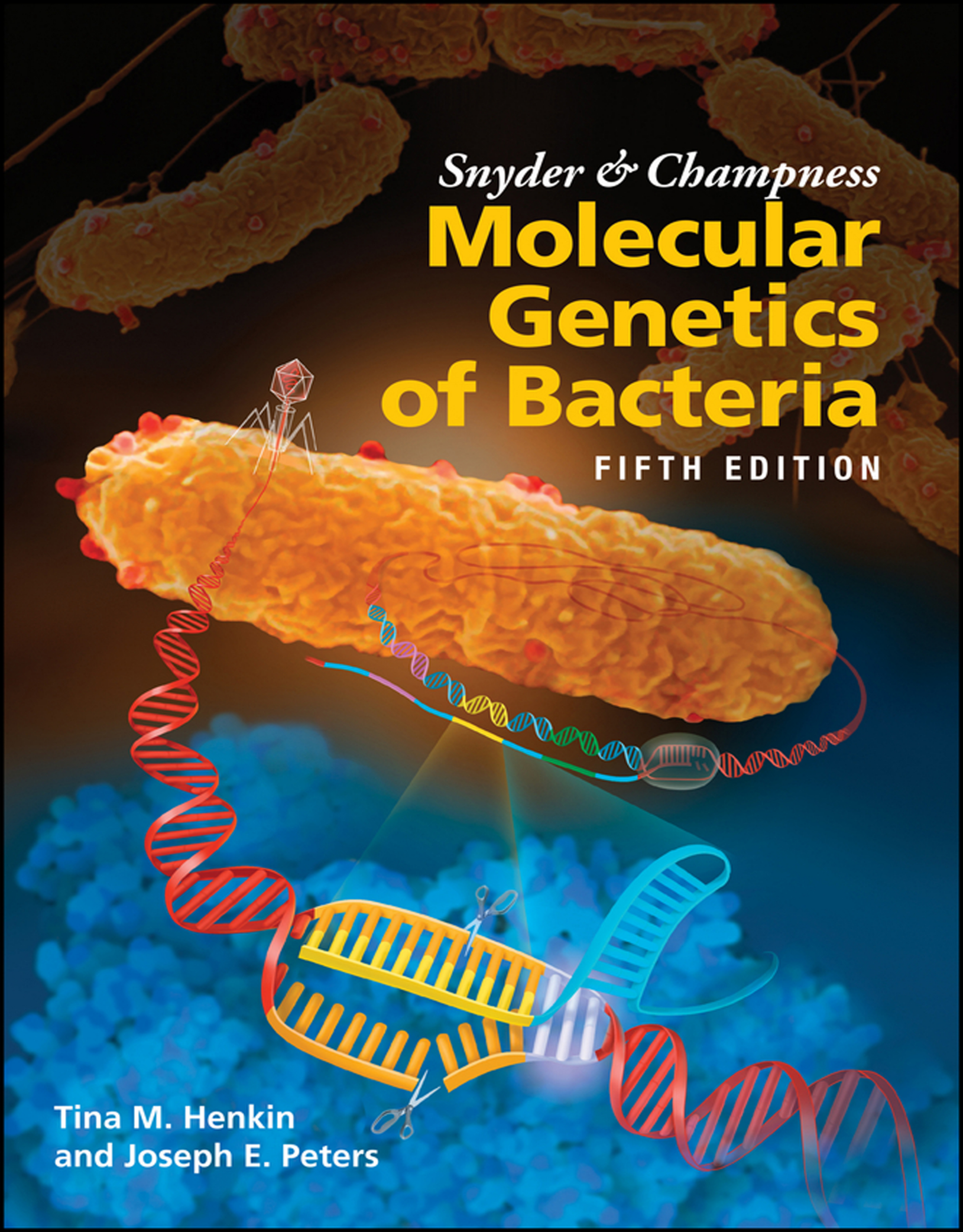


Snyder & Champness

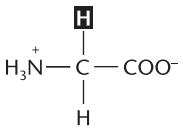
Molecular Genetics of Bacteria

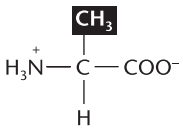
FIFTH EDITION

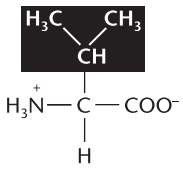
Tina M. Henkin
and Joseph E. Peters

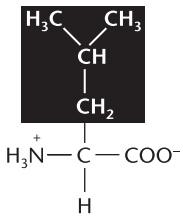
The Amino Acids

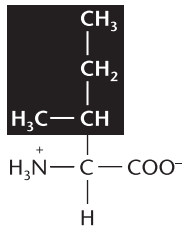
Nonpolar amino acids

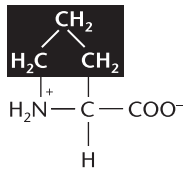

Glycine (Gly, G)

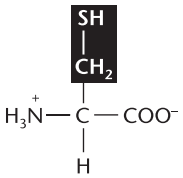

Alanine (Ala, A)

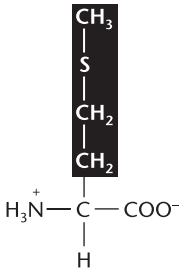

Valine (Val, V)

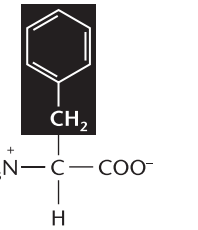

Leucine (Leu, L)

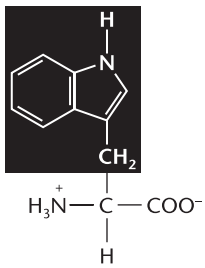

Isoleucine (Ile, I)


Proline (Pro, P)

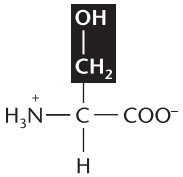

Cysteine (Cys, C)

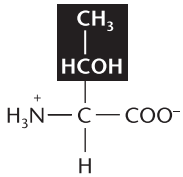

Methionine (Met, M)

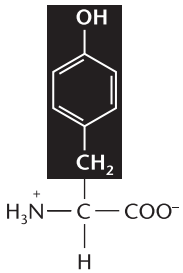

Phenylalanine (Phe, F)

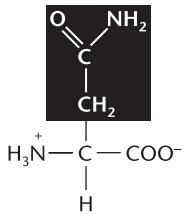

Tryptophan (Trp, W)

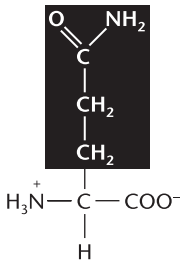
Polar amino acids


Serine (Ser, S)

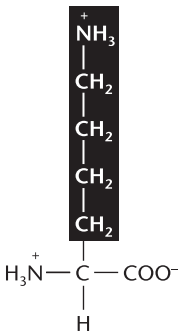

Threonine (Thr, T)

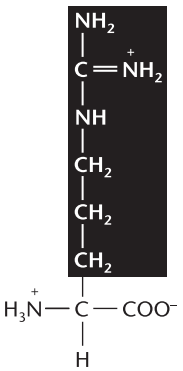

Tyrosine (Tyr, Y)

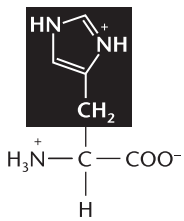

Asparagine (Asn, N)


Glutamine (Gln, Q)

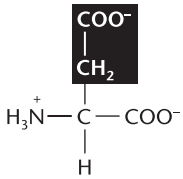
Basic amino acids

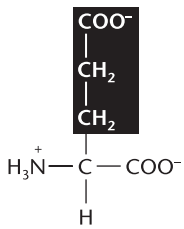

Lysine (Lys, K)


Arginine (Arg, R)


Histidine (His, H)

Acidic amino acids

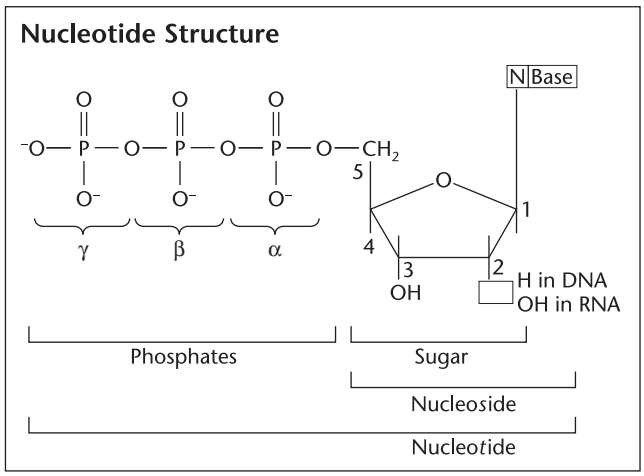

Aspartic acid (Asp, D)


Glutamic acid (Glu, E)

The Genetic Code

First position	Second position U	C	A	G	Third position
	Phe	Ser	Tyr	Cys	U
U	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
	Leu	Pro	His	Arg	U
C	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
	Ile	Thr	Asn	Ser	U
A	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
	Val	Ala	Asp	Gly	U
G	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Nucleotide Structure



Names of Nucleic Acid Subunits

Base	Nucleoside	Nucleotide	Abbreviation RNA	DNA
Adenine	Adenosine	Adenosine triphosphate	ATP	dATP
Guanine	Guanosine	Guanosine triphosphate	GTP	dGTP
Cytosine	Cytidine	Cytidine triphosphate	CTP	dCTP
Thymine	Thymidine	Thymidine triphosphate		dTTP
Uracil	Uridine	Uridine triphosphate	UTP	

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About the Companion Website

This book is accompanied by a companion website:

The URL is **www.wiley.com/go/snyder/mgb5**

The website includes:

Figures from the book

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Molecular Genetics of Bacteria

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Tina M. Henkin
Ohio State University, Columbus, Ohio

Joseph E. Peters
Cornell University, Ithaca, New York

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Contents

Preface

Snyder and Champness Molecular Genetics of Bacteria is a new edition of a classic text updated to address the massive advances in the field of bacterial molecular genetics. We renamed the book as a tribute to the original authors, Larry Snyder and Wendy Champness, who welcomed us as coauthors for the 4th edition and trusted us to continue to build on the strong foundation of their multiple editions in carrying this important text forward. As with the previous editions, we have endeavored to keep the page length approximately the same. This meant making many hard choices of what to remove to make room for exciting new and important material. We are very happy that every illustration is now in full color, which offered us the opportunity to rethink each drawing and clarify and standardize features, which we believe will improve their use by instructors in classroom lectures.

Perhaps the most significant force in molecular genetics research since the last edition has been the plummeting cost of DNA sequencing. This factor has created an explosion of new sequence information of both independent genomes and microbial communities in the form of metagenomics, where DNA is extracted directly from all of the organisms in an environment. This information has vastly expanded our picture of the tree of life and the massive contribution of uncultured species. The broader availability of DNA sequencing at a reasonable price has also left its mark on genomic techniques. These new techniques and new information have had a considerable impact in every chapter and provided the impetus for a new chapter, "Genomes and Genomic Analysis" (chapter 13).

We expanded chapter 1, on DNA structure, DNA replication, and chromosome segregation, to include many advances in our understanding of how chromosomes are managed and the molecular machines that carry out these processes. Our understanding of the nature of FtsK and related DNA-pumping enzymes, the evolving role of SeqA, the mechanism of chromosome partitioning, and the domain structure of the chromosomes also benefited from multiple technological innovations. Chapter 2 focuses on mechanisms of gene expression, from transcription through mRNA turnover, translation, and post-translational effects, including protein targeting, which was moved into this chapter. We reduced the historical aspects of chapter 3, retaining key landmarks such as the important role of the F plasmid discovered by Esther Lederberg, so the chapter now focuses more on practical aspects of genetic analysis.

Newer molecular techniques that have replaced some of the classic approaches (e.g., for generation of targeted chromosomal mutations) are now discussed in the new chapter 13.

Chapter 4 presents a concise understanding of bacterial plasmids as important contributors to the genomic content in bacteria as well as essential tools in molecular biology. Significant additions to the chapter include an expanded discussion of the two major mechanisms of segregation and the ever-broadening view of toxin-antitoxin systems. Toxin-antitoxin systems were first discovered for their role in plasmid stabilization, but while the diversity of molecular mechanisms has expanded, important questions remain concerning the real function of these systems when situated in bacterial chromosomes. Chapter 5, which focuses on conjugation, continues to set its roots in the original conjugal plasmid, the fertility plasmid. We included considerable new information that relates to our recognition that conjugal systems appear to be as common in the form of integrating conjugative elements (ICEs) as they are in stand-alone plasmids. The diversity of ICEs is remarkable, and this chapter strives to provide a foundation for these dynamic elements, which are responsible for the largest known genomic islands transmitted between bacteria.

In chapter 6, we expanded the discussion of natural transformation and its regulation to include additional comparative information about how these systems vary in different groups of organisms. We consolidated the discussion of lytic and lysogenic bacteriophages and their roles in transduction of bacterial DNA as chapter 7. We organized the information on phage biology based on the different functions required for phage infection and replication, and followed this with a discussion of phage genetics, their use in bacterial genetic transfer, and their roles as tools for molecular biology.

We streamlined chapter 8, "Transposition, Site-Specific Recombination, and Families of Recombinases," to make room for additional families of elements including the exciting and still somewhat enigmatic HUH transposons, as well as group II mobile introns, and an advanced appreciation of the interrelationship between mobile elements and host DNA replication. Transposons continue to provide an important tool in genomics, and mobile genetic elements in general provide the most significant mechanisms for the transfer of antibiotic resistance. As the spread of antibiotic resistance is slowly nullifying the effectiveness of antibiotics worldwide, understanding the mechanisms of this spread is more important than ever. Chapter 9, "Molecular Mechanisms of Homologous Recombination," continues to be grounded in the central role that homologous recombination plays in the repair of DNA double-strand breaks. We expanded the chapter to include a better appreciation of the multiple pathways used to load the RecA recombinase onto different types of DNA substrates.

We broadly updated chapter 10, "DNA Repair and Mutagenesis," to reflect our increased mechanistic understanding across many DNA repair systems, as well as information on how mechanisms established in bacterial systems continue to contribute to our understanding of disease in humans. We extensively updated chapter 11, which focuses on mechanisms of gene regulation of individual genes and operons, to include new information as the field continues to advance. In chapter 12, we then applied the principles learned in chapter 11 to global regulatory systems that regulate multiple sets of genes and operons, often in response to multiple regulatory inputs. *Bacillus subtilis* sporulation, a complex developmental system, is presented in depth as a final example that integrates many of the different mechanisms that are introduced in chapters 11 and 12.

Chapter 13, "Genomes and Genomic Analysis," is a new chapter that consolidates relevant topics previously found elsewhere in the book and provides considerable new information on this topic. We provide background on the multiple mechanisms used for DNA sequencing, including the newest generations of high-throughput sequencing strategies. Having hundreds of thousands of bacterial genomes has allowed us to gain a better understanding of how genomes are organized as well as the relationship between core genes and genes acquired by horizontal gene transfer. The chapter also provides basic information on genome annotation and comparative genomics. Chapter 13 further presents an expanded picture of numerous systems that bacteria use to guard against horizontal gene transfer. Although horizontal gene transfer is by far the most important mechanism for evolution in bacteria and archaea, it also provides the greatest vulnerability, with the relentless onslaught of bacteriophages and mobile elements that can sap cellular resources or inactivate important or essential host genes. Significantly, host defense systems also provide the most important tools ever developed for molecular biology. The new chapter provides expanded background on diverse restriction endonucleases and the important roles they play in molecular biology. We cover the variety of tools that are available for cloning and gene assembly, as well as the advantages and disadvantages of these techniques to help guide the investigator. These techniques allow never-imagined possibilities for quickly and accurately constructing synthetic DNA fragments for testing ideas or allowing advances in engineering, including assembling entire bacterial genomes. We greatly expanded the section on CRISPR/Cas systems and chose the Cas9 system, important in many applications in a multitude of model systems and human genome engineering, to illustrate on the book's cover. CRISPR/Cas systems are very diverse, falling into six distinct types and tens of subtypes. We provide the reader with the background needed to understand how these fascinating systems evolved, the role they play in the natural environment, and the massive promise they hold in genome engineering.

Acknowledgments

We continue to be indebted to a large number of individuals for their help, talking through ideas, sharing insights from their area of expertise, or reading over sections for accuracy and clarity. Other individuals have alerted us to errors, suggested areas to include, or provided graphics. However, if errors remain in the text, they are our own.

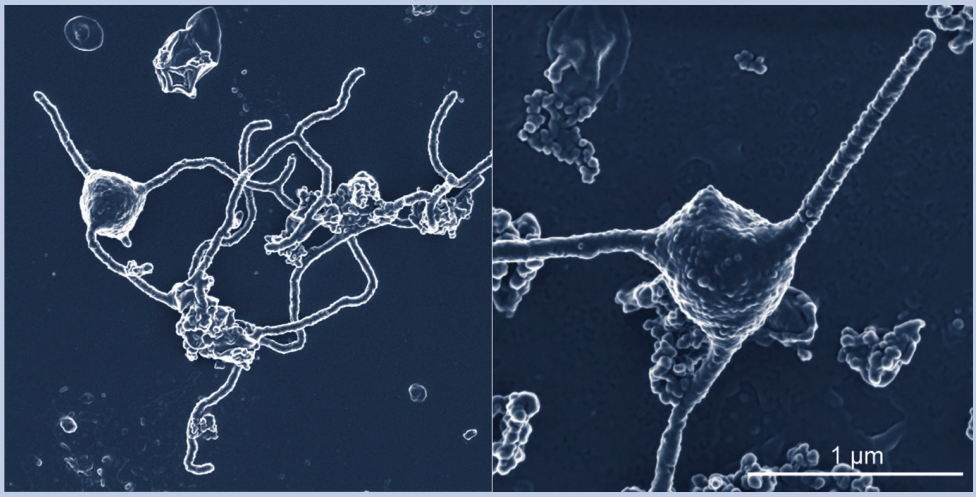
We acknowledge those whose comments on earlier editions have carried over to the current edition. In addition, we thank Esther Angert, Melanie Berkmen, Briana Burton, Druba Chattoraj, Mick Chandler, Pete Christie, Alan Grossman, Alba Guarné, Claudia Guldimann (and the Zürich book club), John Helmann, Laura Hug, Ailong Ke, Bénédicte Michel, Kit Pogliano, Lise Raleigh, Phoebe Rice, Jim Samuelson, Mark Sutton, Anthony Vecchiarelli, Bob Wiess, Steve Winans, Wei Yang, and Steve Zinder.

It was a great pleasure to work with Director Christine Charlip and ASM Press, and we look forward to the new partnership with John Wiley & Sons, Inc. We enjoyed working first with Production Manager Larry Klein and then transitioning to Developmental Editor Ellie Tupper, who coordinated the project to the finish line. We continue to be grateful for the professional and patient work of Patrick Lane of ScEYEence Studios for making our visions come to life in these illustrations.

About the Authors

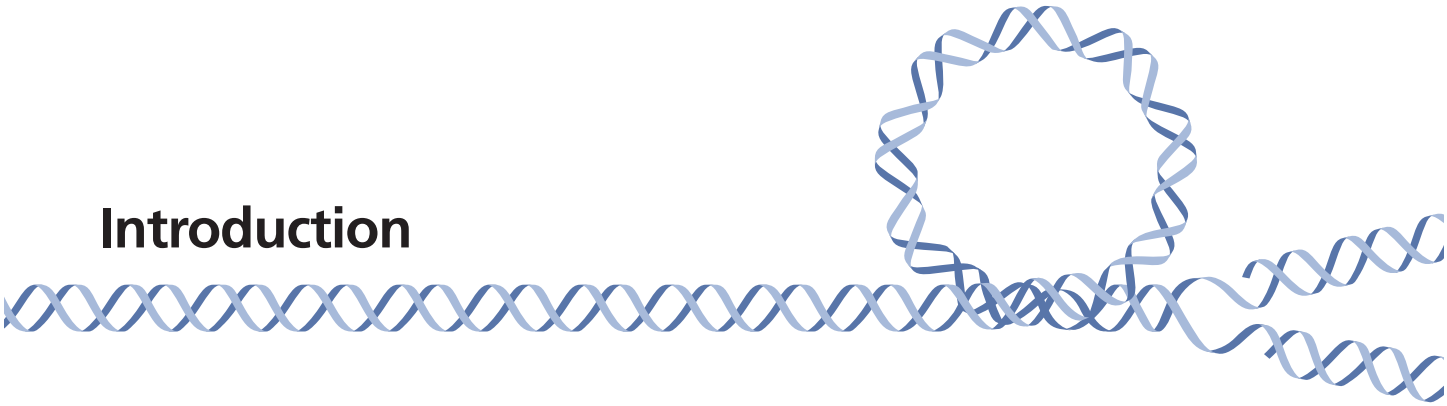
Tina M. Henkin is a Professor of Microbiology, Robert W. and Estelle S. Bingham Professor of Biological Sciences, and Distinguished University Professor at The Ohio State University, where she has been teaching microbiology and bacterial genetics since 1995. She received her B.A. in biology at Swarthmore College and her Ph.D. in genetics at the University of Wisconsin-Madison, and did postdoctoral work in molecular microbiology at Tufts University Medical School. Her research focuses on gene regulation in Gram-positive bacteria, primarily using *Bacillus subtilis* as a model. Her laboratory uncovered the T-box regulatory mechanism, in which the leader RNAs of bacterial genes bind a specific uncharged tRNA to modulate expression of the downstream genes. This work led to the discovery of riboswitch RNAs that bind cellular metabolites to mediate similar regulatory responses. Current work focuses on elucidating the basis for specific ligand recognition and molecular mechanisms for ligand-mediated changes in RNA structure in a variety of riboswitch classes. She is a Fellow of the American Academy of Microbiology, the American Association for the Advancement of Science, and the American Academy of Arts and Sciences, a member of the National Academy of Sciences, and co-winner of the National Academy of Sciences Pfizer Prize in Molecular Biology for her work on riboswitch RNAs.

Joseph E. Peters is a Professor of microbiology at Cornell University, where he has been teaching bacterial genetics and microbiology at the graduate and undergraduate level since 2002. He received his B.S. from Stony Brook University and his Ph.D. from the University of Maryland at College Park. He did postdoctoral work at the Johns Hopkins University School of Medicine, in part as an NSF-Alfred P. Sloan Foundation postdoctoral research fellow in molecular evolution. His research has focused on the intersection between DNA replication, recombination, and repair and how it relates to evolution, especially in the area of transposition. Most recently he has been interested in the evolution of defense systems like CRISPR/Cas systems and how they can be repurposed by mobile elements for new tasks. Research in his lab is funded by the National Science Foundation, the U.S. Department of Agriculture, and the National Institutes of Health. He is the director of graduate studies for the field of microbiology at Cornell.



SEM images of the archaeon "*Candidatus* Prometheoarchaeum syntrophicum" strain MK-D1. Reprinted from Imachi H, et al, ©2020, Springer Nature, CC-BY 4.0, http://creativecommons.org/licenses/by/4.0/.

Introduction



THE GOAL OF THIS TEXTBOOK is to introduce the student to the field of bacterial molecular genetics. From the point of view of genetics and genetic manipulation, bacteria are relatively simple organisms. There also exist model bacterial organisms that are easy to grow and easy to manipulate in the laboratory. For these reasons, most methods in molecular biology and recombinant DNA technology that are essential for the study of all forms of life have been developed around bacteria. Bacteria also frequently serve as model systems for understanding cellular functions and developmental processes in more complex organisms. Much of what we know about the basic molecular mechanisms in cells, such as transcription, translation, and DNA replication, has originated with studies of bacteria. This is because such central cellular functions have remained largely unchanged throughout evolution. Core parts of RNA polymerase and many of the translation factors are conserved in all cells, and ribosomes have similar structures in all organisms. The DNA replication apparatuses of all organisms contain features in common, such as sliding clamps and editing functions, which were first described in bacteria and their viruses, called bacteriophages. Chaperones that help other proteins fold and topoisomerases that change the topology of DNA were first discovered in bacteria and their bacteriophages. Studies of repair of DNA damage and mutagenesis in bacteria have also led the way to an understanding of such pathways in eukaryotes. Excision repair systems, mutagenic polymerases, and mismatch repair systems are remarkably similar in all organisms, and defects in these systems are responsible for multiple types of human cancers.

In addition, as our understanding of the molecular biology of bacteria advances, we are finding a level of complexity that was not appreciated previously. Because of the small size of the vast majority of bacteria, it was impossible initially to recognize the high level of organization that exists in bacteria, leading to the misconception that bacteria were merely "bags of enzymes," where small size allowed passive diffusion to move cellular constituents around. However, it is now clear that movement and positioning within the bacterial cell are highly controlled processes. For example, despite the lack of a specialized membrane structure called the nucleus (the early defining feature of the "prokaryote" [see below]), the genome of bacteria is exquisitely organized to facilitate its repair and expression during DNA replication. In addition, advances facilitated by molecular genetics and microscopy have made it clear that

many cellular processes occur in highly organized subregions within the cell. Once it was appreciated that bacteria evolved in the same basic way as all other living organisms, the relative simplicity of bacteria paved the way for some of the most important scientific advances in any field, ever. It is safe to say that a bright future awaits the fledgling bacterial geneticist, where studies of relatively simple bacteria, with their malleable genetic systems, promise to uncover basic principles of cell biology that are common to all organisms and that we can now only imagine.

However, bacteria are not just important as laboratory tools to understand other organisms; they also are important and interesting in their own right. For instance, they play essential roles in the ecology of Earth. They are the only organisms that can "fix" atmospheric nitrogen, that is, convert N_2 to ammonia, which can be used to make nitrogen-containing cellular constituents, such as proteins and nucleic acids. Without bacteria, the natural nitrogen cycle would be broken. Bacteria are also central to the carbon cycle because of their ability to degrade recalcitrant natural polymers, such as cellulose and lignin. Bacteria and some types of fungi thus prevent Earth from being buried in plant debris and other carbon-containing material. Toxic compounds, including petroleum, many of the chlorinated hydrocarbons, and other products of the chemical industry can also be degraded by bacteria. For this reason, these organisms are essential in water purification and toxic waste clean-up. Moreover, bacteria produce most of the naturally occurring so-called greenhouse gases, such as methane and carbon dioxide, which are in turn used by other types of bacteria. This cycle helps maintain climate equilibrium. Bacteria have even had a profound effect on the geology of Earth, being responsible for some of the major iron ore and other mineral deposits in Earth's crust.

Another unusual feature of bacteria and archaea (see below) is their ability to live in extremely inhospitable environments, many of which are devoid of life except for microbes. These are the only organisms living in the Dead Sea, where the salt concentration in the water is very high. Some types of bacteria and archaea live in hot springs at temperatures close to the boiling point of water (or above in the case of archaea), and others survive in atmospheres devoid of oxygen, such as eutrophic lakes and swamps.

Bacteria that live in inhospitable environments sometimes enable other organisms to survive in those environments through symbiotic relationships. For example, symbiotic bacteria make life possible for *Riftia* tubeworms next to hydrothermal vents on the ocean floor, where living systems must use hydrogen sulfide in place of organic carbon and energy sources. In this symbiosis, the bacteria obtain energy and fix carbon dioxide by using the reducing power of the hydrogen sulfide given off by the hydrothermal vents, thereby furnishing food in the form of high-energy carbon compounds for the worms, which lack a digestive tract. Symbiotic cyanobacteria allow fungi to live in the Arctic tundra in the form of lichens. The bacterial partners in the lichens fix atmospheric nitrogen and make carbon-containing molecules through photosynthesis to allow their fungal partners to grow on the tundra in the absence of nutrient-containing soil. Symbiotic nitrogen-fixing *Rhizobium* and *Azorhizobium* spp. in the nodules on the roots of legumes and some other types of higher plants allow the plants to grow in nitrogen-deficient soils. Other types of symbiotic bacteria digest cellulose to allow cows and other ruminant animals to live on a diet of grass. Bioluminescent bacteria even generate light for squid and other marine animals, allowing illumination, camouflage, and signaling in the darkness of the deep ocean.

Bacteria are also important to study because of their role in disease. They cause many human, plant, and animal diseases, and new diseases are continuously appearing. Knowledge gained from the molecular genetics of bacteria helps in the development of new ways to treat or otherwise control old diseases that can be resistant to older forms of treatment, as well as emerging diseases.

Some bacteria that live in and on our bodies also benefit us directly. The role of our commensal bacteria in human health is only beginning to be appreciated. It has been estimated that of the 10^{14} cells in a human body, only half are human! Of course, bacterial cells are much smaller than our cells, but this shows how our bodies are adapted to live with an extensive bacterial microbiome, which helps us digest food and avoid disease, among other roles, many of which are yet to be uncovered.

Bacteria have also long been used to make many useful compounds, such as antibiotics, and chemicals, such as benzene and citric acid. Bacteria and their bacteriophages are also the source of many of the useful enzymes used in molecular biology.

In spite of substantial progress, we have only begun to understand the bacterial world around us. Bacteria are the most physiologically diverse organisms on Earth, and the importance of bacteria to life on Earth and the potential uses to which bacteria can be put can only be guessed. Thousands of different types of bacteria are known, and new insights into their cellular mechanisms and their applications constantly emerge from research with bacteria. Moreover, it is estimated that less than 1% of the types of bacteria living in the soil and other environments have ever been isolated. Recent culture-independent mechanisms indicate that bacterial diversity is much greater than we ever imagined (see Hug et al., Suggested Reading). In this new picture, it seems that less than half of the major lineages of bacteria have representatives that have been

cultured. Organisms in these uncharacterized groups of bacteria may have all manner of interesting and useful functions. Clearly, studies of bacteria will continue to be essential to our future efforts to understand, control, and benefit from the biological world around us, and bacterial molecular genetics will be an essential tool in these efforts. However, before discussing this field, we must first briefly discuss the evolutionary relationship of bacteria to other organisms.

The Biological Universe

The Bacteria

This textbook comes at a very exciting time in our understanding of the interrelationship of all living things on the planet. After the landmark work of Carl Woese, all organisms on Earth were assigned to three major groups called domains: the bacteria (formerly eubacteria), the archaea (formerly archaebacteria), and the eukaryotes (see Woese and Fox, Suggested Reading). However, it is now clear that two major divisions account for these three groups. Bacteria form one of these divisions, while eukaryotes are now believed to have diverged out of the archaea. Figure 1 shows the microbiologists' view of the living world, where microbes provide most of the diversity and eukaryotes occupy a relatively small niche. This is not a far-fetched concept. Sequence data show that we differ from chimpanzees by only 2% of our DNA sequence, while 25 to 50% of the genes in a typical bacterium are unique to the species. Furthermore, while mammals diverged from each other on the order of millions of years ago, the main bacterial lineages diverged billions of years ago.

Bacteria can differ greatly in their physical appearance under the microscope. Although most are single celled and rod shaped or spherical, some are multicellular and undergo complicated developmental cycles. The cyanobacteria (formerly called blue-green algae) are bacteria, but they have chlorophyll and can be filamentous, which is why they were originally mistaken for algae. The antibiotic-producing actinomycetes, which include *Streptomyces* spp., are also bacteria, but they form hyphae and stalks of spores, making them resemble fungi. Another bacterial group, the *Caulobacter* spp., have both free-swimming and sessile forms that attach to surfaces through a holdfast structure. Some of the most dramatic-appearing bacteria of all belong to the genus *Myxococcus*, members of which can exist as free-living single-celled organisms but can also aggregate to form fruiting bodies, much like slime molds. As mentioned above, bacterial cells are usually much smaller than the cells of higher organisms, but one very large bacterium, *Epulopiscium*, can be over half a millimeter long, longer than even most eukaryotic cells (see Angert, Suggested Reading). In addition, unlike most bacteria that multiply by simple division, *Epulopiscium* gives birth to multiple live progeny. Despite the fact that some bacteria are found in dramatically different shapes and sizes, they cannot be distinguished simply by their physical appearance; instead, it is necessary to use biochemical criteria, such as the sequences of their ribosomal proteins or RNAs (rRNAs), whose sequences are characteristic of the three domains of life.

GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

Bacteria have historically been divided into two major subgroups, the **Gram-negative** and **Gram-positive** bacteria. This division was based on the response to a test called the Gram stain. "Gram-negative" bacteria retain little of the dye and are pink after this staining procedure, whereas "Gram-positive" bacteria retain more of the dye and turn deep blue. The difference in staining typically reflects the fact that Gram-negative bacteria are surrounded by a thinner structure composed of both an inner and an outer membrane, while the structure surrounding Gram-positive bacteria is much thicker, consisting of a single membrane surrounded by a thicker wall. However, this older form of classification is being replaced by talking about the phyla of bacteria as determined by the DNA sequence. The *Firmicutes* are a broad group containing *Bacillus*, clostridia, lactic acid bacteria, and the *Tenericutes*, including the mycoplasmas. Firmicutes have been referred to as low G+C Gram-positive bacteria based on the low percentage of guanine and cytosine (low G+C) compared to adenine and thymine often found in the genome sequence of members of this group (see chapter 1). However, having a low G+C genome is not a universal feature of the *Firmicutes*, which limits the utility of the designation. Another group of bacteria that were classically described as high G+C and Gram positive because they typically possess a higher percentage of guanine and cytosine includes the *Actinobacteria* (actinomycetes), such as *Streptomyces* and *Mycobacterium*.

The Gram designation system of classifying bacteria is particularly weak for capturing the diversity of the numerous phyla that stain Gram negative. While many bacteria historically referred to as Gram negative, such as *Escherichia coli*, *Pseudomonas*, and *Rhizobium*, fall within a broad group known as the *Proteobacteria*, many other characterized and uncharacterized groups also exist. It is also worth pointing out that relying on a staining form of classification is particularly contrived when talking about uncultured bacteria or those that are only capable of growth as symbionts in other organisms. Given all of these considerations, instead of using the Gram-positive and Gram-negative designations as a tool for

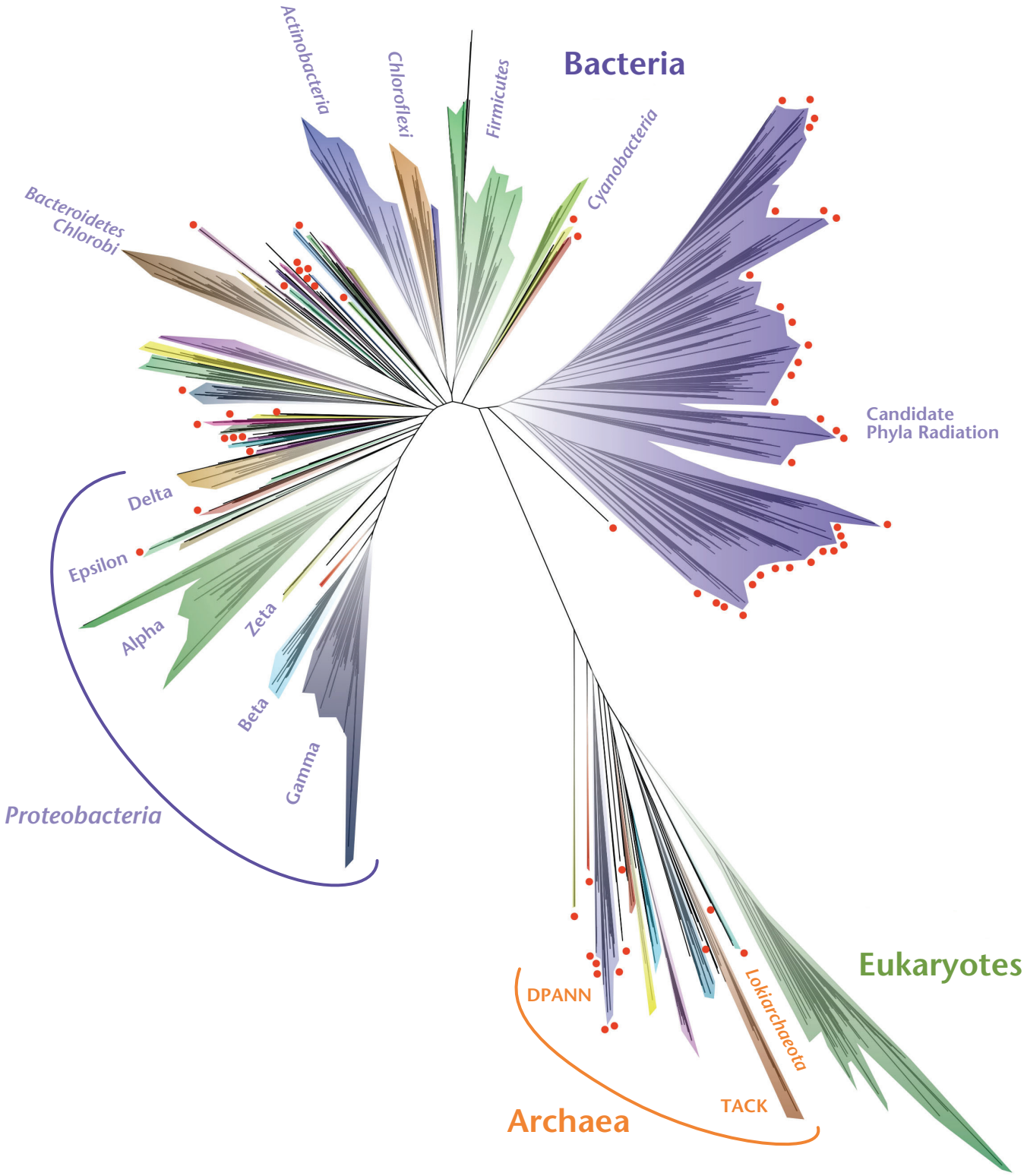


Figure 1 A molecular tree of life capturing diversity using ribosomal proteins from sequenced genomes (see Hug et al., Suggested Reading). Selected major linages within bacteria are indicated, including the *Proteobacteria* and the subgroups Alpha, Beta, Delta, Epsilon, Gamma, and Zeta, the *Firmicutes*, and the Candidate Phyla Radiation, which is almost completely devoid of cultured representatives. For the *Archaea*, two superphyla, TACK and DPANN, are indicated and described in the text. The position of the archaeal *Lokiarchaeota* lineage is indicated. Genome sequences from members of the *Lokiarchaeota* lineage indicate that they possess molecular systems previously believed to be found only in *Eukaryotes*. Red dots indicate lineages that have no cultured representatives. Adapted with permission from Hug L, et al, *Nat Microbiol* **1**:16048 (2016), https://doi.org/10.1038/nmicrobiol.2016.48.