Preface

Bacillus subtilis is one of the best understood prokaryotes in terms of molecular biology and cell biology. Its superb genetic amenability and relatively large size have provided powerful tools to investigate a bacterium in all possible aspects. Recent improvements in fluorescence microscopy techniques have provided novel and amazing insight into the dynamic structure of a single-cell organism. Research on *B. subtilis* has been at the forefront of bacterial molecular biology and cytology, and the organism is a model for differentiation, gene/protein regulation and cell cycle events in bacteria. The aim of this book is to present an overview of the most recent exciting new research fields, and to provide a picture of the major cytological aspects of a bacterium, many of which are highly relevant for a wide variety of bacteria.

Bacillus subtilis is a ubiquitous soil bacterium that can be easily isolated from soil, using starch as an energy source and relatively high salt concentration. Ideally, the soil sample is heated up to 100°C for 30 min, allowing only for enduring spores to be cultured from the sample. *B. subtilis* is unique in that it can choose between at least three different genetic programmes when nutrients or other resources become scarce and/or cell density reaches a critical threshold. To survive or adapt to adverse condition, cells can either enter stationary phase, which is characterized by the formation of single motile cells (exponentially growing cells contain a mixture of mostly non-motile chains of cells and a few motile single cells), can differentiate into enduring and metabolically inactive spores, or, thirdly, can become competent and take up DNA from the environment for acquisition of new genetic material. In all three cases, strikingly different genetic programs are turned on that guide the cell through the differentiation processes. In addition to this, *B. subtilis* shows social behaviour, in that the cells communicate with each other, and form multicellular structures in the form of swarming cells and biofilms. Two component systems, cascades of different sigma factors, regulatory RNAs and specific proteolysis of target proteins form an intricate regulatory network, which is beginning to be unravelled, not only in terms of specific modules, but also in terms of whole complex processes that are connected with each other. Specific mono- and di-nucleotides have gained a lot of interest, as they regulate key steps in bacterial growth and physiology. Most strikingly, it has become clear that many proteins have specific subcellular addresses in bacterial cells. These findings have established the field of 'bacterial cell biology', and *B. subtilis* has been a forerunner in this field. Many vital processes are disturbed if proteins lose their specific localization, but the fundamental question of how proteins are targeted and specifically located in a call lacking intracellular compartments is still unclear for most cases. Therefore, it has become important to also study proteins in terms of their localization within the cell, in addition to analysing their biochemistry and regulation. This book is intended to show that we are beginning to understand the way a bacterial cell functions as a whole entity and in 3D, i.e. how it is spatially organized, and even how bacteria talk to each other, or give their life for the sake of the whole community.

In this book, we will take and inside out approach to look at *Bacillus*, starting with duplication of the chromosome, cell cycle and transcriptional regulation, following its MreB cytoskeleton underneath

the cell membrane, through the membrane, to the cell wall. Finally, we will consider the multi-architectural processes of biofilm formation and sporulation that embrace many of cytological and genetical aspects throughout the cell. New added chapters of the third edition cover the important aspects of motility (Chapter 14) and regulation through nucleotides (Chapter 15). As will become apparent to the reader, many chapters overlap in a variety of aspects, which is due to the fact that most processes addressed in the book are interconnected with each other. For example, the specific localization of the replication machinery (Chapter 1) is an important aspect in DNA repair (Chapter 2) and in ordered chromosome segregation (Chapter 3), and also touches aspects of cell division (Chapter 4). Amazingly, the actin-like MreB cytoskeleton (covered in depth in Chapter 8) is essential for viability, for the ordered insertion of cell wall material (Chapter 10). The structure of short and dynamic filaments appears to connect and coordinate cell cycle events and rod-shaped cell growth, showing that the cytoskeleton was actually a functional prokaryotic invention. Many processes thought to occur throughout the cell or all over the membrane (Chapter 9) have been found to be spatially confined to discrete regions, which has shed light onto processes such as cell division, replication, cell growth and sporulation. Even though transcription and translation are coupled in prokaryotes, these processes occur at defined places within the cells (Chapter 5), apparently facilitating ordered chromosome segregation and efficient synthesis of highly expressed proteins and of stable RNA. Regulation of transcription through RNA molecules (Chapter 6) and regulation of protein activity through proteolysis (Chapter 7) have only recently been recognized as major factors affecting bacterial physiology, and are intertwined with the organization of transcription (Chapter 5), sporulation (Chapter 11) and cell division (Chapter 4). Like many bacteria. *B. subtilis* cells form biofilms, which contain several distinct subpopulations sharing different kinds of labour, and which act rather as a multicellular organism. This new concept in bacteriology is described in Chapter 12. In the second edition, several important recent findings in the rapidly moving fields of research have been included, and the book has had the addition of Chapter 13 dealing with the ability of *B. subtilis* cells to take up DNA from the environment and incorporate it into the chromosome, when sufficient homology exists. This developmental state is called 'competence' and is described in detail. Importantly, the new chapter also explains the molecular basis and mathematics of the phenomenon called bistability, in which two interchangeable *B. subtilis* subpopulations exist in parallel that have distinct physiological states and genetic programmes. This ability allows bacterial populations to do 'bet hedging' and to be able to respond to environmental conditions that may (or may not) change in the near future. The third edition has now captured important new developments from the recent 4 years, and has won two important aspects in the life of a bacterium, motility (Chapter 14) and the regulation of cellular signalling pathways via small nucleotides, of which the important functions played by cyclic di-AMP and cyclic di-GMP have only recently been recognized, and are only slowly being understood at a molecular level. Small nucleotides also affect the lifestyle decision of bacteria whether to become motile or stay sessile, and also motility is under bistable control in *B. subtilis*, tying together several chapters of this new edition.

Clearly, different fields in bacterial cell biology and molecular biology are growing together, providing a more and more integral view of the bacterial cell.

My thank goes out to Gert Bange, who has helped me in editing several chapters of this third edition and to all authors, who have done a great job updating the chapters.

Useful links

Several useful sites on the internet exist that provide tools to study *B. subtilis* in more depth. Foremost, the *Bacillus* sequencing consortium has set up a site in which the whole genome of *B. subtilis* is accessible in a superb way. Subtiwiki is a new site in which all genome data and gene expression analyses can be obtained. Also, genotypes of many strains with gene deletions are accessible via two websites. Finally, most authors of this book have websites for the interested reader to find further information on the various research areas covered in this book.

B. subtilis gene expression database
http://subtiwiki.uni-goettingen.de/wiki/index.php/Main_Page

B. subtilis genome
genolist.pasteur.fr/SubtiList/

B. subtilis stock centre
www.bgsc.org/

List of essential genes
www.pnas.org/cgi/content/full/100/8/4678

H. Murray, replication
http://www.ncl.ac.uk/camb/staff/profile/heathmurray.html#background

J.C. Alonso, DNA repair
http://www.cnb.csic.es/index.php/en/research/research-departments/microbial-biotechnology/genetic-stability

P.L. Graumann, DNA segregation
http://synmikro.com/de/forschung/zellulaere-organisation/peter-graumann.html

F. Gueiros Filho, cell division
http://www2.iq.usp.br/docente/?id=fgueiros

W. Winkler, regulatory RNAs
http://www.wadewinkler.com

P. Lewis, organization of transcription
https://www.newcastle.edu.au/profile/peter-lewis

K. Turgay, proteolysis
https://www.ifmb.uni-hannover.de/turgay.html

R. Carballido-Lopez, cytoskeleton
https://www.micalis.fr/Poles-et-Equipes/Pole-Biosys/ProCeD-Carballido-Lopez

J.M. van Dijl, membrane proteins
www.rug.nl/umcg/faculteit/disciplinegroepen/medischemicrobiologie/index

D.-J. Scheffers, cell wall
http://www.rug.nl/staff/d.j.scheffers/

P. Eichenberger, sporulation
http://biology.as.nyu.edu/object/PatrickEichenberger

E. Gonzales Pastor, biofilm formation https://cab.inta-csic.es/en/investigadores/343/jose-eduardo-gonzalez-pastor