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 cell 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 intercon-
nected 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 divi-
sion (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 segre-
gation and efficient synthesis of highly expressed
proteins and of stable RNA. Regulation of tran-
scription 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 impor-
tant 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 chro-
mosome, 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 develop-
ments from the recent 4 years, and has won two
important aspects in the life of a bacterium, motil-
ity (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 recog-
nized, 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 biol-
ology 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. subti-
lis 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

P.L. Graumann, DNA segregation

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