MICROBIAL PHYSIOLOGY

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SECOND EDITION

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Preface

During the eight- to ten-year span between the writing of the first edition of this text and the present, tremendous strides have been made in the field of microbial physiology. As a result, all of the original chapters have undergone a thorough rewriting and additional chapters have been added to accommodate the many advances in the field. In an effort to provide a more readable text, reference citations have been omitted. However, a variety of recent reviews and journal articles are provided at the end of each chapter.

Microbial genetics has developed so rapidly that many individuals in the field no longer consider it a part of microbial physiology, but a field unto itself. However, advances in our knowledge of the various facets of cell structure and intermediary metabolism have been furthered so tremendously by the application of the techniques and principles of genetics that it is impossible to undertake a discussion of microbial physiology without providing considerable background in genetics.

The authors would like to thank all of those who have provided them with help and encouragement in writing this text. We are especially grateful to the many authors who provided original materials and to Dr. Nyles Charon for reading a portion of the manuscript during the preparative stages.

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Scope of Microbial Physiology

Physiology is the study of the structure and functions of living organisms and their components. At the cellular level, a broad definition will encompass cell structure, metabolism, genetics, and growth. To provide the reader with a broad vista of the scope of this field, the first chapter is devoted to an overview of the major aspects of microbial physiology.

CELL STRUCTURE

Nucleus

Microorganisms range from extremely small, unicellular forms such as bacteria to larger forms such as protozoa, fungi, and algae. Lower forms may be distingished from higher forms on the basis of the degree of intracellular organization. Bacteria are termed procaryotic because of their primitive nucleus. The procaryotic nucleus, sometimes referred to as a **nucleoid** to indicate its primitive form, is not bounded by a nuclear membrane. Higher forms are referred to as eucaryotic because their nucleus is clearly separated from the cytoplasm by a distinct membrane (Fig. 1-1). Eucaryotic organisms contain proteins called histones, ribonucleic acid (RNA), and nonhistone proteins in association with chromosomal DNA. Until relatively recently, it was thought that the procaryotic nucleoid was composed entirely of covalently closed (circular) DNA with no protein in association with it. More sophisticated techniques of separation of the nuclear material from the cell has made it possible to show that the procaryotic nucleoid contains histone-like proteins in association with the DNA.

A major distinction between procaryotic and eucaryotic cells is their mode of chromosome duplication (replication) and cell division. In eucaryotic cells, chromosome duplication is accomplished during an early stage of a

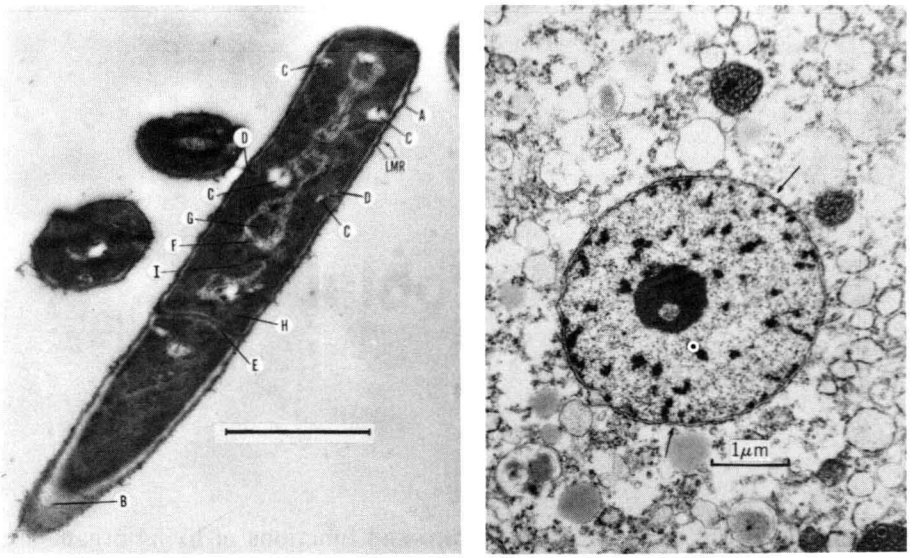

Fig. 1-1. Comparison of cellular structure of procaryotic and eucaryotic cells. (1) Electron micrograph of a longitudinal section of *Bacillus cereus*. (A) Cell wall, (B) oblique section of cell wall showing dense particles and the dense inner layer, (C) four peripheral bodies cut at different levels, (D) centripetally growing transverse cell wall, (E) completed transverse cell wall before thickening, (F) low-density fibrous component of nuclear apparatus, (G) dense body in nuclear apparatus that may be inclusion of cytoplasmic material, (H) small dense particles that appear to be main constituent of cytoplasm, (I) unidentified cytoplasmic inclusion. LMR, limit of resolution of light microscope. Bar equals 1.0 μm. From Chapman, G. B. and J. Hillier. 1953. *J. Bacteriol.* **66**:362. (2) Interphase nucleus of the myxomycete *Arcyria cinerea*. The typical nuclear envelope is interrupted by prominent pores at the arrows. The nucleolus near the center is well defined, while the chromatin is randomly dispersed throughout the nucleus. From Mims, C. W. 1972. *J. Gen. Microbiol.* **71**:53.

process known as mitosis. As mitosis continues, the duplicated chromosomes are separated and guided into new cells to provide an exact copy of the information present in the original DNA (Fig. 1-2).

Division of procaryotic cells takes place in a somewhat different manner. Many details regarding the molecular mechanism involved have not been revealed. However, it is known that the bacterial chromosome is attached to the cell surface at a site referred to as a replication site (Fig. 1-3). As the DNA of the chromosome is duplicated, the cell wall and cell membrane are extended in both directions so that the duplicated chromosomes become separated. Eventually, the duplicated chromosomes are separated from one another by the formation of a cross-wall or septum to produce two new cells. In this manner, each new procaryotic cell receives one copy of the information in the original DNA of the chromosome. Although the mechanism

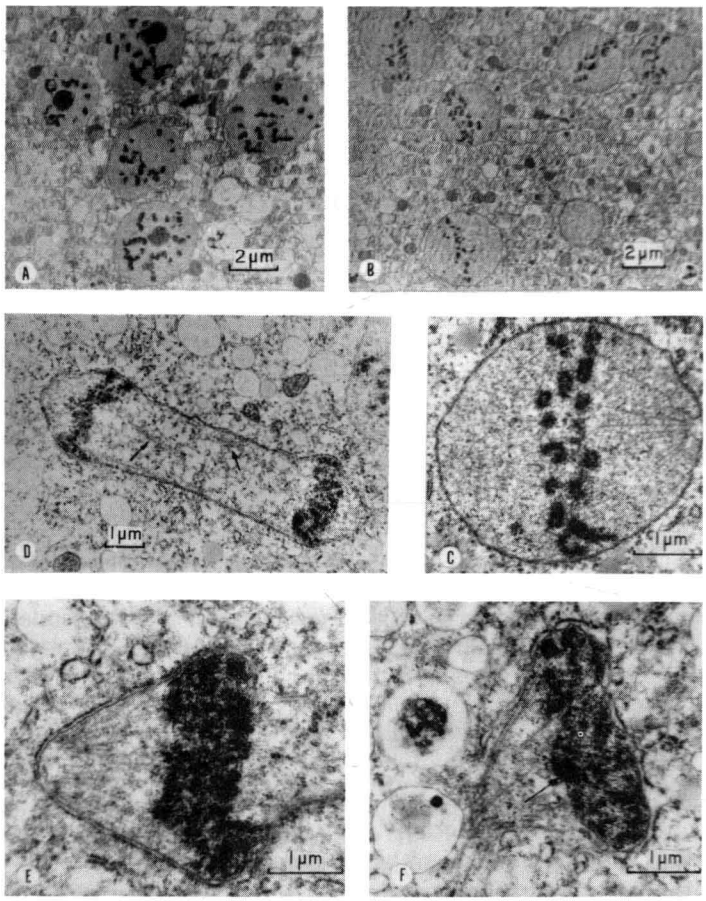

Fig. 1-2. Stages of mitosis in the myxomycete *Arcyria cinerea*. (A) **Prophase nuclei.** Chromatin has begun to condense into chromosomes. Nucleoli are still present. In the nucleus in the upper center, a portion of the spindle apparatus is just barely visible. (B) **Metaphase nuclei.** The nucleoli are no longer present. Division appears to be nearly synchronous. Nuclei are still spherical. (C) **HIgher magnification of a portion of a metaphase nucleus** reveals the presence of spindle fibers that appear to terminate near the inner surface of the nuclear envelope. (D) **Telophase nucleus.** Although the nucleus is greatly elongated, the nuclear envelope persists. Interzonal spindle fibers are visible at the arrows. (E) **Portion of a telophase nucleus shortly after rupture of the nuclear envelope in the interzonal region.** Continuity of the nuclear envelope is still apparent in the polar region. Chromosomal spindle fibers are still visible. (F) **Young daughter nucleus** in which the nucleolus is just visible (arrow). From Mims, C. W. 1972. *J. Gen. Microbiol.* **71**:53.

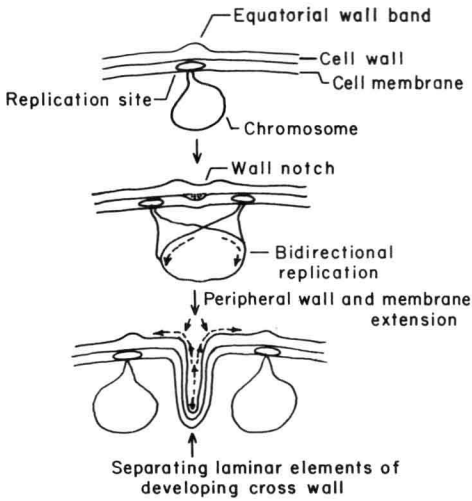

Fig. 1-3. Schematic diagram of the growth stages of a gram-positive bacterial cell. The bacterial chromosome is attached to a replication site embedded in the cell membrane. As the cell wall and cell membrane are extended, a notch develops in the wall. Extension of the surface layers of the cell results in separation of the replication sites as bidirectional replication of the chromosome continues. When chromosomal replication is complete, peripheral extension of the wall and membrane layers continues, and the laminar elements of the developing cross-wall are separated.

of duplication differs considerably, both procaryotic and eucaryotic cells have a rather precise mechanism of transmitting the hereditary information coded in their DNA from one generation to the next.

Cell Surfaces of Microorganisms

Cell Walls. Most microorganisms, whether they are procaryotic or eucaryotic, have a rigid outer structure referred to as a cell wall. In some organisms the outer surface structure of the cell may be rather complex. The cellular contents are bounded by a membrane which, in electron micrographs, appears as a double track. The cytoplasmic membrane is surrounded by the cell wall. In bacteria or yeast, the protective outer wall of the cell can be weakened by treatment of a culture of cells with an antibiotic that specifically inhibits synthesis of a cell wall component or with an enzyme that cleaves chemical bonds holding these components together. As the cell wall is weakened, the cytoplasmic contents round up in a ball-like configuration known as a protoplast. Because protoplasts are more vulnerable to osmotic tension, removal of the wall material must be performed in a medium of sufficient osmolarity to prevent the cell membrane from breaking because of the internal turgor pressure (Fig. 1-4).

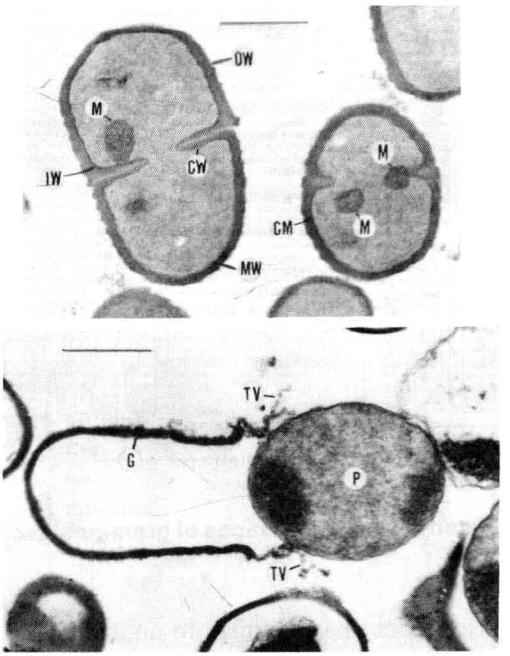

Fig. 1-4. Comparison of cells of *Lactobacillus casei* in the normal state and after treatment with EDTA followed by treatment with lysozyme in the presence of sucrose and 10 mM $MgCl_2$. Normal cells (upper photograph) show a thick outer wall (OW) with a middle cell wall (MW) extending into the area of cross-wall formation (CW). The darkly staining inner wall (IW) is seen in the peripheral region but not in the septum. The cell membrane (CM) adheres to the wall and cross wall but extends into and surrounds the mesosome (M). The protoplast (P) in the lower photograph is still attached to the cell wall ghost (G) by tubular-vesicular elements (TV). Bar equals 500 nm. From Thorne, K. J. I. and D. C. Barker. 1972. *J. Gen. Microbiol.* **70**:87.

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 decolorization with 95% ethanol. Those cells that retain the stain are called **gram-positive**. Those that are decolorized by 95% ethanol and are visible only when stained with a counterstain of contrasting color are referred to as **gram-negative**. Subsequent studies have shown that this fortuitous discovery has considerable significance in that it distinguishes 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 Fig. 1-5, 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, a linear polymer of alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid. A peptide chain is attached to muramic acid. The composition of the poly-