# Preface

*If bacteria are really the cause of anthrax, then we must hypothesize that they can go through a change in life history and assume a condition which will be resistant to alternate drying and moisture. What is more likely, and what has already been indicated by Prof. Cohn, is that the bacteria can form spores which possess the ability to reform bacteria after a long or short resting period.*

Robert Koch, in *Beiträge Biol Pflanzen* 7:277–310, 1876

*In an earlier generation, biochemists regarded spores as inert, resistant little bodies which had few or no enzymes. With what he knows of biochemistry today, an informed student, aware of the spore's potential for rapid germination from an inert state and its resistance to adverse external conditions, would postulate a far more elaborate composition for a spore.*

Arthur Kornberg, James A. Spudich, David L. Nelson, and Murray P. Deutscher, in *Annu Rev Biochem* 37:51–78, 1968

*In no organism are questions of cell fate, cell differentiation, and morphogenesis more accessible to experimental investigation than in the spore-forming bacterium* Bacillus subtilis.

Patrick Stragier and Richard Losick in *Annu Rev Genet* 30:297–241, 1996

The first two publications reporting that some bacteria have the ability to form dormant spores appeared in 1876 as back-to-back articles in the plant journal *Beiträge zur Biologie der Pflanzen*. Remarkably, each report was authored by a founding father in the field of microbiology. The first paper, which described sporulation in the bacterium *Bacillus subtilis*, was written by Ferdinand Cohn, who is now remembered as the first bacterial taxonomist. The second paper, contributed by Robert Koch, focused on the etiology of anthrax, revealing the role played by spores of *Bacillus anthracis* in causing the disease. Koch's discoveries on spores (and his following work on tuberculosis), along with studies from his archrival Louis Pasteur, established the basis for the germ theory of disease. Pasteur himself stumbled on spores on many occasions: sometimes as a nuisance, when his famous experiments to disprove spontaneous generation of bacteria were challenged by Felix Pouchet (it was later understood that the hay infusions that Pouchet had used for his experiments were contaminated by spores that were resistant to boiling); sometimes more auspiciously, when Pasteur developed a vaccine against anthrax. The study of spore formation is thus as old as microbiology itself. Major conceptual and technological advances in the field of microbiology have impacted the study of sporeformers ever since. Similarly, many techniques and hypotheses pioneered in spore-forming bacteria continue to influence microbial research at large.

Molecular mechanisms of sporulation were studied in detail during the second half of the 20th century. Progress was significantly aided by the realization, from the work of Spizizen and Anagnostopoulos, that *B. subtilis* cells were naturally competent, thus opening the door for manipulations of the *B. subtilis* genome. A combination of genetics-, biochemistry-, and electron microscopy-based approaches led to a deep understanding of spore structure and a clear description of the different stages of the spore formation process. As an example, a review by Piggot and Coote from 1976 (i.e., a century after Cohn and Koch) listed over 30 genetic *spo* loci, i.e., genes or operons, whose mutation affected the efficiency of sporulation without disturbing the bacterium's ability to grow. Each locus was named using roman numerals referring to the stage of blockage in the sporulation cycle of the corresponding mutants. These stages were originally defined by Ryter, Schaeffer, and collaborators, who used electron microscopy to investigate the morphology of sporulating cells.

Biochemical and cytological analysis of sporulation mutants played a key role in cementing the position of *B. subtilis* as an excellent model system to investigate basic developmental mechanisms. Importantly, spores formed by *B. subtilis* and related bacteria are endospores, in the sense that spore maturation must occur inside a mother cell. The mother cell and the forespore (i.e., the cell that will develop into a spore) both result from an asymmetric division of the sporulating cell near the cell pole. Thus, sporulating cells provide a relatively simple system for the study of cellular differentiation. More specifically, how can the asymmetric division of a progenitor cell be exploited to produce two daughter cells that will experience distinct fates? Many studies during that period focused on understanding the mechanisms of intercellular communication and the cell-specific activation of the sporulation sigma factors that control the parallel lines of gene expression established in the forespore and the mother cell.

In *The Bacterial Spore: From Molecules to Systems* (which is published exactly 140 years after the founding papers by Cohn and Koch), we have attempted to summarize this vast amount of knowledge. One of our goals was to integrate several decades of research about the process of spore formation in the model organism *B. subtilis* with other efforts to characterize medically, evolutionarily, and ecologically relevant species of spore-forming Firmicutes (primarily *Bacillaceae* and clostridia). The publication of the full genome sequence of *B. subtilis* in 1996 represented a milestone, which was later followed by the release of a multitude of complete genome sequences of other spore-forming bacteria. Comparative genomics (chapter 1) led to a broader understanding of evolution in spore-forming species (chapters 2 and 4), while the surge of metagenomics is likely to enrich our view of the ecology of spore-forming species (chapter 3).

Our book is subtitled *From Molecules to Systems* to indicate our desire to provide a link between classic molecular genetics approaches (chapters 8 to 11) and more recent efforts that view the spore as a system (chapters 5 and 6). As an illustration of the systems biology of spores, transcriptomic studies have revealed that several hundred genes are expressed during sporulation. Strikingly, this is an order of magnitude higher than the number of sporulation genes estimated from genetic screens. Furthermore, a bridge between traditional methods and novel technologies is provided by fluorescence microscopy-based methods, which have become very common in recent years (chapter 7).

Among pathogenic sporeformers, *B. anthracis* has always occupied a central position (chapters 12 through 14); however, pathogenic *Clostridium* species (chapters 15 and 16) have also garnered considerable attention including, for instance, *Clostridium difficile*, a bacterium that has recently caused nosocomial infections in alarming numbers. In contrast to being agents of infectious diseases, spore-forming bacteria can also be used for practical applications. For example, in an attempt to generate new vaccine delivery systems, it was shown that spore surfaces can be useful for antigen display (chapter 17). In addition, spores are excellent candidates for use in bioremediation, in particular the removal of metal contaminants (chapter 18).

In spite of this vast trove of information, it goes without saying that an enormous amount remains to be discovered; each chapter in this book defines important open questions for future research. As just one example, one of the most intriguing gaps in our knowledge of spore-forming bacteria is a detailed understanding of their natural ecology. We still do not know the exact roles that the diverse stress responses produced by these bacteria play in their incompletely characterized ecosystems. This is especially true of sporulation, the lifestyle that so powerfully caught the attention of Robert Koch and Ferdinand Cohn many years ago. We hope that the open questions posed in these chapters are as intriguing to our readers as are the detailed answers, and that they will inspire continued study for many more years to come.

We wish to thank all of the extremely skilled people at ASM Press who have been instrumental in bringing this book forward. In particular, we wish to acknowledge our colleagues Ellie Tupper and Eleanor Riemer, whose efforts have been especially important.