the procaryotic cell cycle, including current models of chromosome partitioning and septation; updated and expanded coverage of biofilms and quorum sensing.

Part III

Chapter 8—A new section providing an overview of metabolism and a framework for the more detailed discussions of metabolism that follow; chemotaxis is introduced as an example of regulation of a behavioral response by covalent modification of enzymes.

Chapter 9—Reorganized discussion of chemoorganotrophic metabolism to illustrate the connections among the pathways used

and how these pathways supply the materials needed for anabolism; addition of a discussion of rhodopsin-based phototrophy.

Chapter 10—Reorganized to more clearly correlate N-, P-, and S-assimilation mechanisms with the synthesis of amino acids and nucleotides; discussion of peptidoglycan synthesis is included in the discussion of polysaccharide biosynthesis.

Part IV

Chapter 11—Reorganized to focus solely on genome structure and replication, gene structure, and gene expression.

Chapter 12—Focuses exclusively on the regulation of gene expression; reorganized according to level at which regulation occurs; updated and expanded discussion of riboswitches and regulation by small RNA molecules.

Chapter 13—Covers mutation, repair, and recombination in the context of processes that introduce genetic variation into populations.

Part V

Chapter 14—Begins with, and then builds upon, a concept map describing the principal steps involved in the construction of recombinant DNA molecules with emphasis that recombinant DNA technology is not confined to a few model and industrial microorganisms.

Chapter 15—Rewritten to explore the many ways in which genomics has changed microbiology. Expanded sections on bioinformatics and functional genomics, and a new section introduces environmental genomics (metagenomics).

Part VI

Chapter 16—A new section describing virus reproduction in general terms, so that this chapter can now stand alone as an introduction to viruses.

Part VII

Chapter 19—Rewritten and re-titled *Microbial Evolution, Taxonomy, and Diversity;* the chapter now opens with an in-depth discussion of the origin of life. Discussion of molecular techniques and their importance in microbial taxonomy has also been expanded.

Chapter 20—In keeping with recent discoveries describing the ubiquity of archaea, the seventh edition presents the differences between microbes in the bacterial and archaeal domains in chapter 3. Thus chapter 20 now presents a more in-depth look at some of the specifics of archaeal physiology, genetics, taxonomy, and diversity.

Chapter 25—The protist chapter has been completely rewritten in accordance with the 2005 reclassification of the *Eucarya* by the International Society of Protistologists. Emphasis is placed on medically and environmentally important protists. Thus the chapter entitled *The Algae* found in previous editions has been eliminated and photosynthetic protists are now covered in chapter 25.

Part VIII

Chapter 27—Rewritten and re-titled *Biogeochemical Cycling and Introductory to Microbial Ecology*. Expanded coverage of biogeochemical cycling now includes the phosphorus cycle. Discussion on microbial ecology emphasizes the importance and application of culture-independent approaches. Discussion of water purification and wastewater treatment has been moved to chapter 41, *Applied and Industrial Microbiology*.

Chapter 28—Expanded and reorganized to cover the microbial communities found in the major biomes within marine and freshwater environments. The role of the oceans in regulating global warming is introduced.

Chapter 29—Reorganized to first introduce soils as an environment, is followed by more in-depth and updated treatment of mycorrhizae, the rhizobia, and plant pathogens. Approaches to studying the subsurface environment and new discoveries in this growing field are now included.

Chapter 30—Microbial interactions previously introduced in chapter 27 have been moved to this chapter, where they are presented along with human-microbe interactions (previously presented with innate immunity), helping to convey the concept that the human body is an ecosystem.

Part IX

Chapter 31—Reorganized and updated "nonspecific host resistance" as its own chapter (normal microflora is now in chapter 30); enhanced sections on natural antimicrobial substances.

Chapter 32—Reorganized and updated to enhance linkages between innate and acquired immune activities; integrated medical immunology concepts.

Chapter 33—Most virulence mechanisms have been either updated and/or expanded; added section on host defenses to microbial invasion to link infectious disease processes with host immunity.

Part X

Chapter 34—Content focuses on mechanism of action of each antimicrobial agent; added section on anti-protozoan drugs.

Chapter 35—Now includes both clinical microbiology and immunology; reorganized and updated to reflect current clinical laboratory practices.

Chapter 36—New focus on the important role of epidemiology in preventative medicine, thus vaccines are now covered in this chapter (formerly found in chapter 32); new section on bioterorrism preparedness added.

Chapter 37—Reorganized and updated to reflect viral pathogenesis; select (potential bioterrorism) agents highlighted; influenza section augmented to include the most current information regarding avian influenza; HIV etiology, pathogenesis and treatment sections updated; new section on viral zoonoses.

Chapter 38—Expanded coverage of bacterial pathogenesis; select (potential bioterrorism) agents highlighted; new sections on group B streptococcal disease and bacterial zoonoses.

Chapter 39—Reorganized and updated to reflect disease transmission routes (similar to chapters 37 and 38); new sections on cyclospora and microsporidia.

Part XI

Chapter 40—Expanded discussion of lactic acid bacteria, probiotics: chocolate fermentation now featured in a Techniques & Applications box.

Chapter 41—Revised to include water purification and wastewater treatment. New section on nanotechnology; expanded section on the biochemistry of bioremediation.

Tools For Learning

Chapter Preview

- Each chapter begins with a preview—a list of important concepts discussed in the chapter.

Concept Maps

- Many chapters include a concept map, new to this edition, that outlines critical themes.

Cross-Referenced Notes

- In-text notes in blue type refer students to other parts of the book to review.

Review and Reflection Questions within Narrative

- Review questions throughout each chapter assist students in mastering section concepts before moving on to other topics.

Figure 8.1 Overview of Metabolism.

Figure 14.1 Steps in Cloning a Gene.

Figure 12.1 Gene Expression and Common Regulatory Mechanisms in the Three Domains of Life.

Special Interest Essays

- Interesting essays on relevant topics are included in each chapter. Readings are organized into these topics: Historical Highlights, Techniques & Applications, Microbial Diversity & Ecology, Disease, and Microbial Tidbits.

Microbial Tidbits

35.2 Biosensors: The Future Is Now

The 120-plus-year-old pathogen detection systems based on culture and biochemical phenotyping are being challenged. Fueled by the release of anthrax spores in the U.S. postal system, government agencies have been calling for newer technologies for the near-immediate detection and identification of microbes. In the past, detection technologies have traded speed for cost and complexity. The agar plate technique, refined by Robert Koch and his contemporaries in the 1880s, is a trusted and highly efficient method for the isolation of bacteria into pure cultures. Subsequent phenotyping biochemical methods, often using differential media in a manner similar to that used in the isolation step, then identifies common bacterial pathogens. Unfortunately, reliable results from this process often take several days. More rapid versions of the phenotyping systems can be very efficient, yet still require pure culture inoculations. The rapid immunological tests offer faster detection responses but may sacrifice sensitivity. Even DNA sequence comparisons, which are extremely accurate, may require significant time for DNA amplification and significant cost for reagents and sensitive readers. As usual, necessity has begat invention.

The more recent microbial detection systems, many of which are still untested in the clinical arena, sound like science fiction gizmos, yet promise a new age for near-immediate detection and identification of pathogens. These technologies are collectively referred to as "biosensors," and if the biosensor is integrated with a computer microchip for information management, it is then called a "biochip." Biosensors should ideally be capable of highly specific recognition so as to discriminate between nearest relatives, and "communicate" detection through some type of transducing system. Biosensors that detect specific DNA sequences, expressed proteins, and metabolic products have been developed that use optical (mostly fluorescence), electrochemical, or even mass displacement, to report detection. The high degree of recognition required to reduce false-positive results has demanded the uniquely specific, receptor-like capture that is associated with nucleic acid hybridization and antibody binding. Several microbial biosensors employ single-stranded DNA or RNA sequences, or antibody, for the detection component. The transducing or sensing component of biosensors may be markedly different, however. For example, microcantilever systems detect the increased mass of the receptor-bound ligand; the surface acoustic wave device detects change in specific gravity; the bulk quartz resonator monitors fluid density and viscosity; the quartz crystal microbalance measures frequency change in proportion to the mass of material deposited on the crystal; the micromirror sensor uses an optical fiber waveguide that changes reflectivity; and the liquid crystal-based system reports the reorientation of polarized light. Thus the specific capture of a ligand is reflected in the net change measured by each system and results in a signal that announces the initial capture event. Microchip control of the primary and subsequent secondary signals has resulted in automation of the detection process. The reliable detection of pathogens in complex specimens will be the real test as each of these technologies continues to compete for a place in the clinical laboratory.

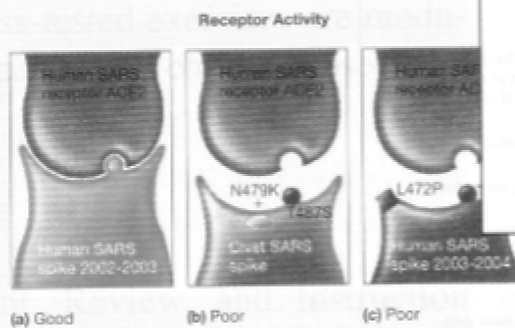
Microbial Diversity & Ecology

18.1 SARS: Evolution of a Virus

In November 2002, a mysterious pneumonia was seen in the Guangdong Province of China, but the first case of this new type of pneumonia was not reported until February 2003. Thanks to the ease of global travel, it took only a couple of months for the pneumonia to spread to more than 25 countries in Asia, Europe, and North and South America. This newly emergent pneumonia was labeled Severe Acute Respiratory Syndrome (SARS) and its causative agent was identified as a previously unrecognized member of the coronavirus family, the SARS-CoV. Almost 10% of the roughly 8,000 people with SARS died. However, once the epidemic was contained, the virus appeared to "die out," and with the exception of a few mild, sporadic cases in 2004, no additional cases have been identified. From where does a newly emergent virus come? What does it mean when a virus "dies out"?

We can answer these questions thanks to the availability of the complete SARS-CoV genome sequence and the power of molecular modeling. Coronaviruses are large, enveloped viruses with positive-strand RNA genomes. They are known to infect a variety of mammals and birds. Researchers suspected that SARS-CoV had "jumped" from its animal host to humans, so samples of animals at open markets in Guangdong were taken for nucleotide sequencing. These studies revealed that cat-like animals called masked palm civits (*Paguma larvata*) harbored variants of the SARS-CoV. Although thousands of civits were then slaughtered, further studies failed to find widespread infection of domestic or wild civits. In addition, experimental infection of civits with human SARS-CoV strains made these animals ill, making the civit an unlikely candidate for the reservoir species. Such a species would be expected to harbor SARS-CoV without symptoms so that it could efficiently spread the virus.

Bats are reservoir hosts of several zoonotic viruses (viruses spread from animals to people) including the emerging Hendra and Nipah viruses that have been found in Australia and East Asia, respectively. Thus it was perhaps not too surprising when in 2005, two groups of international scientists independently demonstrated that Chinese horseshoe bats (genus *Rhinolophus*) are the natural reservoir of a SARS-like coronavirus. When the genomes of the human and bat SARS-CoV are aligned, 92% of the nucleotides are identical. More revealing is alignment of the translated amino acid sequences of the proteins encoded by each virus. The amino acid sequences are 96 to 100% identical for all proteins except the receptor-binding spike proteins, which are only 64% identical. The SARS-CoV spike protein mediates both host cell surface attachment and membrane fusion. Thus a mutation of the spike protein allowed the virus to "jump" from bat host cells to those of another species. It is not clear if the SARS-



Host Range of SARS-CoV Is Determined by Several Amino Acid Residues in the Spike Protien. (a) The spike protien of the SARS-CoV that caused the SARS epidemic in 2002–2003 fits tightly to the human host cell receptor ACE2. (b) The civit SARS-CoV has two different amino acids at positions 4[illegible] and 487. This spike protein binds very poorly to human ACE2, thus the receptor is only weakly activated. (c) The spike protein on the human SARS-CoV that was isolated from patients in 200[illegible] and 2004 also differs from that seen in the epidemic-causing SARS-CoV by two amino acids. This SARS-CoV variant caused on[illegible] mild, sporadic cases.

within the RBD, only four differ between civit and human. Two[illegible] these amino acids appear to be critical. As shown in the **Box figu[illegible]** compared to the spike RBD in the SARS-CoV that caused [illegible] 2002–2003 epidemic, the civit spike has a serine (S) substituted [illegible] a threonine (T) at position 487 (T487S) and a lysine (K) at posit[illegible] 479 instead of asparagine (N), N479K. This causes a 1,000-fold [illegible]crease in the capacity of the virus to bind to human ACE2. Furth[illegible]more, the spike found in SARS-CoV isolated from patients in 20[illegible] and 2004 also has a serine at position 487 as well as a proline (P) [illegible] leucine (L) substitution at position 472 (L472P). These amino a[illegible] substitutions could be responsible for the reduced virulence of [illegible] virus found in these more recent infections. In other words, th[illegible] mutations could be the reason the SARS virus "died out."

Meanwhile a SARS vaccine based on the virulent 2002–20[illegible] strain is being tested. This raises additional questions. Does [illegible]

Techniques & Applications

40.3 Chocolate: The Sweet Side of Fermentation

Chocolate could be characterized as the "world's favorite food," and yet few people realize that fermentation is an essential part of chocolate production. The Aztecs were the first to develop chocolate fermentation, serving a chocolate drink made from the seeds of the chocolate tree, *Theobroma cocao* [Greek *theos*, god and *broma*, food, or "food of the gods"]. Chocolate trees now grow in West Africa as well as South America.

The process of chocolate fermentation has changed very little over the past 500 years. Each tree produces a large pod that contains 30 to 40 seeds in a sticky pulp (see **Box Figure**). Ripe pods are harvested and slashed open to release the pulp and seeds. The sooner the fermentation begins, the better the product, so fermentation occurs on the farm where the trees are grown. The seeds and pulp are placed in "sweat boxes" or in heaps in the ground and covered, usually with banana leaves.

Like most fermentations, this process involves a succession of microbes. First, a community of yeasts, including *Candida rugosa* and *Kluyveromyces marxianus*, hydrolyze the pectin that covers the seeds and ferment the sugars to release ethyl alcohol and Co_2. As the temperature and the alcohol concentration increase, the yeasts are inhibited and lactic acid bacteria increase in number. The mixture is stirred to aerate the microbes and ensure an even temperature distribution. Lactic acid production drives the pH down; this encourages the growth of bacteria that produce acetic acid as a fermentation end product. Acetic acid is critical to the production of fine chocolate because it kills the sprout inside the seed and releases enzymes that cause further degradation of proteins and carbohydrates, contributing to the overall taste of the chocolate. In addition, acetate esters, derived from acetic acid, are important for the development of good flavor. Fermentation takes five to seven days. An experienced chocolate grower will know when the fermentation is complete—if it is stopped too soon the chocolate will be bitter and astringent. On the other hand, if fermentation lasts too long, microbes start growing on the seeds instead of in the pulp. "Off-tastes" arise when the gram-positive bacterium *Bacillus* and the filamentous fungi *Aspergillis*, Penicillium, and *Mucor* hydrolyze lipids in the seeds to release short-chain fatty acids. As the pH begins to rise, the bacteria of the genera *Pseudomonas*, *Enterobacter*, and *Escherichia* also contribute to bad tastes and odor.

After fermentation, the seeds, now called beans, are spread out to dry. Ideally this is done in the sun, although drying ovens are also used. The oven-drying method is considered inferior because the beans can acquire a smoky taste. The dried beans are brown and lack the pulp. They are bagged and sold to chocolate manufacturers, who first roast the beans to further reduce the bitter taste and kill most of the microbes (some *Bacillus* spores may remain). The beans are then ground and the nibs—the inner part of each bean—are removed. The nibs are crushed into a thick paste called a chocolate liquor, which contains cocoa solids and cocoa butter, but no alcohol. Cocoa solids are brown and have a rich flavor, and cocoa butter has a high fat con-

Historical Highlights

5.1 The Discovery of Agar as a Solidifying Agent and the Isolation of Pure Cultures

The earliest culture media were liquid, which made the isolation of bacteria to prepare pure cultures extremely difficult. In practice, a mixture of bacteria was diluted successively until only one organism, as an average, was present in a culture vessel. If everything went well, the individual bacterium thus isolated would reproduce to give a pure culture. This approach was tedious, gave variable results, and was plagued by contamination problems. Progress in isolating pathogenic bacteria understandably was slow.

The development of techniques for growing microorganisms on solid media and efficiently obtaining pure cultures was due to the efforts of the German bacteriologist Robert Koch and his associates. In 1881 Koch published an article describing the use of boiled potatoes, sliced with a flame-sterilized knife, in culturing bacteria. The surface of a sterile slice of potato was inoculated with bacteria from a needle tip, and then the bacteria were streaked out over the surface so that a few individual cells would be separated from the remainder. The slices were incubated beneath bell jars to prevent airborne contamination, and the isolated cells developed into pure colonies. Unfortunately many bacteria would not grow well on potato slices.

At about the same time, Frederick Loeffler, an associate of Koch, developed a meat extract peptone medium for cultivating pathogenic bacteria. Koch decided to try solidifying this medium. Koch was an amateur photographer—he was the first to take photomicrographs of bacteria—and was experienced in preparing his own photographic plates from silver salts and gelatin. Precisely the same approach was employed for preparing solid media. He spread a mixture of Loeffler's medium and gelatin over a glass plate, allowed it to harden, and inoculated the surface in the same way he had inoculated his sliced potatoes. The new solid medium worked well, but it could not be incubated at 37°C (the best temperature for most human bacterial pathogens) because the gelatin would [illegible]. Furthermore, some bacteria digested the gelatin.

About a year later, in 1882, agar was first used as a solidif[illegible] agent. It had been discovered by a Japanese innkeeper, Mi[illegible] Tarazaemon. The story goes that he threw out extra seaweed [illegible] and discovered the next day that it had jelled during the cold w[illegible] night. Agar had been used by the East Indies Dutch to make je[illegible] and jams. Fannie Eilshemius Hesse (*see figure 1.7*), the New Je[illegible] born wife of Walther Hesse, one of Koch's assistants, had lea[illegible] of agar from a Dutch acquaintance and suggested its use whe[illegible] heard of the difficulties with gelatin. Agar-solidified medium [illegible] an instant success and continues to be essential in all area[illegible] microbiology.

Disease

1.2 Koch's Molecular Postulates

Although the criteria that Koch developed for proving a causal relationship between a microorganism and a specific disease have been of great importance in medical microbiology, it is not always possible to apply them in studying human diseases. For example, some pathogens cannot be grown in pure culture outside the host; because other pathogens grow only in humans, their study would require experimentation on people. The identification, isolation, and cloning of genes responsible for pathogen virulence have made possible a new molecular form of Koch's postulates that resolves some of these difficulties. The emphasis is on the virulence genes present in the infectious agent rather than on the agent itself. The molecular postulates can be briefly summarized as follows:

1. The virulence trait under study should be associated much more with pathogenic strains of the species than with nonpathogenic strains.
2. Inactivation of the gene or genes associated with the suspected virulence trait should substantially decrease pathogenicity.
3. Replacement of the mutated gene with the normal wild-type gene should fully restore pathogenicity.
4. The gene should be expressed at some point during the infection and disease process.
5. Antibodies or immune system cells directed against the gene products should protect the host.

The molecular approach cannot always be applied because of problems such as the lack of an appropriate animal system. It also is difficult to employ the molecular postulates when the pathogen is not well characterized genetically.

Chapter Summaries

- End-of-chapter summaries are organized by numbered headings and provide a snapshot of important chapter concepts.

has been used to study the interactions between the *E. coli* GroES and GroEL chaperone proteins, to map plasmids by locating restriction enzymes bound to specific sites, to follow the behavior of living bacteria and other cells, and to visualize membrane proteins (**figure 2.29**).

1. How does a confocal microscope operate? Why does it provide better images of thick specimens than does the standard compound microscope?
2. Briefly describe the scanning probe microscope and compare and contrast its most popular versions—the scanning tunneling microscope and the atomic force microscope. What are these microscopes used for?

Summary

2.1 Lenses and the Bending of Light

a. A light ray moving from air to glass, or vice versa, is bent in a process known as refraction.
b. Lenses focus light rays at a focal point and magnify images (**figure 2.2**).

2.2 The Light Microscope

a. In a compound microscope like the bright-field microscope, the primary image is formed by an objective lens and enlarged by the eyepiece or ocular lens to yield the final image (**figure 2.3**).
b. A substage condenser focuses a cone of light on the specimen.
c. Microscope resolution increases as the wavelength of radiation used to illuminate the specimen decreases. The maximum resolution of a light microscope is about 0.2 μm.
d. The dark-field microscope uses only refracted light to form an image (**figure 2.7**), and objects glow against a black background.
e. The phase-contrast microscope converts variations in the refractive index and density of cells into changes in light intensity and thus makes colorless, unstained cells visible (**figure 2.9**).
f. The differential interference contrast microscope uses two beams of light to create high-contrast, three-dimensional images of live specimens.
g. The fluorescence microscope illuminates a fluorochrome-labeled specimen and forms an image from its fluorescence (**figure 2.12**).

2.3 Preparation and Staining of Specimens

a. Specimens usually must be fixed and stained before viewing them in the bright-field microscope.
b. Most dyes are either positively charged basic dyes or negative acidic dyes and bind to ionized parts of cells.
c. In simple staining a single dye is used to stain microorganisms.
d. Differential staining procedures like the Gram stain and acid-fast stain distinguish between microbial groups by staining them differently (**figure 2.15**).
e. Some staining techniques are specific for particular structures like bacterial capsules, flagella, and endospores (**figure 2.14**).

2.4 Electron Microscopy

a. The transmission electron microscope uses magnetic lenses to form an image from electrons that have passed through a very thin section of a specimen (**figure 2.19**). Resolution is high because the wavelength of electrons is very short.
b. Thin section contrast can be increased by treatment with solutions of heavy metals like osmium tetroxide, uranium, and lead.
c. Specimens are also prepared for the TEM by negative staining, shadowing with metal, or freeze-etching.
d. The scanning electron microscope (**figure 2.23**) is used to study external surface features of microorganisms.

2.5 Newer Techniques in Microscopy

a. The confocal scanning laser microscope (**figure 2.25**) is used to study thick, complex specimens.
b. Scanning probe microscopes reach very high magnifications that allow scientists to observe biological molecules (**figures 2.27** and **2.29**).

Key Terms

acidic dyes 26
acid-fast staining 26
atomic force microscope 36
basic dyes 26
bright-field microscope 18
capsule staining 26
chemical fixation 26
chromophore groups 26
confocal scanning laser microscope (CSLM) 34
dark-field microscope 21
differential interference contrast (DIC) microscope 23
differential staining 26
endospore staining 26
eyepieces 18
fixation 25
flagella staining 28
fluorescence microscope 23
fluorescent light 23
fluorochromes 24
focal length 18
focal point 18
freeze-etching 30
Gram stain 26
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refraction 17
refractive index 17
resolution 18
scanning electron microscope (SEM) 30
scanning probe microscope 35
scanning tunneling microscope 35
shadowing 29
simple staining 26
substage condenser 18
transmission electron microscope (TEM) 29
working distance 20

End-of-Chapter Material

- *Key Terms* highlight chapter terminology and list term location in the chapter.
- *Critical Thinking Questions* supplement the questions for review and reflection found throughout each chapter; they are designed to stimulate analytical problem solving skills.
- *Learn More* includes a short list of recent and relevant papers for the interested student and professor. Additional references can be found at the Prescott website at *www.mhhe.com/prescott7*.

Critical Thinking Questions

1. If you prepared a sample of a specimen for light microscopy, stained with the Gram stain, and failed to see anything when you looked through your light microscope, list the things that you may have done incorrectly.
2. In a journal article, find an example of a light micrograph, a scanning or transmission electron micrograph, or a confocal image. Discuss why the figure was included in the article and why that particular type of microscopy was the method of choice for the research. What other figures would you like to see used in this study? Outline the steps that the investigators would take in order to obtain such photographs or figures.

Learn More

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Rochow, T.G. 1994. *Introduction to microscopy by means of light, electrons, X-rays, or acoustics*. New York: Plenum.

Scherrer, Rene. 1984. Gram's staining reaction, Gram types and cell walls of bacteria. *Trends Biochem. Sci.* 9:242–45.

Stephens, D. J., and Allan, V. J. 2003. Light microscopy techniques for live cell imaging. *Science* 300:82–6.

Please visit the Prescott website at www.mhhe.com/prescott7 for additional references.

Student Resources

Student Study Guide

The **Student Study Guide** is a valuable resource that provides learning objectives, study outlines, learning activities, and self-testing material to help students master course content.

Laboratory Exercises in Microbiology

The seventh edition of ***Laboratory Exercises in Microbiology*** by John P. Harley has been prepared to accompany the text. Like the text, the laboratory manual provides a balanced introduction in each area of microbiology. The class-tested exercises are modular and short so that an instructor can easily choose those exercises that fit his or her course.

ARIS

McGraw-Hill's ARIS—Assessment, Review, and Instruction System for *Prescott, Harley, and Klein's Microbiology, www.mhhe.com/prescott7*. This online resource provides helpful study materials that support each chapter in the book. Features include:

Self-quizzes
Animations (with quizzing)
Flashcards
Clinical case studies
Additional course content and more!

Instructor Resources

ARIS (www.mhhe.com/prescott7)

McGraw-Hill's ARIS—Assessment, Review, and Instruction System for *Prescott, Harley, and Klein's Microbiology* is a complete, online tutorial, electronic homework, and course management system. Instructors can create and share course materials and assignments with colleagues with a few clicks of the mouse. All PowerPoint lectures, assignments, quizzes, and tutorials are directly tied to text-specific materials. Instructors can also edit questions, import their own content, and create announcements and due dates for assignments. ARIS has automatic grading and reporting of easy-to-assign homework, quizzing, and testing. All student activity within McGraw-Hill's ARIS is automatically recorded and available to the instructor through a fully integrated grade book that can be downloaded to Excel. Contact your local McGraw-Hill Publisher's representative for more information on getting started with ARIS.

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- **Art Library**—Color-enhanced, digital files of all illustrations in the book can be readily incorporated into lecture presentations, exams, or custom-made classroom materials. The large, bolded labels make the images appropriate for use in large lecture halls.
- **TextEdit Art Library**—Every line art piece is placed into a PowerPoint presentation that allows the user to revise, move, or delete labels as desired for creation of customized presentations or for testing purposes.
- **Photo Library**—Like the Art Library, digital files of all the photographs from the book are available.
- **Table Library**—Every table that appears in the book is provided in electronic form.
- **Animations Library**—Full-color presentations involving key process figures in the book have been brought to life via animations. These animations offer flexibility for instructors and were designed to be used in lecture or for self-study. Instructors can pause, rewind, fast forward, and turn audio off/on to create dynamic lecture presentations.
- **PowerPoint Lecture Outlines**—These ready-made presentations combine art and lecture notes for each of the 41 chapters of the book. The presentations can be used as they are, or they can be customized to reflect your preferred lecture topics and organization.
- **PowerPoint Outlines**—The art, photos, and tables for each chapter are inserted into blank PowerPoint presentations to which you can add your own notes.

Instructor Testing and Resource CD-ROM

This cross-platform CD contains the *Instructor's Manual and Test Bank*, both available in Word and PDF formats. The *Instructor's Manual* contains chapter overviews, objectives, and answer guidelines for Critical Thinking Questions. The Test Bank provides questions that can be used for homework assignments or the preparation of exams. The computerized test bank allows the user to quickly create customized exams. This user-friendly program

allows instructors to search for questions by topic, format, or difficulty level; edit existing questions or add new ones; and scramble questions and answer keys for multiple versions of the same test.

Transparencies

A set of 250 full-color acetate transparencies is available to supplement classroom lectures. These have been enhanced for projection and are available to adopters of the seventh edition.

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