MICROBIAL PHYSIOLOGY

Fourth Edition

Albert G. Moat
John W. Foster
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WILEY-LISS
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Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data:

Moat, Albert G.
Microbial physiology / Albert G. Moat, John W. Foster, Michael P. Spector.–4th ed.
p.cm.
Includes bibliographical references and index.
ISBN 0-471-39483-1 (paper: alk. paper)
1. Microorganisms — Physiology. I. Foster, John Watkins. II. Spector, Michael P.
III. Title.

QR84.M64 2002
571.29–dc21 2002071331

Printed in the United States of America.

10 9 8 7 6 5 4 3 2 1

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PREFACE

The field of microbial physiology has expanded at an incredibly rapid pace since the last edition of this text. The development and implementation of new, highly sophisticated, techniques to study the molecular genetics and physiology of an ever broadening range of microbes has prompted us to write a fourth edition to this book. To give full measure to the extraordinary advances made in microbial physiology we have found it necessary to reorder, separate, and add new material. However, in doing so we have attempted to remain true to the goal of the first edition of "Moat's Notes" and each subsequent edition by targeting discussions to undergraduate and beginning graduate students while providing sufficient detail useful to established microbial physiologists. This new edition continues the tradition of addressing the physiology of a variety of microbes and not just *Escherichia coli*. We have updated chapters on bacterial structures, intermediary metabolism, genetics and growth; and added chapters discussing the genomic and proteomic methodologies employed by the new breed of microbial physiologist. We have reorganized, updated and expanded chapters on microbial stress responses and bacterial differentiation and have added a chapter on host-parasite interactions that correlates microbial physiology with microbial pathogenesis. We hope that the reader, be they an advanced undergraduate entering the field or a professor who has been in the field for forty years, will come to better appreciate the elegant simplicities and the intricate complexities of microbial physiology, while at the same time realizing that there is still much to be learned.

The authors would like to thank the many students, colleagues, and family who provided help and encouragement as we compiled this new edition. We are particularly

thankful to those who granted us permission to use figures or illustrations and/or provided us with original materials for this purpose.

ALBERT G. MOAT
JOHN W. FOSTER
MICHAEL P. SPECTOR

Huntington, West Virginia
Mobile, Alabama

CHAPTER 1

INTRODUCTION TO MICROBIAL PHYSIOLOGY

THE *ESCHERICHIA COLI* PARADIGM

Microbial physiology is an enormous discipline encompassing the study of thousands of different microorganisms. It is, of course, foolhardy to try to convey all that is known on this topic within the confines of one book. However, a solid foundation can be built using a limited number of organisms to illustrate key concepts of the field. This text helps set the foundation for further inquiry into microbial physiology and genetics. The gram-negative organism *Escherichia coli* is used as the paradigm. Other organisms that provide significant counterexamples to the paradigm or alternative strategies to accomplish a similar biochemical goal are also included. In this chapter we paint a broad portrait of the microbial cell with special focus on *E. coli.* Our objective here is to offer a point of confluence where the student can return periodically to view how one aspect of physiology might relate to another. Detailed treatment of each topic is provided in later chapters.

CELL STRUCTURE

As any beginning student of microbiology knows, bacteria come in three basic models: spherical (coccus), rod (bacillus), and spiral (spirillum). They do not possess a membrane-bound nucleus as do eukaryotic microorganisms; therefore, they are prokaryotic. In addition to these basic types of bacteria, there are other more specialized forms described as budding, sheathed, and mycelial. Figure 1-1 presents a schematic representation of a typical (meaning *E. coli*) bacterial cell.

The Cell Surface

The interface between the microbial cell and its external environment is the cell surface. It protects the cell interior from external hazards and maintains the integrity of the cell

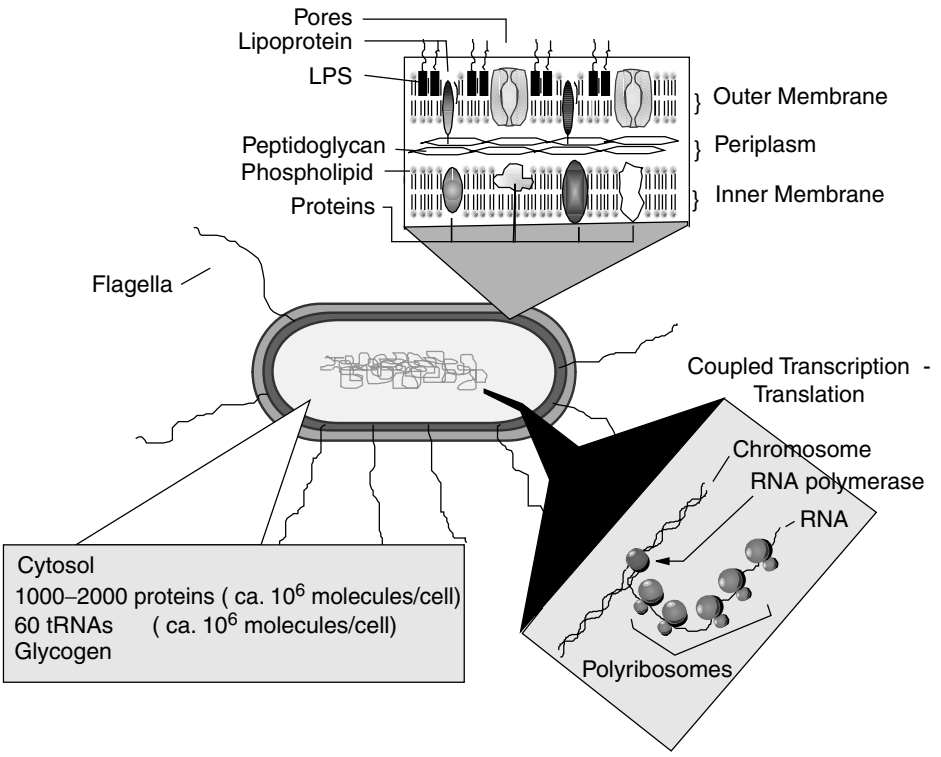


Fig. 1-1. Diagrammatic representation of a "typical" bacterial cell (*Escherichia coli*). Portions of the cell are enlarged to show further details.

as a discrete entity. Although it must be steadfast in fulfilling these functions, it must also enable transport of large molecules into and out of the cell. These large molecules include carbohydrates (e.g., glucose), vitamins (e.g., vitamin B_{12}), amino acids, and nucleosides, as well as proteins exported to the exterior of the cell. The structure and composition of different cell surfaces can vary considerably depending on the organism.

Cell Wall. In 1884, the Danish investigator Christian Gram devised a differential stain based on the ability of certain bacterial cells to retain the dye crystal violet after decoloration with 95% ethanol. Cells that retained the stain were called gram positive. Subsequent studies have shown that this fortuitous discovery distinguished two fundamentally different types of bacterial cells. The surface of gram-negative cells is much more complex than that of gram-positive cells. As shown in the schematic drawings in Figure 1-2, the gram-positive cell surface has two major structures: the cell wall and the cell membrane. The cell wall of gram-positive cells is composed of multiple layers of peptidoglycan, which is a linear polymer of alternating units of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). A short peptide chain is attached to muramic acid. A common feature in bacterial cell walls is cross-bridging between the peptide chains. In a gram-positive organism such as *Staphylococcus aureus*, the cross-bridging between adjacent peptides may be close to 100%. By contrast, the frequency of cross-bridging in *Escherichia coli* (a gram-negative organism) may be as low as 30% (Fig. 1-3). Other components — for example, lipoteichoic acid (only

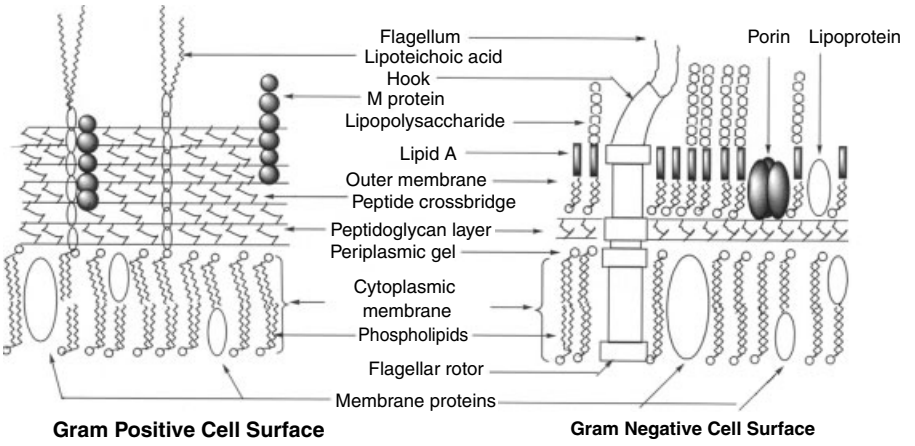


Fig. 1-2. Composition of the cell surfaces of gram-positive and gram-negative bacteria. Not all structures shown are found in all organisms. For example, M protein is only used to describe a structure in some of the streptococci. Also, not all organisms have flagella.

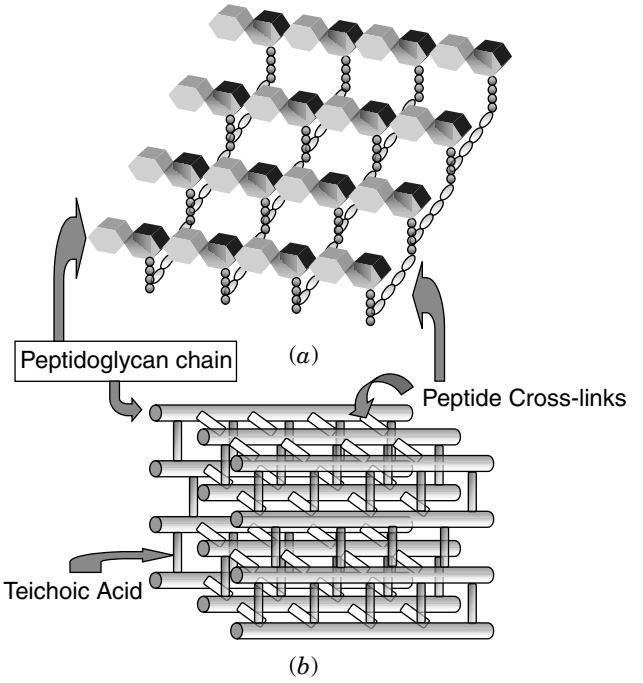


Fig. 1-3. Diagrammatic views of bacterial peptidoglycan. (*a*) Monolayer of peptidoglycan. Lightly shaded hexagons represent N-acetylglucosamine; darkly shaded hexagons represent N-acetylmuramic acid; vertically arranged spheres represent the peptide side chains; horizontal ovals represent the amino acid cross-bridges between peptide chains. (*b*) Diagrammatic representation of the multilayered peptidoglycan in the gram-positive cell wall. Long horizontal bars denote the chains of N-acetylglucosamine and N-acetylmuramic acid. Short horizontal bars indicate peptide cross-bridges and vertical bars represent teichoic acid.

present in gram-positive organisms) — are synthesized at the membrane surface and may extend through the peptidoglycan layer to the outer surface.

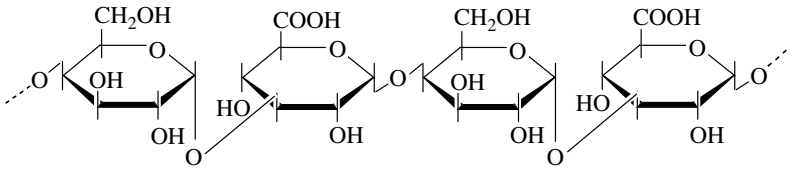
The peptidoglycan layer of a gram-negative cell is generally a single monolayer. An outer membrane surrounding the gram-negative cell is composed of phospholipids, lipopolysaccharides, enzymes, and other proteins, including lipoproteins. The space between this outer membrane and the inner membrane is referred to as the **periplasmic space**. It may be traversed at several points by various enzymes and other proteins (Fig. 1-2).

Membranes. The cytoplasmic membrane of both gram-positive and gram-negative cells is a lipid bilayer composed of phospholipids, glycolipids, and a variety of proteins. The proteins in the cytoplasmic membrane may extend through its entire thickness. Some of these proteins provide structural support to the membrane while others function in the transport of sugars, amino acids, and other metabolites.

The outer membrane of gram-negative cells contains a relatively high content of **lipopolysaccharides**. These lipid-containing components represent one of the most important identifying features of gram-negative cells: the **O antigens,** which are formed by the external polysaccharide chains of the lipopolysaccharide. This lipid-containing component also displays **endotoxin** activity — that is, it is responsible for the shock observed in severe infections caused by gram-negative organisms. Bacterial cell surfaces also contain specific carbohydrate or protein receptor sites for the attachment of **bacteriophages**, which are viruses that infect bacteria. Once attached to these receptor sites, the bacteriophage can initiate invasion of the cell.

Gram-positive and gram-negative cells have somewhat different strategies for transporting materials across the membrane and into the cell. The cytoplasmic membrane of gram-positive organisms has immediate access to media components. However, chemicals and nutrients must first traverse the outer membrane of gram-negative organisms before encountering the cytoplasmic membrane. Gram-negative cells have **pores** formed by protein triplets in their outer membrane that will permit passage of fairly large molecules into the periplasmic space. Subsequent transport across the inner or cytoplasmic membrane is similar in both gram-positive and gram-negative cells.

Capsules. Some bacterial cells produce a capsule or a **slime layer** (Fig. 1-4) of material external to the cell. Capsules are composed of either polysaccharides (high-molecular-weight polymers of carbohydrates) or polymers of amino acids called polypeptides (often formed from the D- rather than the L-isomer of an amino acid). The capsule of *Streptococcus pneumoniae* type III is composed of glucose and glucuronic acid in alternating β-1, 3- and β-1, 4- linkages:



This capsular polysaccharide, sometimes referred to as pneumococcal polysaccharide, is responsible for the virulence of the pneumococcus. *Bacillus anthracis*, the anthrax

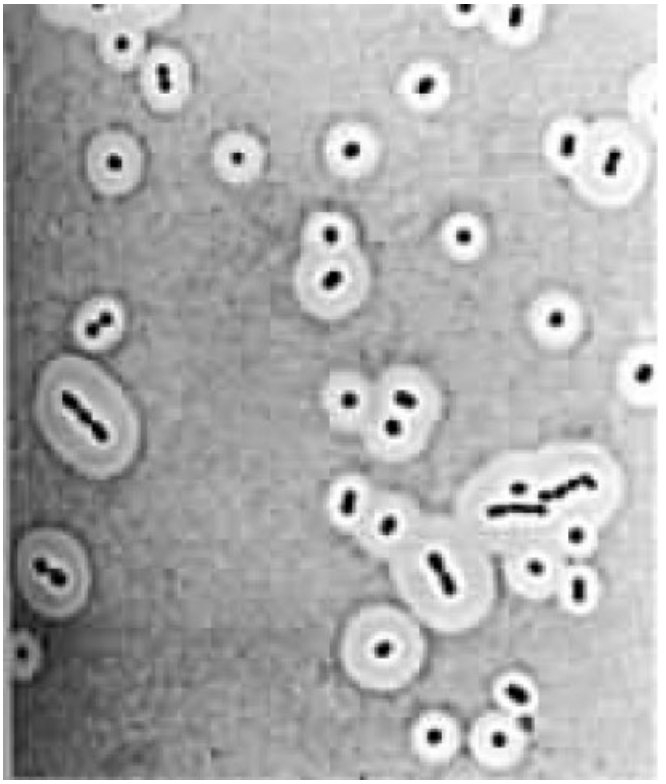


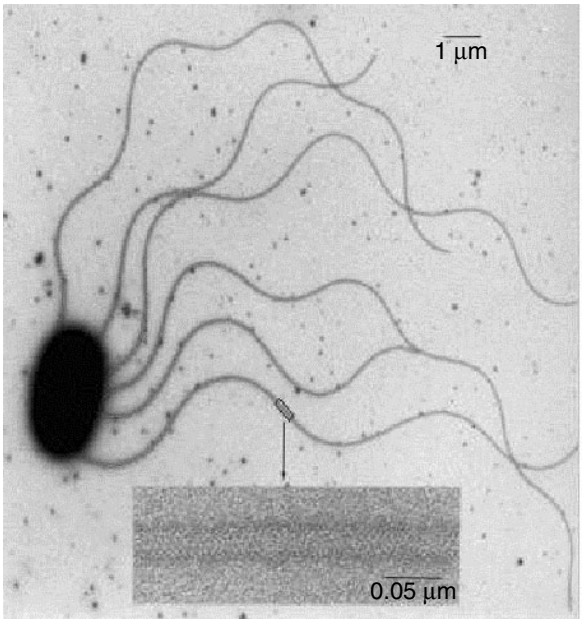
Fig. 1-4. Capsules of *Streptococcus pneumoniae*.

bacillus, produces a polypeptide capsule composed of D-glutamic acid subunits, which is a virulence factor for this organism.

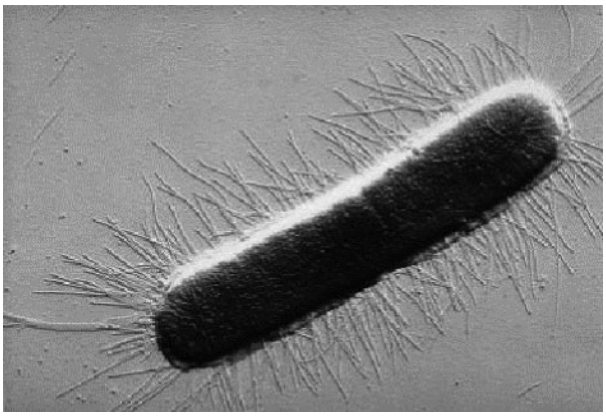
Organs of Locomotion. Many microorganisms are motile — that is, able to move from place to place in a concerted manner — especially in an aqueous environment. In the case of bacteria, this motility is accomplished by means of simple strands of protein (flagellin) woven into helical organelles called flagella. The bacterial flagellum is attached at the cell surface by means of a basal body (Fig. 1-5a). The basal body contains a motor that turns the flagellum, which propels the organism through the liquid environment.

Pili or Fimbriae. Many bacteria possess external structures that are shorter and more rigid than flagella. These structures have been termed pili (from Latin meaning "hair") or fimbriae (from Latin meaning "fringe"). These appendages also appear to arise from a basal body or granule located either within the cytoplasmic membrane or in the cytoplasm immediately beneath the membrane (Fig. 1-5b). Generalized or common pili play a role in cellular adhesion to surfaces or to host cells.

Ribosomes. The cytoplasm of all cells has a fine granular appearance observed in many electron micrographs. Tiny particles called ribosomes are responsible for this look. Ribosomes contain approximately 65% RNA and 35% protein (see Fig. 1-1).



(*a*)



(*b*)

Fig. 1-5. Microbial appendages. (*a*) Flagella of *Salmonella typhimurium.* (*b*) Pili of *Escherichia coli.* (*Source*: Pili Image courtesy Indigo Instruments. Visit http://www.indigo.com.) Reprint permission is granted with this footer included.

The ribosome orchestrates the polymerization of amino acids into proteins (i.e., protein synthesis). At higher magnification under the electron microscope the ribosome particles are spherical. In properly prepared specimens the ribosomes are observed as collections or chains held together on a single messenger RNA (mRNA) molecule and are referred to as **polyribosomes** or simply polysomes.

The more or less spherical ribosome particle, when examined by sucrose gradient sedimentation, has been found to have a svedberg coefficient of 70S. (A svedberg unit denotes the rate of sedimentation of a macromolecule in a centrifugal field and

is related to the molecular size of that macromolecule.) The prokaryotic ribosome may be separated into two lower-molecular-weight components: one of 50S and another of 30S. Only the complete 70S particle functions in polypeptide synthesis. By comparison, the ribosomes of eukaryotic cells are associated with the endoplasmic reticulum, are larger (80S), and are composed of 40S and 60S subunits. The function of both 70S and 80S ribosomes in protein synthesis is identical. Curiously, eukaryotic mitochondria characteristically display 70S ribosomes — not the 80s particles that you would expect — because mitochondria probably evolved from endosymbiotic prokaryotic cells, a hypothesis supported by extensive analyses comparing bacterial and mitochondrial genomes.

SYNTHESIS OF DNA, RNA, AND PROTEIN

The chromosome of *E. coli* is a single, circular, double-stranded DNA molecule whose nucleotide sequence encodes all the information required for cell growth and structure. The major molecular events required for propagating the species start with the chromosome and include DNA replication, transcription, and translation. In bacteria, replication involves the accurate duplication of chromosomal DNA and the formation of two daughter cells by **binary fission**. In binary fission the cell grows until a certain mass-to-DNA ratio is achieved, at which point new DNA is synthesized and a centrally located cross-wall is constructed that will ultimately separate the two daughter cells.

A simplified view of **DNA replication** in *E. coli* is shown in the diagram in Figure 1-6. The double-stranded DNA molecule unwinds from a specific starting point (**origin**). The new DNA is synthesized opposite each strand. The enzyme involved in

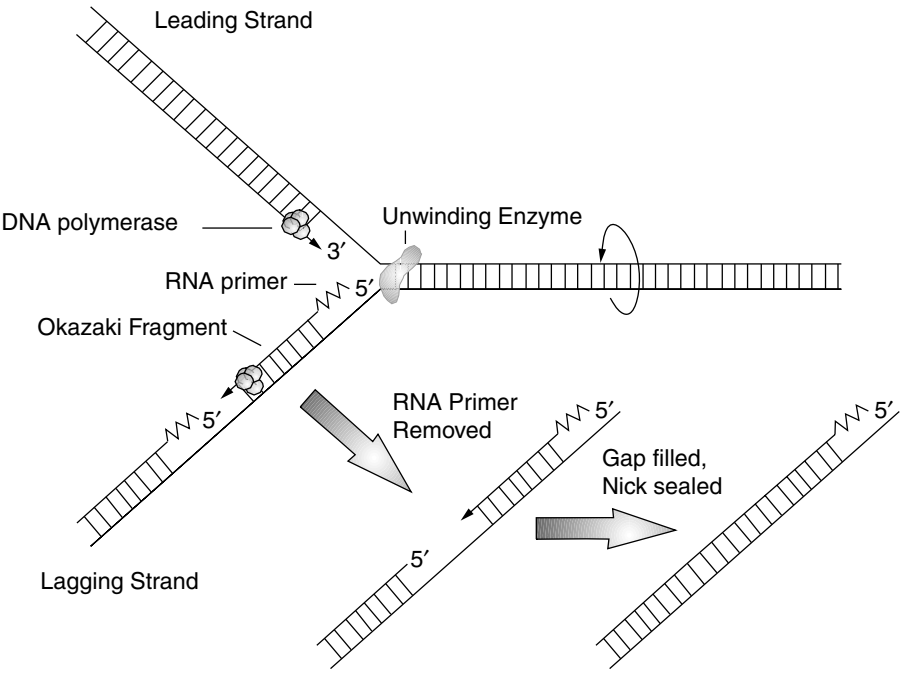


Fig. 1-6. Simplified depiction of DNA replication.

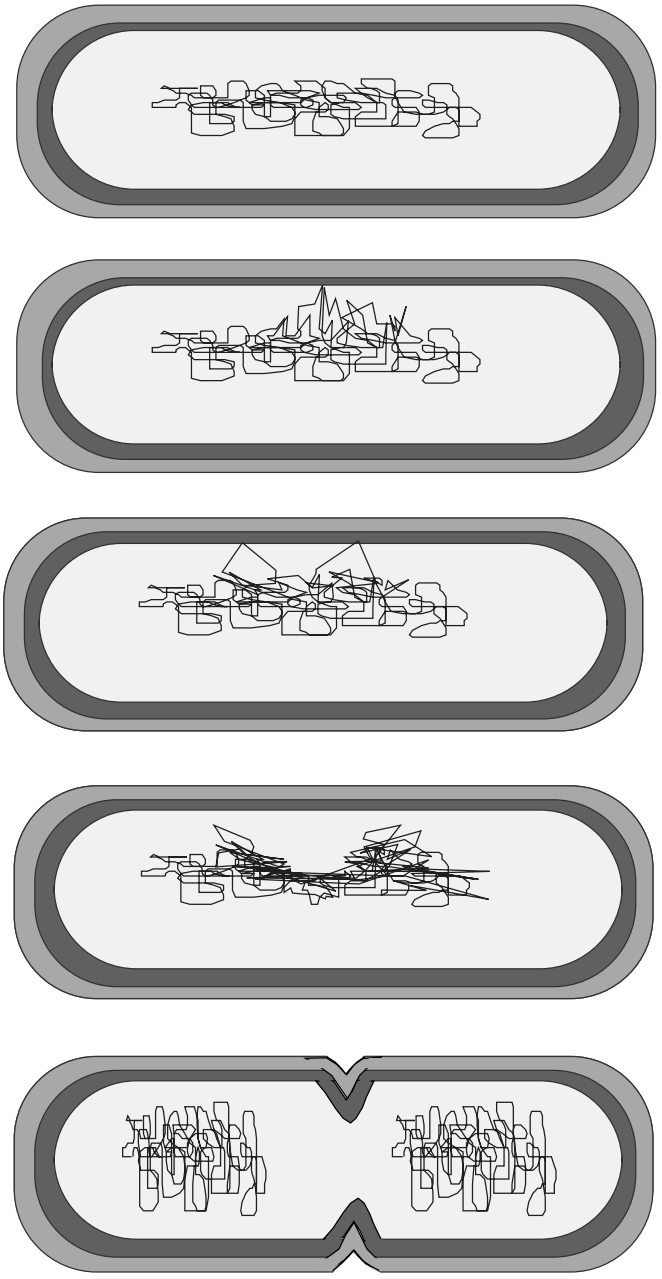


Fig. 1-7. Segregation of the bacterial chromosome.

replication (**DNA polymerase**) uses a parent strand as a template, placing adenine residues opposite thymine, and cytosine residues opposite guanine. New DNA is synthesized in both directions from the origin and continues until both replication forks meet at the **terminus** 180° from the origin. At this point, cell division proceeds with cross-wall formation occurring between the two newly synthesized chromosomes