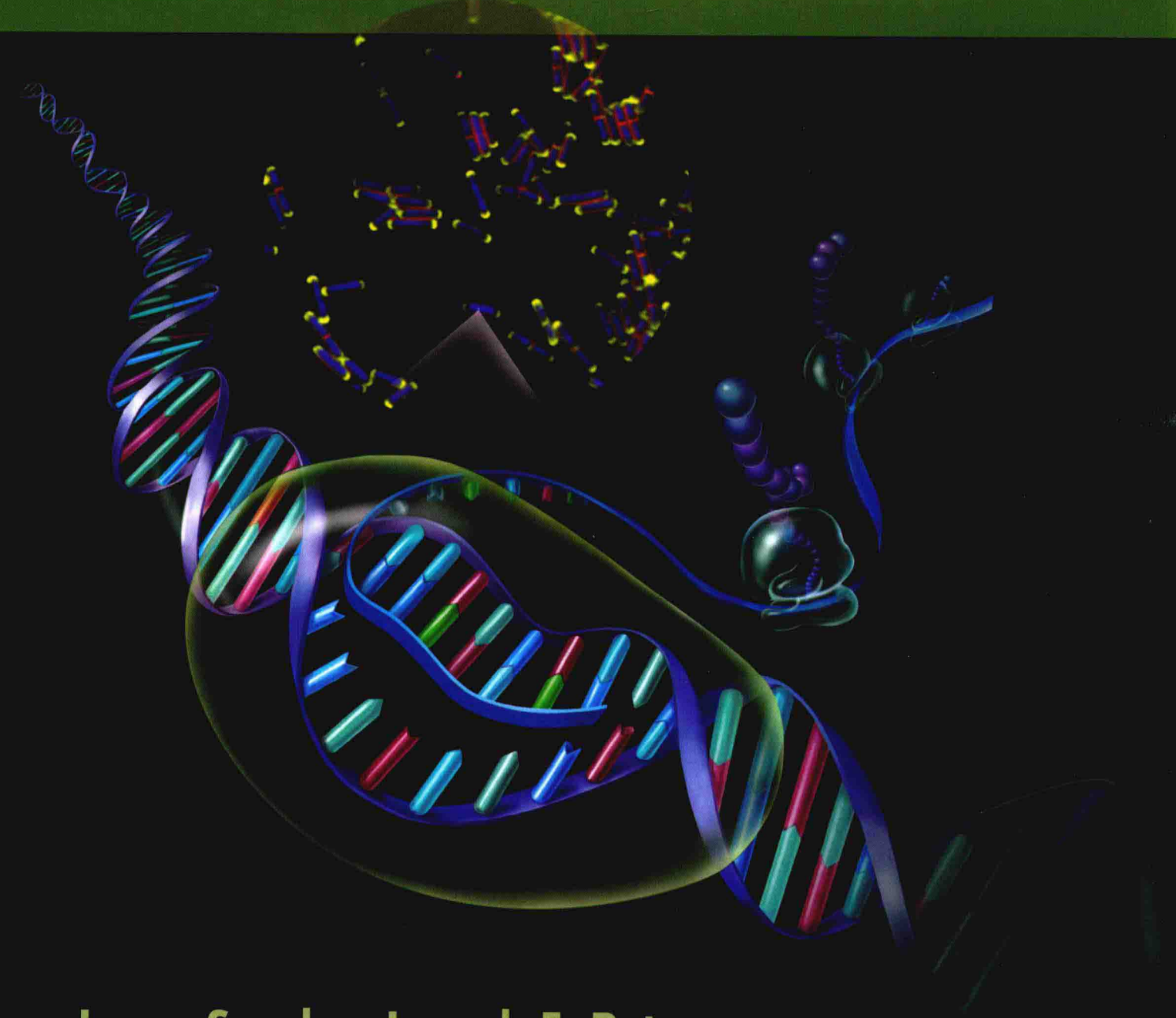
4TH EDITION

Molecular Genetics of Bacteria



Larry Snyder, Joseph E. Peters,
Tina M. Henkin, and Wendy Champness

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Larry Snyder
Department of Microbiology and Molecular Genetics
Michigan State University
East Lansing, Michigan

Joseph E. Peters
Department of Microbiology
Cornell University
Ithaca, New York

Tina M. Henkin
Department of Microbiology
Ohio State University
Columbus, Ohio

Wendy Champness
Department of Microbiology and Molecular Genetics
Michigan State University
East Lansing, Michigan

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Cover photo: Fluorescence micrograph of *Bacillus subtilis* cells showing the location of the cell membrane (red), DNA (blue), and ConE mating protein fused to green fluorescent protein (yellow-green). The ConE protein is a component of the conjugative DNA translocation channel required for horizontal transfer of the integrating conjugative element ICE*Bs1*. The ConE protein is concentrated at the cell poles, but additional protein is localized around the entire cell periphery. The lateral distribution enables cells to transfer ICEBs1 side to side, and the high concentration at the poles may contribute to the very efficient transfer of ICEBs1 observed in chains of cells from pole to pole. See chapter 5 for details. Photo courtesy of Melanie Berkmen and Alan Grossman. Modified from M. B. Berkmen et al., *J. Bacteriol.* **192:**38–45, 2010.

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Preface

WITH THE ADDITION OF TWO NEW COAUTHORS, the fourth edition of the textbook *Molecular Genetics of Bacteria* has been substantially revised and some new sections have been added. We tried to do this without increasing the length of the book, which, at more than 700 pages, was already quite long. While the book retains the same number and order of chapters, many topics have been moved or integrated more completely into the text to reflect a more modern perspective. The purpose was to convey more accurately how one approaches questions in modern bacterial genetics, using the full repertoire of methods now available. Also, to make room for the new material, we made the philosophical decision to condense or eliminate descriptions of methods where they seemed unnecessarily detailed for a textbook.

Chapter 1, on DNA structure, DNA replication, and chromosome segregation, was expanded to include updates in our understanding, including how replication proceeds through obstacles typically found during normal DNA replication in bacteria, while some aspects of repair-associated replication were moved to later chapters. The chapter was also significantly expanded with new information about how numerous cell processes coordinate for the efficient processing and organizing of chromosomes after DNA replication. Scientists now more fully appreciate how sequences "hidden" in the structure guide a variety of systems that aid in repairing, segregating, packaging, and pumping the chromosome for exquisite genome stability in bacteria. In chapter 2, which covers bacterial gene expression, the translation section has been reorganized to follow the same order as the transcription section. It begins with initiation of translation and then discusses elongation followed by termination, rather than following the more historical order with the genetic code coming first. We reasoned that this order makes more sense since most students already have had some exposure to translation and the genetic code. More information on RNA degradation is now included, and the sections on gene regulation have been moved to chapter 12. The protein transport section has been moved from chapter 2 to chapter 14 (see below), where it can be better integrated with other topics of protein export. Chapter 3, on bacterial genetic analysis, also

now takes a less historical approach. Rather than beginning with a review of classical genetic analysis and then contrasting it with bacterial genetic analysis as in previous editions, the chapter now begins with bacterial genetic analysis, again assuming that students have already had some general genetics. Furthermore, rather than putting more recently developed methods such as site-specific mutagenesis, recombineering, etc., into a separate section, we have integrated throughout the text all the methods available nowadays to use in a genetic analysis. The discussion of mapping by Hfr crosses has been sharply condensed, since it is likely that no one will ever again perform the laborious task of constructing the genetic map of a bacterium. The relative ease of DNA sequencing now allows the placing of mutations on the sequenced genomes of bacteria by direct comparison of sequences rather than by Hfr mapping. Transduction and transformation (including electroporation) are used extensively for genetic manipulations, so their use is still covered in some detail. Chapter 4 has been updated with more information about how plasmids are typically used in the laboratory setting in work with model organisms and beyond as well, including updates on our understanding of partitioning systems. Chapter 5 was extensively updated to illustrate the hodgepodge organization of conjugal elements and advances in our understanding of conjugation and to more fully integrate the important role of integrating conjugative elements in bacterial genomes (including a focus on one of these elements from *B. subtilis* on the front cover). Chapter 6 is updated throughout and focuses on similarities and differences between different transformation systems. The bacteriophage chapters (chapters 7 and 8) have been updated, and new material has been added. Some highlights from phage genomics are now covered, as are phage defense mechanisms, including CRISPR. The section on phage lysis is expanded, as is the text box on phage display, whose power is now demonstrated with some current uses. The interaction between lysogenic phages and genetic islands has been updated and moved into the text, as have some more recently developed techniques using lysogenic phages, for example, in detecting protein-protein interactions. Chapter 9, which covers transposable elements and site-specific recombination, has been updated to clarify the basic molecular biology of these elements, and it includes updated sections describing how they are used in the laboratory today. Chapter 10 was significantly reorganized to stress the role of homologous recombination in the repair of DNA double-strand breaks that occur at interruptions in the template DNA during replication. The role of homologous recombination in repair explains the underpinning of the evolution of the process and also clarifies how the process works in concert with DNA replication. A more comprehensive treatment for how DNA double-strand breaks are repaired across different types of bacteria, using systems found in all domains of life, is also included. Chapter 11 was updated to discuss many advances in the field of repair, including an expanded understanding of the regulation of multiple DNA polymerases found in bacteria with the SOS response. Chapters 12 and 13 have been reorganized so that chapter 12 is now focused on mechanisms of regulation of individual genes and operons and chapter 13 is mostly concerned with examples of global regulatory systems that utilize these mechanisms. There is also more emphasis on posttranscriptional regulation in both chapters, and global regulatory mechanisms in *Escherichia coli* are contrasted with those in *Bacillus subtilis*. Chapter 14 is probably the most changed chapter. It now contains our entire discussion of protein export, including the Sec and Tat systems as well as the secretion systems of gram-negative (i.e., *Proteobacteria*) and gram-positive (i.e., *Firmicutes*) bacteria. Most notably, it now contains a new section on bacterial cell biology, including cell wall synthesis and cell division and their regulation, as well as a new box on the evolution of cytoskeletal filaments, and it introduces the use of *Caulobacter crescentus* as a model system for these studies. Chapter 14 finishes with sporulation in *B. subtilis*, probably the best understood bacterial developmental system.

As in earlier editions, we do not mention the names of most investigators who have made major contributions to bacterial molecular genetics. We include only those names that have become icons in the field because they are associated with certain seminal experiments (e.g., Meselson and Stahl or Luria and Delbrück), models (e.g., Jacob and Monod), or structures (e.g., Watson and Crick). Many other names are available in the suggested reading lists, where we give some of the original references to the developments under discussion, and in the credit lines for sources of figures and tables, which are given at the end of the book.

Again we are indebted to a number of people who helped us in various ways. Some read sections of the book at our request and made valuable suggestions. Some, who have used the book for teaching, have pointed out ways to make it more useful for them and their students. Others have noticed factual errors or errors of omission and have pointed out references that helped us check our facts. In addition to those who commented on earlier editions, many of whose contributions have carried over, this list includes Dennis Arvidson, Dominique Belin, Melanie Berkmen, Helmut Bertrand, Lindsay Black, Rob Britton, Yves Brun, Rich Calendar, George Chaconas, Dhruba Chattoraj, Carton Chen, Todd Ciche, Laszlo Csonka, Gary Dunny, Marie Elliot, Laura Frost, Barbara Funnell, Peter Geiduschek,

Graham Hatfull, John Helmann, Ann Hochschild, Susan Lovett, Ken Marinas, Norman Pace, Steven Sandler, Joel Schildbach, Linda Sherwood, Chris Waters, Robert Weiss, Joanne Willey, Steve Winans, Ry Young, and Steve Zinder. Special thanks go to Lee Kroos, who agreed to update an entire section. Yet others furnished original figures that we could incorporate into the text; some of them are mentioned in the figure credits. However, in the end, any mistakes and omissions were all ours.

As with the first three editions, it was a great pleasure to work with the professionals at ASM Press. The former director of ASM Press, Jeff Holtmeier, helped us prepare for the fourth edition. We have been fortunate to continue to work with Kenneth April, the production manager, who coordinated the entire project. We have also had the good fortune to work again with two of the same professionals who did a masterful job with the first three editions: Susan Brown Schmidler, who created the book and cover design; Terese Winslow, who created the cover illustration; and Elizabeth McGillicuddy, who copyedited the manuscript. We also thank Patrick Lane of ScEYEnce Studios for bringing an attractive aestheticism to the rendering of our hand-drawn illustrations into the final figures.

Larry Snyder
Joe Peters
Tina Henkin
Wendy Champness

About the Authors

Larry (Loren R.) Snyder, PhD, is a professor emeritus of microbiology and molecular genetics at Michigan State University, where he taught microbial genetics and microbiology to undergraduate and graduate students for about 40 years. He received his BS in mathematics and zoology at the University of Minnesota in Duluth and his PhD in biophysics at the University of Chicago before doing postdoctoral work at the International Laboratory of Genetics and Biophysics in Naples, Italy, and at the Curie Institute and Faculty of Sciences at the University of Paris as a Jane Coffin Childs postdoctoral fellow. He was a visiting professor at Harvard University and the University of Tel Aviv. Most of his research was on the interaction between bacteriophage T4 and its host, *Escherichia coli*, and was supported by the National Science Foundation (NSF) and the National Institutes of Health (NIH). At Michigan State, he served as acting chair of the Department of Microbiology for one year and was a founding co-principal investigator of the NSF Center for Microbial Ecology and director of the Howard Hughes Medical Institute Undergraduate Research Program. He was awarded the College of Natural Science Alumni Association Meritorious Faculty Award in 2002.

Joseph E. Peters, PhD, is an associate professor of microbiology at Cornell University, where he has been teaching bacterial genetics and microbiology since 2002. He received his BS from Stony Brook University and his PhD 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 of DNA replication, recombination, and repair and how it relates to evolution, especially in the area of transposition. Research in his laboratory is funded by the NSF and NIH. He is the chair of the advisory board for the NSF-funded *E. coli* Genetic Stock Center, the chair of the American Society for Microbiology's Division of Genetics and Molecular Biology, and the director of graduate studies for the field of microbiology at Cornell.

Tina M. Henkin, PhD, is a professor of microbiology, chair of the Department of Microbiology, and Robert W. and Estelle S. Bingham Professor of Biological Sciences at Ohio State University, where she has been teaching bacterial genetics and microbiology since 1995. She received her BA in biology at Swarthmore College and her PhD in genetics at the University of Wisconsin—Madison, and she did postdoctoral work at the Tufts University School of Medicine. Her research focuses on gene regulation and regulatory RNAs in bacteria. Research in her laboratory is funded by the NIH. 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 a cowinner of the National Academy of Sciences Pfizer Prize in Molecular Biology for her work on riboswitch RNAs.

Wendy Champness, PhD, is a professor emerita of microbiology and molecular genetics at Michigan State University, where she taught microbial genetics and microbiology to undergraduate and graduate students for more than 25 years. She received her BS and PhD degrees at Michigan State, where she was an NSF predoctoral fellow. She did postdoctoral work at the Massachusetts Institute of Technology as a Jane Coffin Childs postdoctoral fellow and was a visiting scientist at the John Innes Research Centre in Norwich, United Kingdom, and at the University of Tel Aviv. Most of her research was on the regulation of antibiotic synthesis genes in *Streptomyces*, and research in her laboratory was supported by grants from the NSF and NIH. She was a charter member of the NSF Center for Microbial Ecology at Michigan State and was a member of the editorial board of the *Journal of Bacteriology* for 12 years as well as an associate editor of the journal *Microbiology*.

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