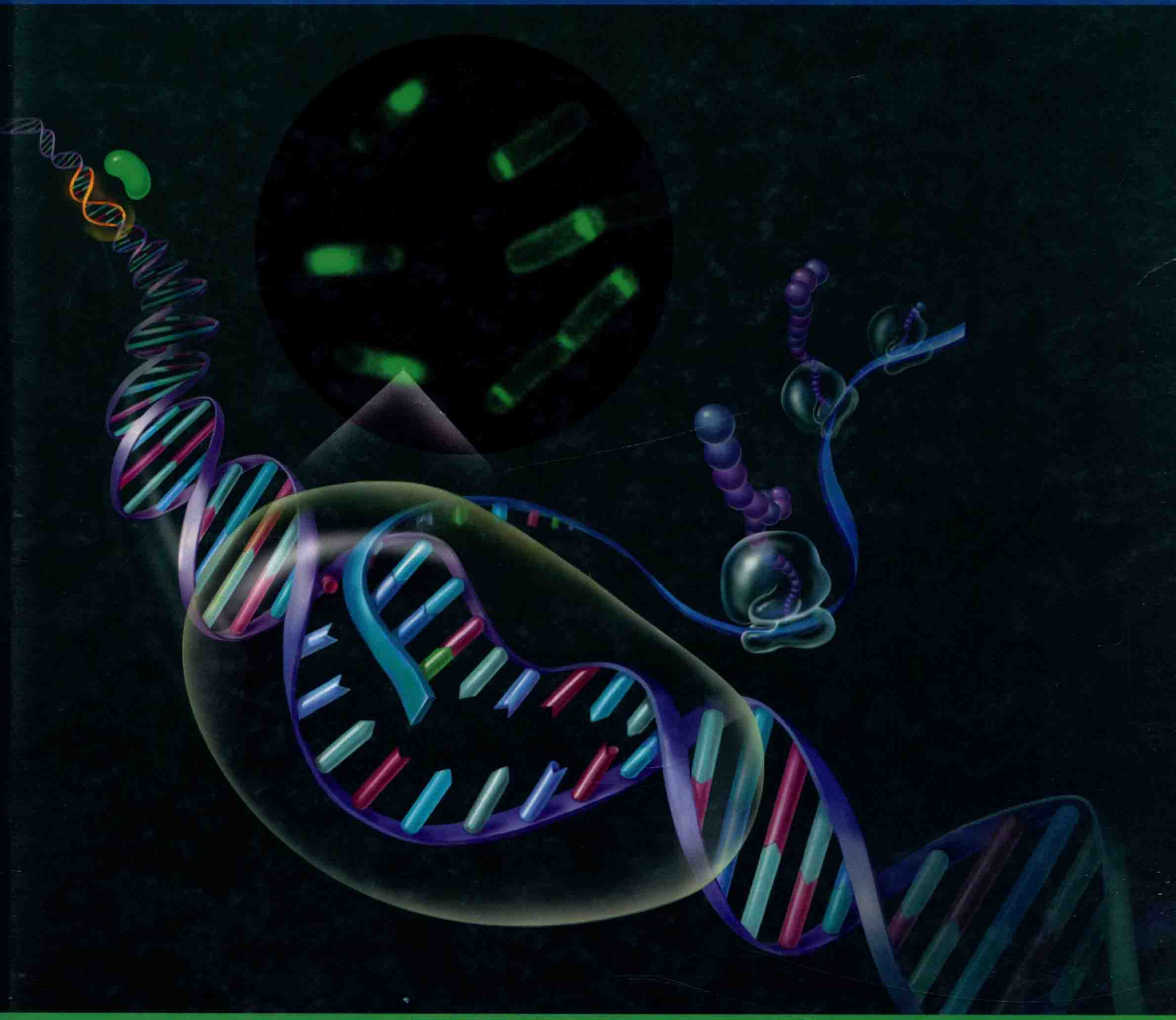
SECOND EDITION

Molecular Genetics *of* Bacteria



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Molecular Genetics *of* Bacteria

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Cover photograph (courtesy of Richard Losick and Masaya Fujita, Department of Molecular and Cellular Biology, Harvard University) illustrates the cellular localization of a Bacillus subtilis *sporulation-specific transcription factor, σ^E, as visualized with protein fusions to the "green fluorescent protein." Pro-σ^E first localizes to the septal and cytoplasmic membranes of sporulating cells at the stage of polar septation as shown in the cells on the right side of the insert. To the left, the mature σ^E is present in the cytoplasm in the large chamber of the sporangium, where it directs mother-cell-specific transcription.*

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Preface

We were motivated to prepare a second edition of our textbook *Molecular Genetics of Bacteria* because of the favorable response to the first edition and because of the important advances made in this field since the original edition was published. The second edition retains much of the organization and style of the first edition. The order of topics is unchanged as is the emphasis on experimental approaches: features popular with most instructors using the first edition. Each chapter retains a chapter summary, problems (with answers), and suggested readings, all of which are updated. We continue to use "boxes" to present material of related interest to each topic without breaking the continuity of the narrative. The material in these boxes can serve as a starting point for special reports or to link with material from other courses that the students might be taking. One change in the organization of the second edition is that much of the material in the last four chapters of the first edition, which presented some more sophisticated experiments and some current applications, has been updated and incorporated into the earlier chapters. As a result, the chapters usually begin with a descriptive treatment of each topic and end with some relatively technical molecular genetic experiments that led to the knowledge. This organization allows instructors to assign only early sections of each chapter if appropriate but retains the usefulness of the textbook for more advanced undergraduate and beginning graduate classes. This allows the book to be more concise since each subject need be introduced only once. We added a final chapter that illustrates comprehensively how the techniques and concepts of bacterial molecular genetics discussed in earlier chapters have been used to study biological phenomena such as protein translocation and sporulation.

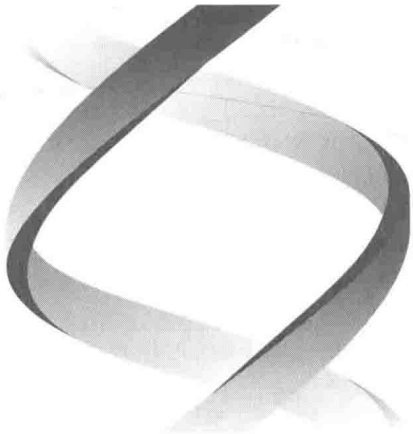
The material in each chapter is substantially updated and reflects the exciting developments in the field of bacterial molecular genetics and emphasizes the relationships of prokaryotic and eukaryotic cell biology and

development. In particular, important progress has been made since the first edition in understanding chromosome segregation and cell division in bacteria; the close relationship of protein secretion and conjugation both between bacteria and from bacteria into eukaryotes; the intimate relationship between the "three Rs"—replication, recombination, and repair; the universality of repair mechanisms and mutagenic DNA polymerases and their roles in cancer; the techniques of genomics, microarrays, and bioinformatics and their applications in bacterial molecular genetics; as well as many other important updates. With these changes, the textbook will continue to provide an appropriate up-to-date treatment of bacterial molecular genetics for undergraduate and beginning graduate courses and will also continue to provide a good foundation and reference guide for scientists in the many fields of biology and engineering who depend on the concepts and techniques developed with prokaryotes.

An extraordinary number of researchers have made major contributions to the field of bacterial molecular genetics. We could not reasonably expect students to learn even a fraction of their names, and we could not possibly do justice to all of their important contributions. Therefore, 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 a structure (e.g., Watson and Crick). We redress our omissions somewhat in the suggested readings, where we give some of the original references to the developments under discussion.

In writing the second edition, we have benefited from the help and advice of a large number of colleagues around the world. Many instructors using the first edition pointed out errors and communicated advice. Many additional colleagues generously read or discussed material in their areas of specialty. We would particularly like to thank our colleague at Michigan State University, Lee Kroos, for his careful reading of the new *Bacillus subtilis* sporulation section. We also benefited from the input of many of our own undergraduates, who used the first edition in our classes. We especially thank Andrea Hartlerode and Heather Hall, who pointed out areas they found confusing or in need of additional explanation. However, we take full responsibility for any mistakes, misconceptions, or omissions.

As before, it was a great pleasure to work with the professionals at ASM Press, who repeated their magic act of transforming our primitive text and illustrations into a printed book. For the first edition, as neophyte authors, we depended on the expert advice of the former director of ASM Press, Patrick Fitzgerald. In preparing the second edition, we have been indebted to the current director, Jeff Holtmeier, for his encouragement, enthusiasm, and patience. We have also had the good fortune to work again with a number of the same professionals who did a masterful job with the first edition, including Susan Birch, Production Manager, who oversaw the entire process; Yvonne Strong, who copyedited the manuscript and illustrations; Susan Brown Schmidler, who created the book and cover design; and Terese Winslow, who created the cover illustration. Finally, we especially thank Berta Steiner, president of Bermedica Production, Ltd., who managed this publication project and supervised the work of Precision Graphics artists who rendered our hand-drawn sketches into clear attractive figures.



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Boxes

Introduction

THE GOAL OF THIS TEXTBOOK is to introduce the student to the field of bacterial molecular genetics. Bacteria are relatively simple organisms, and some are quite easy to manipulate in the laboratory. For these reasons, many methods in molecular biology and recombinant DNA technology have been developed around bacteria, and these organisms often 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 translation and replication, has originated with studies in bacteria. This is because such central cellular functions have remained largely unchanged throughout evolution. Ribosomes have a similar structure in all organisms, and many of the translation factors are highly conserved. The DNA replication apparatus of all organisms contains features in common such as sliding clamps and editing functions, which were first described in bacteria and their phages. Chaperones that help other proteins fold and topoisomerases that change the topology of DNA were first discovered in bacteria and phages. 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 similar in all organisms and have recently been implicated in some types of human cancers.

Also, recent evidence indicates that the cell biology of bacteria might be much more complex and more like that of eukaryotes than previously believed. For a long time it has been possible to observe the seemingly purposeful movement of constituents on the cytoskeleton within eukaryotic cells. However, bacterial cells, being much smaller, were thought to be merely "bags of enzymes" and to rely on mere diffusion to move their cellular constituents around. Now new technologies make it possible to observe movement within bacterial cells, revealing, for example, that some proteins

involved in cell division and partitioning oscillate from one end of the cell to the other during the cell cycle (see chapter 1). Bacteria even have proteins related to the proteins of the cytoskeleton, including a cell division protein called FtsZ, which is similar structurally to the tubulins that make up microtubules, and a protein called MreB, which helps give bacterial cells their structure and forms filaments like actin (see Jones et al., and van den Ent et al., Suggested Reading). It has been speculated that we might be entering another stage in biology similar to the early days of molecular genetics, when studies with bacteria led the way to the discovery of new principles of cell biology that are common to all organisms.

However, bacteria are not just important as laboratory tools to understand higher orgamisms; they are important and interesting in their own right. For instance, they play an essential role in the ecology of the 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 of the Earth because of their ability to degrade recalcitrant natural polymers such as cellulose and lignin. Bacteria and some types of fungi thus prevent the 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 cleanup. Moreover, bacteria produce most of the naturally occurring so-called greenhouse gases, such as methane and carbon dioxide, which are in turn degraded by other types of bacteria. This cycle helps maintain climate equilibrium. Bacteria have even had a profound effect on the geology of the Earth, being responsible for some of the major iron ore and other types of deposits in the Earth's crust.

Another unusual feature of bacteria is their ability to live in extremely inhospitable environments, many of which are devoid of life except for bacteria. These organisms are the only ones living in the Dead Sea, where the water's salt concentration is very high. Some types of bacteria live in hot springs at temperatures close to the boiling point of water, 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 tubular worms next to hydrothermal vents on the ocean floor, where the atmosphere is hydrogen sulfide rather than oxygen. In this symbiosis, the bacteria 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. 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 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. Chemiluminescent bacteria even generate light for squid and other marine animals, allowing individuals to find each other in the darkness of the deep ocean.

Bacteria are also worth studying because of their role in disease. They cause many human, plant, and animal diseases, and new ones are continuously appearing. Knowledge gained from the molecular genetics of bacteria will help in the development of new ways to treat or otherwise control these diseases.

Bacteria and their phages (i.e., viruses that infect bacteria) are also the source of many useful substances such as many of the enzymes used in biotechnology and other industries. Moreover, bacteria make antibiotics and chemicals such as benzene and citric acid.

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 at. 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. Who knows what interesting and useful functions the undiscovered bacteria might have. Clearly, studies with 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. But before discussing this field, we must first briefly discuss the evolutionary relationship of the bacteria to other organisms.

The Biological Universe

The Eubacteria

According to a present view, all organisms on Earth belong to three major divisions: the eubacteria, the archaea (formerly archaebacteria), and the eukaryotes (Figure 1). Most of the familiar bacteria such as *Escherichia coli, Streptococcus pneumoniae,* and *Staphylococcus aureus* are **eubacteria.** These organisms can differ greatly in