RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY (2007) 10:157-168
DOI: 10.2436/20.1501.01.23 ISSN: 1139-6709 **www.im.microbios.org**

INTERNATIONAL MICROBIOLOGY

The hidden side of the prokaryotic cell: rediscovering the microbial world

Ricardo Guerrero,[1]* Mercedes Berlanga[2]

[1]Department of Microbiology, Faculty of Biology, University of Barcelona, Barcelona, Spain
[2]Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

Received 15 June 2007 · Accepted 25 July 2007

Summary. How many different forms of life exist and how they are evolutionarily related is one of the most challenging problems in biology. In 1962, Roger Y. Stanier and Cornelis B. van Niel proposed "the concept of a bacterium" and thus allowed (micro)biologists to divide living organisms into two primary groups: prokaryotes and eukaryotes. Initially, prokaryotes were believed to be devoid of any internal organization or other characteristics typical of eukaryotes, due to their minute size and deceptively simple appearance. However, the last few decades have demonstrated that the structure and function of the prokaryotic cell are much more intricate than initially thought. We will discuss here two characteristics of prokaryotic cells that were not known to Stanier and van Niel but which now allow us to understand the basis of many characteristics that are fully developed in eukaryotic cells: First, it has recently become clear that bacteria contain all of the cytoskeletal elements present in eukaryotic cells, demonstrating that the cytoskeleton was not a eukaryotic invention; on the contrary, it evolved early in evolution. Essential processes of the prokaryotic cell, such as the maintenance of cell shape, DNA segregation, and cell division, rely on the cytoskeleton. Second, the accumulation of intracellular storage polymers, such as polyhydroxyalkanoates (a property studied in detail by Stanier and colleagues), provides a clear evolutionary advantage to bacteria. These compounds act as a "time-binding" mechanism, one of several prokaryotic strategies to increases survival in the Earth's ever-changing environments. [**Int Microbiol** 2007; 10(3):157-168]

Key words: *The Microbial World* · Roger Y. Stanier · prokaryotic internal membranes · cytoskeleton · polyhydroxyalkanoates

Introduction

The year 2007 marks the 50th anniversary of the publication of *The Microbial World* [46], a textbook that revolutionized the teaching of microbiology in many countries. Recently, another textbook, *Microbe* [42], has been published that is intimately connected with *The Microbial World*, not only with respect to its focus—an in-depth study of the structure, physiology, and genetics of prokaryotes—but also from the point of view of its authors, Moselio Schaechter, John L. Ingraham, and Frederick C. Neidhardt. Both the American Society for Microbiology, at its 107th General Meeting held in Toronto, May 21-25, 2007, and the Spanish Society for Microbiology, during its 21st National Congress, held in Seville on September17-20, 2007, organized special sessions devoted to these two books.

During the fifty years that have elapsed since the first edition of *The Microbial World*, there has been a second Golden Age of microbiology [27]. The first Golden Age was initiated by the discovery and, subsequently, the ability to eradicate those microorganisms responsible for the clinically most important infectious diseases. In the second Golden Age, microbiologists acquired a detailed understanding of the metabolism, structure, and genetics of microorganisms, with major contributions made by the authors of *The Microbial World* (Roger Y. Stanier, Michael Doudoroff, and Edward A. Adelberg), and of *Microbe* (Schaechter, Ingraham and Neidhardt, a generation later). The third Golden Age, which began in the late 1990s, is the application of this detailed knowledge of the prokaryotic genome (as of August, 2007, 523 genomes of *Bacteria* and 46 of *Archaea* have been published) to the study of the proteome and metagenome [7].

***Corresponding author:** R. Guerrero. E-mail: rguerrero@iec.cat

This article is based on the contribution of RG to Symposium 321, "Perspectives of the Microbial World," held at the 107th General Meeting of the American Society for Microbiology (Toronto, Canada, May 24, 2007).

This article commemorates the 50th anniversary of the publication of *The Microbial World* and pays homage to the authors of *Microbe*, both for their investigations, carried out over the past five decades, into the essential genetic and physiological processes of prokaryotes, and for their role in the training of several generations of microbiologists from all over the world.

The legacy of an idea: the Delft School

The effort to define comprehensively the place of bacteria in the living world was the leitmotif of the Delft School, with an emphasis on the ecological aspects related to biochemistry. Delft, a small city in the Netherlands, is well-known for its distinctive blue and white porcelain and for being the birthplace of the painter Jan Vermeer. But Delft also played a significant role in the history of microbiology. There, microbes (first, protists in 1674, and then bacteria, in 1683) were discovered by Antonie van Leeuwenhoek (1632-1723), the founding father of microbiology. It was also in Delft that Martinus W. Beijerinck (1851-1931) established the scientific principles of prokaryotic physiology and ecology. His successor in the chair of microbiology was Albert J. Kluyver (1888-1956). Kluyver's disciple, Cornelis B. van Niel (1897-1985), transmitted the ideas of the Delft School to the USA following his move to that country in 1929.

Beijerinck carried out what can be considered as the first direct experimental investigation of Darwin's principle of natural selection [2]. He proposed that most microorganisms were cosmopolitan and that their presence or absence could be predicted and produced under the appropriate environmental conditions (light, temperature, pH, ion concentration, etc.). He also showed that the cause of the "mosaic", or "spots" (*Flecken* in German, in the original article) marring tobacco leaves was a filterable, diffusible, and precipitable agent that multiplied only in living cells. He called this infective agent a *contagium vivum fluidum*, or virus, a Latin word used previously to refer to any agent of infectious disease.

Kluyver, considered the father of comparative biochemistry, and his assistant, van Niel, postulated the metabolic unity of life and proposed the use of microorganisms to elucidate the biochemical pathways and energy transformations characteristic of all living beings. Their model showed that all forms of life are related through the recycling of matter and by the network of ecosystems to which they belong [14].

The work of Lourens G.M. Baas Becking (1895-1963), who adhered to the methods of the Delft School of microbiology, must also be cited. Baas Becking was a student at Delft University and attended van Niel's courses but he soon transferred to Utrecht University, where he studied biology, with a major focus on botany. Baas Becking was strongly influenced by Beijerink's work, which established the basis for a general view of the role of bacteria in the cycle of nutrients in the biosphere, and thus of the interactions between life and Earth. He invoked the concept of Gaia more than 30 years before Lovelock proposed his Gaia hypothesis [40] and his ideas, which he summarized as "everything is everywhere, but the environment selects," played an important role in modern studies of the biogeography of microorganisms and the assembly of natural communities [8].

Van Niel trained several other important microbiologists, including Robert E. Hungate (1906-2004), Michael Doudoroff (1911-1975), and Stanier. In Pacific Grove, California, van Niel held what became a famous university course that promoted the study of microorganisms in nature. This course eventually developed into the annual summer courses on microbial ecology held in Woods Hole, Massachusetts, which have maintained the spirit established by van Niel [41,45] (Fig. 1).

What's in two names? *The Microbial World* and *Microbe*

Roger Yates Stanier (Victoria, BC, Canada, 1916–Paris, France, 1982) was one of the main representatives of the Delft School in the United States. His studies included the taxonomic and nutritional properties of the cytophaga, enzyme induction and regulation, aromatic degradative pathways, the regulation of bacteriochlorophyll synthesis by non-sulfur purple bacteria, protection by carotenoids against photooxidative damage, polyhydroxyalkanoate (PHA) accumulation in bacteria [9], the molecular basis of streptomycin dependence, the life cycle of *Caulobacter*, and the taxonomy of pseudomonads [49]. Stanier was born in Canada and maintained strong ties to his native country during his entire life. In 1971, after 24 years at the University of California at Berkeley, he and his French wife, Germaine, who was also a microbiologist, moved to Paris, to the Institut Pasteur. There, Stanier focused on the physiology and taxonomy of cyanobacteria (formerly named "blue-green algae") and in doing so changed forever the adscription of those "algae," from the field of botany to the turf of microbiology. Stanier was also a leading proponent and contributor to general microbiology. His widely used textbook, *The Microbial World,* first published in 1957, captured the conceptual approach to microbiology that had been masterly and concisely formulated in 1956 by Kluyver and van Niel in *The Microbe's Contribution to Biology* [21,24; Int. Microbiol. devoted a special issue to the Kluyver and van Niel's book, in September 2006, 9(3)].

Adelberg [1] remembers the writing-genesis of *The Microbial World* as follows: "We began meeting evenings, to prepare chapter outlines and assign the writing of various

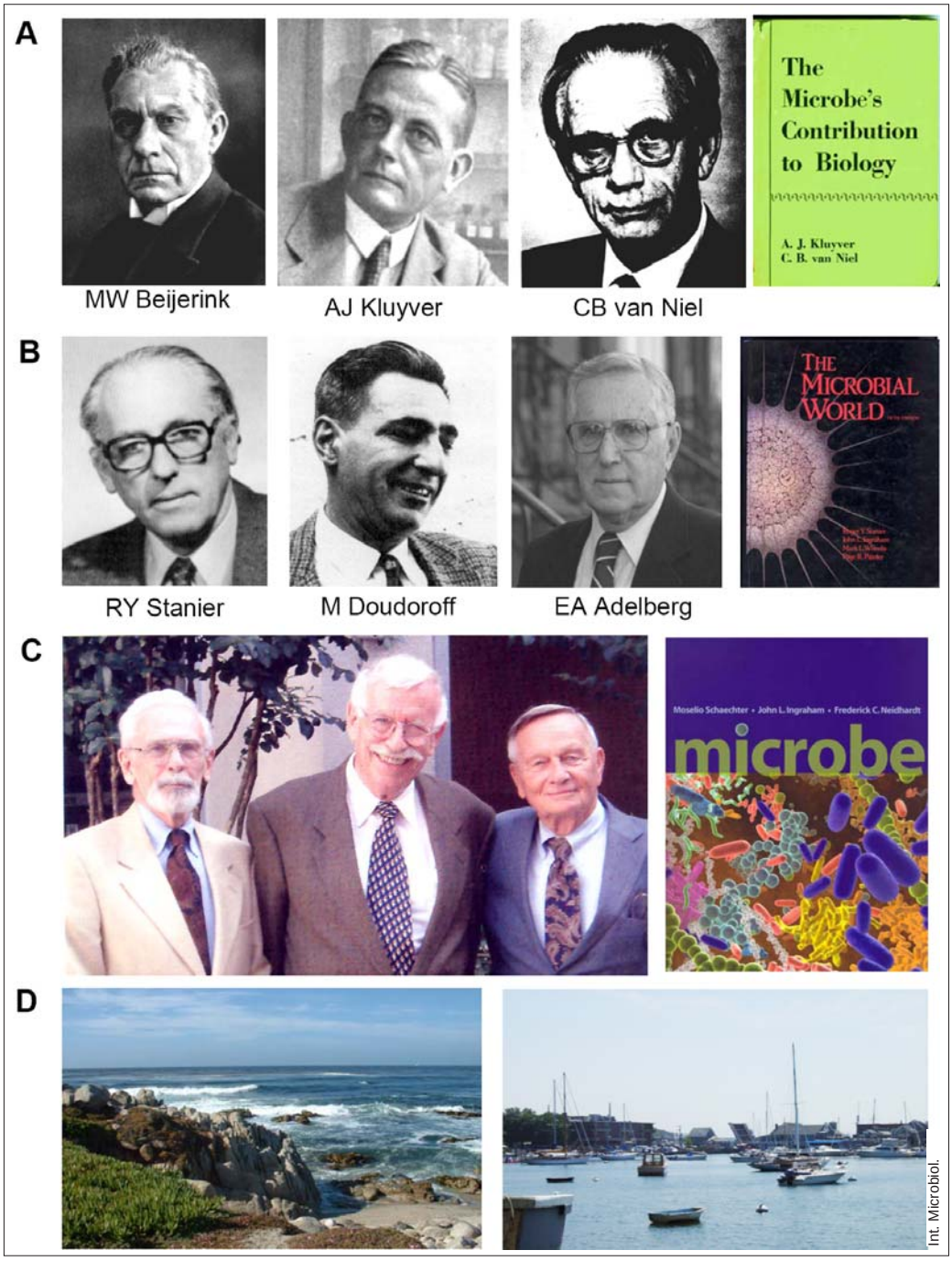


Fig.1. (**A**) Founders of the Delft School. (**B**) Disciples of the Delft School and the autthors of the book *The Microbial Word*. (**C**) Authors of the book *Microbe*. From left to right: F.C. Neidhardt, M. Schaechter, J.L. Ingraham. (**D**) The summer course on microbial ecology originally took place in Pacific Grove, CA, USA, and now is held in Woods Hole, MA, USA. On the right, three significant books for the teaching of microbiology.

chapters to one or another of us. The book was to have three parts, the first of which—dealing with the major groups of microorganisms—Roger proposed be called The Microbial World. We hadn't given much thought to the title for the book itself, but it probably would have been rather conventional, such as Introduction to Microbiology or something of the sort. In the midst of our writing, however, Prentice Hall called to say that a Science Book Club had come into being, and that our book might have a shot at being adopted by it if it had a jazzier title. And so the whole book became *The*

Table 1. Succesive editions of *The Microbial World*, and their Spanish translations

Original version	Spanish version	Translators
RY Stanier, M Doudoroff, EA Adelberg (1957) *The Microbial World*. Prentice-Hall		
RY Stanier, M Doudoroff, EA Adelberg (1963) *The Microbial World*. 2nd ed. Prentice-Hall	(1965) *El mundo de los microbios*. Ed. Aguilar	Isabel García Acha; Manuel Losada; Julio R. Villanueva
RY Stanier, M Doudoroff, EA Adelberg (1970) *The Microbial World*. 3rd ed. Prentice-Hall	(1977) *Microbiología*. Ed. Aguilar	Isabel García Acha; Enrique Cerdá Olmedo; Claudio Fernández Heredia; Manuel Losada; Julio R. Villanueva
RY Stanier, EA Adelberg, John L Ingraham (1976) *The Microbial World*. 4th ed. Prentice-Hall	(1984) *Microbiología*. Ed. Reverté	Isabel García Acha; Ricardo Guerrero; César Nombela; Julio R. Villanueva Directed by: Julio R. Villanueva Coordination: Ricardo Guerrero
RY Stanier, JL Ingraham, Mark L Wheelis, Page R Painter (1986) *The Microbial World*. 5th ed. Prentice-Hall	(1992) *Microbiología*. Ed. Reverté	Mariano Gacto; Isabel García Acha; Ricardo Guerrero; Julio R. Villanueva Directed by: Julio R. Villanueva Coordination: Ricardo Guerrero

Microbial World, a phrase that caught on and is still popular today. […] By June of 1955 [chapters] were ready, and we agreed to spend the summer editing them. This we did by meeting every day from early morning until late afternoon on the patio of Roger's hillside house stopping only for brown-bag lunches. […] Roger's chapters needed little, if any, change, but Mike and I had to rewrite much of our material to bring it up to Roger's standards. In the end, the style of the writing bore Roger's imprint throughout the entire book. Needless to say, this word-by-word editing process took up the entire summer, which proved to be one of the greatest learning experiences (and one of the most enjoyable) of my life."

The 2nd edition of *The Microbial World* (published in 1963) introduced an essential idea to modern microbiology: the concept of a bacterium [47]. The book declared that, "in fact, this basic divergence in cellular structure, which separates the bacteria and blue-green algae from all other cellular organisms, represents the greatest single evolutionary discontinuity to be found in the present-day world" [48]. In the second edition, the classification of the living world consisted of the three Kingdoms—plants, animals, and protists—proposed by Ernst Haeckel (1834-1919) in 1866. The protists included both eukaryotic (upper protists) and prokaryotic (lower protists or monera, according again to Haeckel) microorganisms. In addition, emphasis was placed on the further classification of prokaryotes into two groups: those of "atypical" morphology (cyanobacteria, myxobacteria, spirochetes, actinomycetes—at present, actinobacteria) and those belonging to the "eubacteria" ("conventional" bacteria, or cells with a well-defined morphology, essentially rods and cocci). A 3rd edition was published in 1970. In the 4th edition (1976), Ingraham replaced Doudoroff (who died in 1975) and he became the main author for the 5th and last edition (1986), when Stanier became seriously ill during the book's preparation (Table 1). (See the Editorial by Schaechter et al., this issue, pp. 153-156 [43].)

The early translation into Spanish of the 2nd edition of the book, by Isabel García-Acha, Manuel Losada, and Julio R. Villanueva (*El mundo de los microbios*, Ed. Aguilar, Madrid, 1965), had a transcendent influence on the universities and research centers of Spain and Latin America, where the book was widely used for many years. By then, the three translators were already prominent researchers at the Spanish National Research Council (CSIC), Villanueva and his wife García-Acha, in Madrid, and Losada, in Seville. Their translation of the 2nd edition and involvement in successive ones brought a new concept of microbiology to the Spanish-speaking world. Due to the delays incurred in translating the book's early editions, the 4th and 5th Spanish editions introduced data and sections prepared by the Spanish team that had not been published in the previous translations of *The Microbial World*. Those updates were praised by Ingraham: "We welcome the excellent updated Spanish version of the 4th edition of *The Microbial World*, intended for students and professional microbiologists on both sides of the Atlantic. This edition is one of the many contributions that Spanish-speaking microbiologists have made to the science of Microbiology. We hope that this book contributes to the dissemination of the field both in Spain and Latin America. We deeply acknowledge the translators and the publisher for the excellent work done and the intense efforts they have made." (*Microbiología*, Ed. Reverté, 1984, p. VII. In Spanish in the original.)

In 2006, another microbiology textbook appeared, *Microbe*, published by the ASM Press. But, a book with a so simple title would, by any other name, be just as valuable. This modest title certainly does not reflect the wealth of

knowledge on the main concepts of microbiology that is contained in the book. Wherever there is life, there are microbes. Therefore, the study of microbes is central to the study of all living things, and microbiology is essential to the study and understanding of all life on Earth. The notoriety of microbes as agents of disease has tended to overshadow their vital role in nature. Despite the attacks of pathogens, humans and other living beings have evolved to coexist with—and even to depend on—microbes. As the authors of *Microbe* point out, the intimate relationship between humans and microbes is an aspect of life that we have hardly appreciated. The book describes the unique characteristics (anatomy, cell cycle, sensing of the environment, cell–cell communication, etc.) of microorganisms as well as their activities (metabolism) in nature—including the human body and other colonizable habitats—regardless of whether they are "good" or "bad" from the human point of view.

But, what is indeed in the name of a microbiology book? In 1957, *The Microbial World* broke with the style and contents of previous textbooks of our discipline. In 1970, Brock's *Biology of Microorganisms* [5] presented new ideas on both the ecological implications of microorganisms and the teaching of the discipline. *Microbe* is the third and most recent "non-canonical" title of a microbiology textbook.

Although several other, very good microbiology textbooks are available, *Microbe* is as special as the living forms it describes. The book—whose translation into Spanish is under way—is truly a "labour of love", a product of the passionate interest that its authors, Schaechter, Ingraham, and Neidhardt, have in the microbial world, to which they have devoted years of intense research. These three microbiologists also have been frequent contributors to many of the most influential reference works and textbooks on microbiology of the second half of the twentieth century. With *Microbe*, the authors confirm what Francis Bacon (1561-1626) wrote in the Preface of his *Maxims of the Law*: "I could hold every man a debtor to his profession; from the which as men of course do seek to receive countenance and profit, so ought they of duty to endeavour themselves by way of amends to be a help and ornament thereunto."

Desperately seeking the nucleus: do bacteria have one?

Several microbiological discoveries after the 1940s established microbiology as a true biological science. The new field of molecular biology was based on the newly acquired understanding of microbial genetics. In 1943, Salvador E. Luria (1912-1991) and Max L. Delbrück (1906-1981) showed that, upon exposure to an agent capable of killing bacteria, some of the cells survive and generate descendants resistant to the agent. These mutations were shown to be spontaneous and not induced by the presence of the agent in the medium. In 1944, Oswald T. Avery (1877-1955) and his collaborators showed the existence of DNA transfer ("transformation") in *Streptococcus* (Diplococcus) *pneumoniae*. In 1946, Joshua Lederberg (b. 1925) and Edward L. Tatum (1909-1975) described the direct transfer of genetic material from one strain of *Escherichia coli* to another ("conjugation"). Finally, in 1952, Norton Zinder (b. 1928) and Lederberg reported the transfer of genetic material from one bacterial strain to another by means of a phage ("transduction"). In the 1950s, the importance of microbial molecular biology was recognized. Thus, if cells as "simple" as bacteria have a huge panoply of biochemical pathways, a bona fide genetic system, and characteristic behavior, why do bacteria lack a distinctive internal cytoplasmic structure? Or, simply stated, where are the "nuclei" of these active and promiscuous cells?

Science develops as a continuous interaction between facts and ideas. The progress made in our understanding of the natural world is usually preceded by technological innovations that allow us to newly measure and observe the world around us, and to conceive experiments that were not possible before. Nonetheless, technology, despite its unquestionable utility, is merely a tool, one that depends on adequate intellectual preparation. Chance only favors the prepared mind, as Louis Pasteur said. Stanier and van Niel fathomed the prokaryote/eukaryote differences based on comparisons of the respective cell structures. In the late 1940s and early 1950s, the transmission electron microscope provided researchers with the means to inspect the previously unobserved bacterial cytoplasm. As a consequence, the presence of structures similar to those distinctive of the eukaryotic nucleus, such as a nuclear membrane or a kind of simple "chromosomes", were postulated. For example, Mudd and Smith write: "The preparative procedure described permits parallel electron and light cytological observations to be made to complement one another in learning the effects of successive steps of cytological processing and in analyzing the nature of the structures under observation. In the nuclear sites after appropriate processing Feulgen-positive bodies are found that stain with chromatin dyes and behave characteristically toward nucleic-acid-splitting enzymes. Expressed in the idiom of the cytologist, bacteria possess vesicular nuclei containing chromatin." [Mudd S, Smith AG (1950) Electron and light microscopic studies of bacterial nuclei. J Bacteriol 59:561-573]. Other works claimed to have demonstrated the presence of a nucleus in different bacteria [Knaysi G (1942) The demonstration of a nucleus in the cell of a *Staphylococcus*. J Bacteriol 43:365-384; Knaysi G, Baker RF (1947) Demonstration, with the electron microscope, of a nucleus in *Bacillus mycoides*. J Bacteriol 53: 539-553; Mudd S, Smith AG (1950) Electron and light micro-

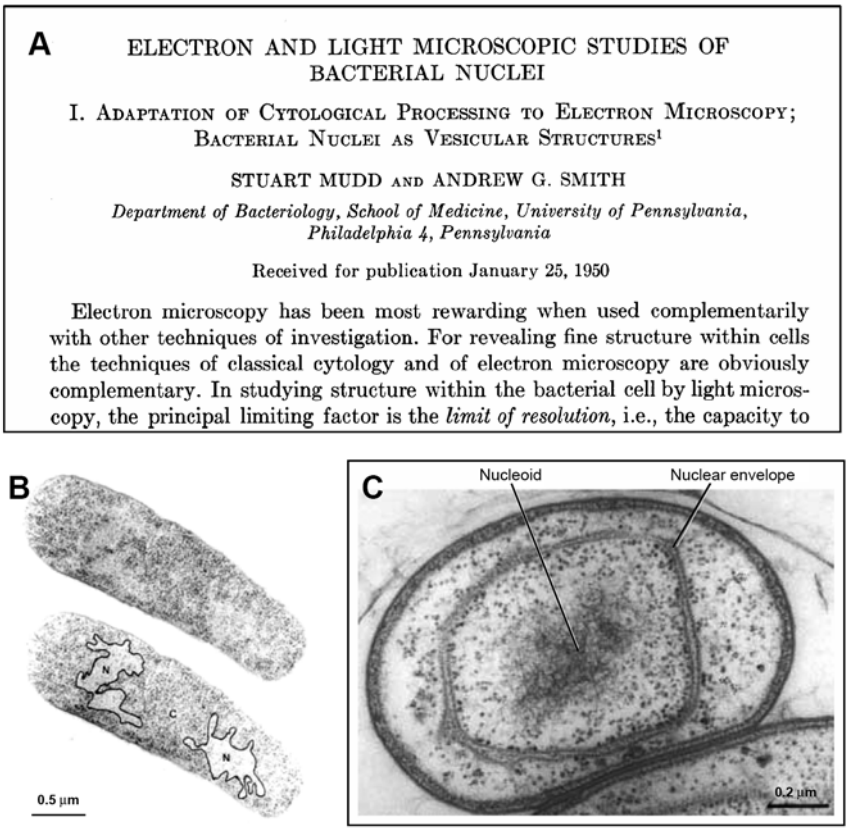


Fig. 2. (**A**) An example of the articles published in the J. Bacteriol. in the late 1940s and early 1950s that described a nucleus in bacteria. (**B**) Electron micrograph of a thin section in *Escherichia coli* cells. The clear area corresponds to the nucleoid (N) that has been outlined in the lower picture. (**C**) An electron micrograph of *Gemmata obscuriglobus* that shows a nucleoid surrounded by a nuclear envelope. (B from E. Kellemberg, C from Lindsay et al. Arch. Microbiol. 175:413, 2001. Both reproduced from *Microbe,* ASM Press, 2006.)

scopic studies of bacterial nuclei. J Bacteriol 59:561-573] (Fig. 2). Nevertheless, upon thoroughly observation and improved techniques, electron microscopy ultimately disaproved the presence of a "true" nucleus in prokaryotic cells.

The concept of "prokaryote" is organizational, not phylogenetic, and was challenged soon after the emergence of molecular approaches to bacterial phylogeny. In 1977, a deep dichotomy among the prokaryotic groups *Eubacteria* and *Archaebacteria* emerged based on the study of their rRNA [54]. In the 1990s, the proposal was made to rename the eukaryotes, eubacteria, and archaebacteria as *Eukarya*, *Bacteria*, and *Archaea*. Initial genetic analyses indicated that archaea were more closely related to eukaryotes than bacteria were. Carl R. Woese and Norman R. Pace have insisted that the major eukaryotic organelles—mitochondria and chloroplasts—are bacterial in origin, but that the nucleus is not. The nuclear line of descent is as ancient as the archaeal line and is not derived from either archaea or bacteria. Thus, according to Woese and Pace, the prokaryote/eukaryote model of biological diversity and evolution is invalid and the prokaryote/eukaryote concept should be abandoned [35]. However, recent comparisons of fully sequenced microbial genomes have added a twist to this story: eukaryotes contain both archaeal and bacterial genes. Archaeal genes tend to encode processes involving DNA and RNA: they are "informational" genes. Bacterial genes are responsible for metabolism or housekeeping chores: they are "operational" genes [23]. Margulis et al. [29] proposed that the last eukaryotic common ancestors (LECA) originated by the recombination of bacteria and archaeal DNA, and the nucleus evolved by prokaryotic recombination and membrane hypertrophy, analogous to *Gemmata obscuriglobus.* The nucleus remained attached to bacterial motility structures and became the microtubular cytoskeleton, including the mitotic apparatus. Those authors also suggested that the mitochondria should have evolved after the nucleus did. How the eukaryotic cell came to be is one of the greatest riddles of biology. It is a tale so complex that no single gene (no matter how important rDNA may be) is able to tell the whole story. Only entire genomes can.

As early as 1984, there were researchers who suggested that some planctomycetes had internal membranes related to genetic material. Sophisticated electron microscopy techniques confirmed the existence of those membranes, even revealing double ones resembling the eukaryotic nucleus (Fig. 2). Do those observations refute the dogma that prokaryotes have no internal membranes? [10]. Furthermore, recent advances in microbiology have demonstrated that bacterial cells possess an organized and dynamic subcellular architecture. Compartmentalization in the eukaryotic cell is well-established, but in prokaryotes it is less obvious since

most prokaryotes do not need intracytoplasmic membranes to maintain vital functions. But compartmentalization in prokaryotes does exist. Moreover, membranes, the nucleoid, or the genophore (chromosomes), multi-enzyme complexes, storage granules, and cytoskeletal elements are involved in compartment formation, not only operationally but also physically and structurally. Like their eukaryotic counterparts, bacteria employ a full complement of cytoskeletal proteins, localize proteins and DNA to specific subcellular addresses at specific times, and use intercellular signaling to coordinate multicellular events. This dynamic organization regulates complex cellular events in both space and time [11,12]. Therefore, if prokaryotes are so complex and have the seeds of many eukaryotic structures and functions, where is the border that separates the two "anatomies" and strategies of life? What is actually a bacterium?

The earliest scaffold: the cytoskeleton in prokaryotes

Traditional representations of the prokaryotic cytoplasm show an amorphous, unstructured amalgamation of proteins and ribosomes basking in a dense liquid with a randomly arranged chromosome placed in the center. This image of the prokaryotic cell is incorrect. One of the most significant advances in microbial biology in recent years has been the discovery of broadly conserved cytoskeletal elements in bacteria. Although the absence of a cytoskeleton was one of the features used to distinguish prokaryotes from eukaryotes, bacteria do contain many of the cytoskeletal elements that are found in eukaryotic cells, such as microtubule, actin, and intermediate-filament homologs (i.e., MreB, FtsZ and crescentin), which have significant functions in diverse cellular processes (Table 2). The cytoskeleton was not a eukaryotic invention but appeared early in evolution [32]. Although the cytoskeleton mediates cytokinesis and chromosome segregation in both bacteria and eukaryotes, the functions of its specific elements are reversed. Cytokinesis is driven by the actin-based contractile ring in eukaryotes and by the FtsZ tubulin homolog in bacteria, while DNA segregation uses the MreB and ParM actin homologs in prokaryotes and the microtubule-based spindle in eukaryotes (Fig. 3). This apparent inversion of actin and tubulin functions could represent convergent evolution: perhaps the last universal common ancestor of bacteria and eukaryotes had both actin and tubulin but did not yet dedicate these proteins to specific functions. Alternatively, an inversion of actin and tubulin function may have occurred in one of the two lineages [11].

FtsZ, a 37- to 43-kDa protein, is almost ubiquitous in bacteria, archaea, and chloroplasts. Although the amino acid sequence similarity between tubulin and FtsZ is low and confined to regions forming the active sites, the tertiary structures of these proteins are surprisingly similar. FtsZ acts as the central organizer of prokaryotic cytokinesis. It forms a ring structure (the Z-ring) at the cell-division site. During cell division, the Z-ring assembles and constricts at the division site, directing the peptidoglycan synthesis that is required for the formation of new cell poles. In addition, FtsZ ring formation triggers the assembly of the cell-division machinery, which comprises at least seven different proteins (such as FtsA, ZipA, ZapA, and EzrA) depending on the bacterial species [25]. Given the prokaryotic origin of chloroplasts and mitochondria, it was perhaps not surprising to discover that host cells recruit FtsZ to function as organelle division proteins. What was surprising was the absence of α-proteobacteria-related *FtsZ* genes in the sequenced genomes of model fungi, animals, and plants, which indicated that FtsZ no longer played a role in mitochondrial division. Instead, a second group of large self-assembling guanosine triphosphatases (GTPases), the dynamin-related proteins (DRPs), function in mitochondrial fission in these organisms. Recent findings have shown, however, that mitochondria and chloroplasts universally require dynamin-related GTPases to divide. This mechanistic link provides fundamental insights into the molecular

Table 2. Main bacterial cytoskeletal proteins

Name	Known function(s)	Eukaryotic homolog
FtsZ	Cell division (septum formation)	Tubulin
BtubA/BtubB	Unknown function. To date only identified in the genus *Prosthecobacter* from the division *Verrucomicrobia*	Tubulin
FtsA	Stabilization of the Z-ring; recruiment of proteins to the division zone	Actin
MreB/Mbl	Cell shape in rods; chromosome segregation; cell polarity in *Caulobacter*	Actin
ParM	Plasmid segregation	Actin
Crescentin	Cell shape in *Caulobacter*	Intermediate filaments

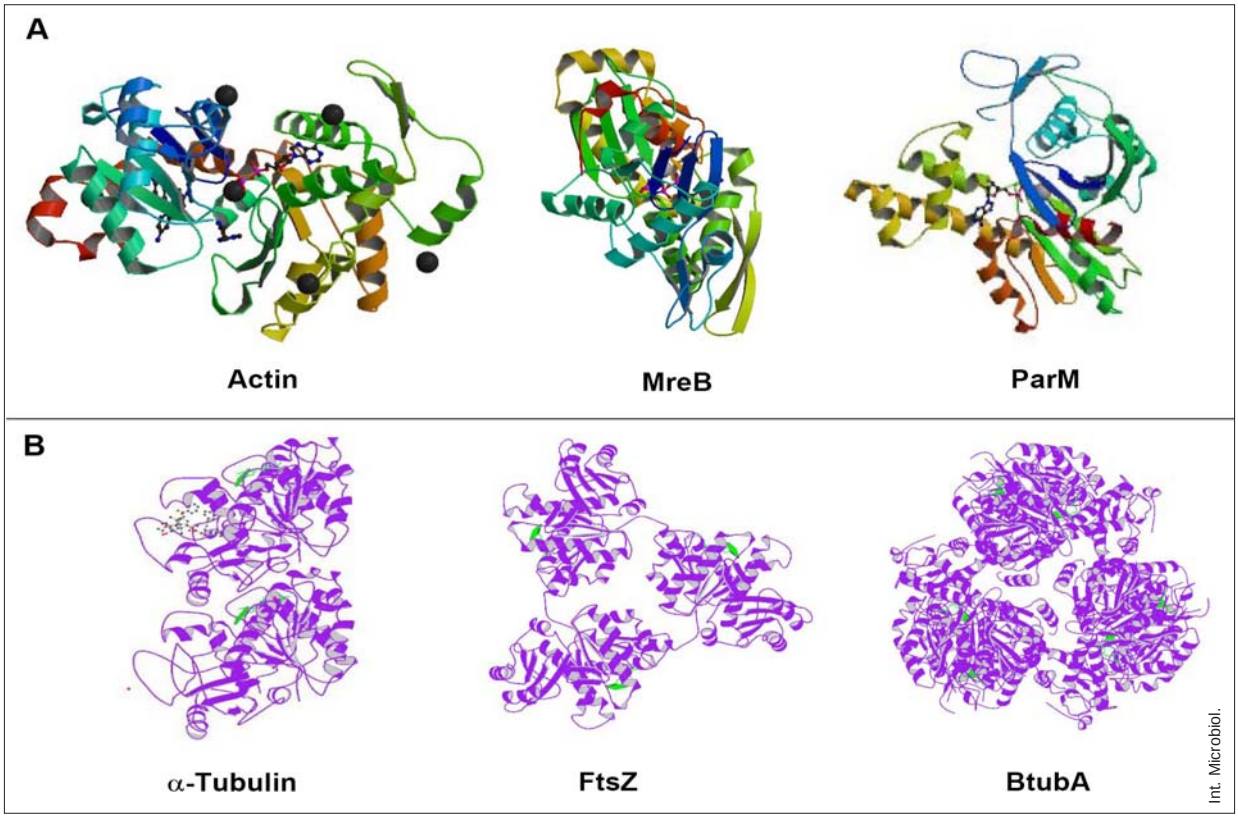


Fig. 3. Structural comparison of prokaryotic and eukaryotic counterpart cytoskeletal proteins. Protein structures were downloaded from the Protein Data Bank (PDB) [http://www.rcsb.org/pdb/home/home.do]. (**A**) Uncomplexed actin (PDB no. 1J6Z); *Thermotoga maritima* MreB (PDB no. 1JCG); *Escherichia coli* (PDB no. 1MWM). (**B**) *Bos taurus* α-tubulin (PDB no. 1JFF); *Methanococcus jannaschii* FtsZ (PDB no. 1FSZ); *Prosthecobacter dejongeii* BtubA (PDB no. 2BTO).

events driving the division, and possibly the evolution, of eukaryotic organelles [34].

The actin-like protein MreB was originally discovered based on its role in establishing the rod shape, as deletion of the *mreB* gene in *Escherichia coli* determined a round or irregular cell morphology. *mreB* is also present in *Bacillus subtilis*, which contains two additional *mreB* homologs: *mbl* (*mreB*-like) and *mreBH*. Notably, most spherical bacterial species lack *mreB*, whereas it is well-represented among bacteria with more complex shapes. Bacterial cytoskeletal elements such as MreB (or Mbl in *B. subtilis*) govern cell shape by localizing cell-wall synthesis (insertion of nascent peptidoglycan) to specific subcellular locations during growth and division [4]. Mollicutes do not have a cell wall, but they do have a defined shape and are able to glide (*Mycoplasma*) or swim (*Spiroplasma*) despite the absence of flagella. An internal cytoskeletal ribbon found only in *Spiroplasma* may be responsible for this organism's ability to swim [52].

Eukaryotic cells use a tubulin-based cytoskeleton to segregate their chromosomes during mitosis. In bacteria, this task is accomplished by MreB. In vivo, MreB forms helical cables that traverse the length of the cell in all bacterial organisms examined, such as *E. coli*, *B. subtilis*, and *Caulobacter crescentus*. MreB filaments are dynamic structures that are continuously remodeled throughout the cell cycle and that move away from the mid-cell towards opposite cell poles. Depletion of MreB in *B. subtilis* and in *C. crescentus* leads to a defect in chromosome segregation [22].

The most recently discovered member of the bacterial cytoskeletal family is crescentin, a *Caulobacter* coiled-coil protein whose biochemical properties and domain structure resemble those of intermediate filaments. Crescentin polymerizes on the inner curvature of comma-shaped *Caulobacter* and is not found in other bacteria, plants, or fungi [11].

With the discovery of bacterial homologs for each of the eukaryotic cytoskeletal families, the list of bacterial cytoskeletal types was thought to be complete. It is now clear, however, that bacteria have additional cytoskeletal families without evident eukaryotic counterparts [12]. It was recently found that the linear organization of magnetosomes in the cytoplasm of magnetotactic bacteria is caused by the attachment of magnetosomes via MamJ to a cytoskeleton-like structure. This shows one of the highest structural levels in a prokaryotic cell and confers optimal function in magnetotaxis. Gene *mamJ* is co-transcribed with the *mamK* gene, which was previously hypothesized to encode a cytoskeletal protein because of the

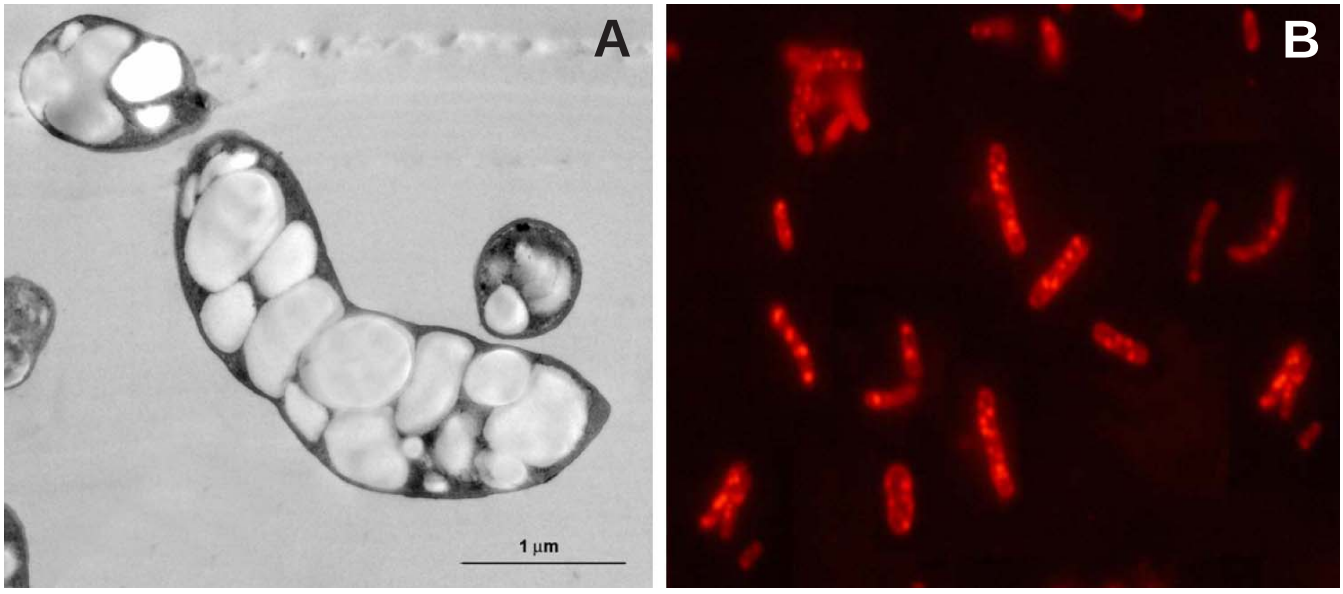
latter's striking similarity to actin-like MreB proteins in other bacteria [44]. Clearly, the degree of subcellular organization in prokaryotes has been underestimated.

Fortune tellers: intracellular polymers

The adaptations of the central pathways evolved by microorganisms have facilitated microbial growth under a wide range of ecological circumstances. The incorporation of resources and the production of metabolic compounds by spatially separated microbial populations is the driving force in the formation of chemical and physical gradients. Nutrient limitation (sources of C, N, P, especially) normally leads to a decrease or inhibition of metabolic activity (synthesis of biomass); but a lack of energy substrates (electron donors) forces a population to switch to another type of metabolism, or may even cause a change in its composition [6]. Prokaryotes have evolved numerous mechanisms of resistance to these and other stress conditions. For example, many microorganisms have an inherent ability to form resting stages (e.g., cysts and spores), which allows them to survive in desiccated environments [30]. Others, such as the spirochete *Spirosymplokos deltaiberi*, swell and form refractile bodies on exposure to air [28].

The accumulation of intracellular storage polymers, such as PHAs, which serve as an endogenous source of carbon and energy during starvation, is another bacterial strategy that increases survival in a changing environment [3,33] and may offer protection against other adverse factors [16]. Intracellular storage polymers thereby forecast the future ("time-binding"), since their synthesis reflects the fact that a microorganism is anticipating adverse environmental conditions. By accumulating compounds (polymers of C, N, P) in the cytoplasm when the environment offers plenty of resources (more than cells need to grow), bacteria are able to prevent famine. Since the environment constantly changes, conditions of stress (nutritional scarcity, dehydration, non-permissive temperature, etc.) are the rule rather than the exception. Upon the eventual return to "favorable" environmental conditions, cells that were "ants" have more possibilities to thrive than those that were "grasshoppers." Thus, "time-binding" represents a Darwinian selective advantage for the evolution and maintenance of autopoietic systems. It constitutes another prokaryotic strategy to increase survival in the ever-changing environment that has constantly marked the rhythm of the dance of life on Earth.

The polymerization of soluble intermediates into insoluble molecules does not change the osmotic state of the cell, thus avoiding leakage of these nutrient-rich compounds out of the cell. In addition, PHA-producing bacteria have the advantage of nutrient storage at a relatively low maintenance cost and with a secured return of energy [3,26] (Fig. 4). Bacterial survival in the absence of exogenous carbon sources depends on the intracellular content of polyhydroxybutyrate (PHB), the best-studied PHA. For *Legionella pneumophila,* a correlation between the amount of accumulated PHB and long-term survival (up to 600 days) in the absence



Int. Microbiol.

Fig. 4. (**A**) Transmission electron micrograph of the strain MAT-28 from Ebro Delta microbial mats. (**B**) The same strain stained with Nile Red dye, observed by epifluorescence microscopy. PHA granules fluoresce bright orange within the cells. (Photographs made by M. Berlanga and A. Barberán, University of Barcelona.)

Table 3. Biological functions and biotechnological applications of polyhydroxyalkanoates (PHAs)

Biological functions
Carbon and energy reserve, increasing survival by PHA accumulating bacteria
Possible relationship between synthesis of PHA and nitrogen fixation by symbiotic bacteria
PHA synthesis may facilitate the acquisition of competence in natural transformer cells
Environmental markers of physiological status of the population
Biotechnological applications
Packaging industry
Food packaging and beverage bottles
Water-resistant surfaces covering paper or cardboard
Shampoo bottles
Medical applications
Tissue engineering (bone plates, surgical sutures, etc.)
Cardiovascular uses (pericardial patches, artery augments, heart valves, etc.)
Drugs carriers to controlled release inside the body
Agricultural applications
Controlled release of insecticides
Carriers of bacterial nitrogen-fixing inoculants in soils growing maize and wheat
PHA synthesis in transgenic plants
PHA available industrially
BIOPOL®, initially manufactured by Zeneca/ICI, then Monsanto and now by Metabolix Inc (USA): Co-polymer of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
Nodax™, Procter & Gamble (USA): A co-polymer of 3-hydroxybutyrate (PHB) and small quantity of medium chain length monomers
DegraPol®, AB Medica SPA (Italy): A block-copolyester urethane (chemically synthesized from PHB-diol and α,ω-dihydroxy-poly(caprolactone-block-diethyleneglycol-block--caprolactone)
Antibiotic Simplex®, AKZ, New Zealand (a division of Pfizer Labs): PHB and methacrylate

of an exogenous carbon source was found. It has been shown that *Wautersia eutropha* H16 (=*Cupriavidus necator*, =*Ralstonia eutropha*, =*Alcaligenes eutrophus*) can grow even in the absence of any exogenous carbon source by utilizing previously accumulated PHB. Microbial mats are an excellent source of PHA-accumulating bacteria, probably due to the fluctuation of flooded and dry conditions that characterize these environments [3].

Depending on the number of carbon atoms of the monomers, PHAs are classified as short- (3–5 C-atoms) or medium- (6 or more C-atoms) chain-length PHAs. In many bacteria, PHAs consist of more than one type of monomer (copolyesters). The high number of monomers and the variable monomeric composition of PHAs result in an enormous variation of their physical and chemical characteristics. This makes PHAs an excellent source of biodegradable plastic compounds, an application of potentially great industrial interest (Table 3).

PHAs accumulate as intracellular granules, which are coated with a monolayer of phospholipids and proteins. These granule-associated proteins play a major role in the synthesis and degradation of PHA and in granule formation. Four types of granule-associated proteins are found: (i) PHA synthase; (ii) PHA depolymerases and 3HB-oligomer hydroxylase; (iii) phasins (PhaPs), which are thought to be the major structural proteins of the membrane surrounding the inclusion; and (iv) the regulator of phasin expression, PhaR. PHA begins to form as amorphous, nearly spherical granules that gradually fill the cells and force them to expand. The final number of PHA granules in a typical PHA-producing cell of *W. eutropha* is ten on average. The typical granule has a diameter of about 500 nm when growth ceases [20,39].

The physicochemical properties of a polyester strongly impact its biodegradability. The most important factors are stereoregularity, crystallinity, monomeric composition, and accessibility of the polymer surface. PHA in vivo and outside of bacteria is present in two different biophysical states. Intracellular PHA is amorphous whereas extracellular PHA is a partially crystalline polymer. It has been shown that highly ordered structures, i.e., highly crystalline materials, have lower biodegradability. Intracellular degradation is the active degradation (mobilization) of an endogenous storage reservoir by the accumulating bacterium itself. The source of extracellular polymer is PHA that is released by accumulating cells after death and cell lysis [38].

Our knowledge about the formation of PHA inclusions and mobilization is still very limited. PHA formation starts from soluble substrates, which are finally found in the insoluble inclusions [20,50]. But, where does PHA synthesis start? Most of the images of PHB granules show them to be distributed more or less randomly in the bacterial cell, and it has long been assumed that PHB granules are located in the cytoplasm. However, these images were of cells at the end of growth and/or at the end of the PHB accumulation phase;

consequently, they are not useful to predict the initiation site of a PHB granule. In several species of bacteria, PHB granules at the early stage of formation are located either close to the cytoplasmic membrane or are attached to it; only larger granules (>300 nm in diameter) are clearly detached from the membrane [15,18]. In *Rhodospirillum rubrum*, *W. eutropha*, and recombinant *E, coli,* PHB granules at the early stages frequently are found near the cell poles and cell wall. PHB synthesis may thus initiate not randomly but at discrete regions in bacteria [17,37]. However, Tian et al. [51] observed that the nascent granules arose from the center of the cell, and during the early stages of PHB production dark-stained mediation elements have been observed. These could serve as cytoskeleton-like scaffolds, providing sites for the synthase to initiate granule formation. The darkly staining mediation elements gradually disappear as the granules increase in size.

The accumulation of PHB in *W. eutropha* increases the cell volume from 1.208 to 3.808 μm^3 and the buoyant density from 1.110 to 1.145 $pg/\mu m^3$ [36]. The cell volume changes linearly with PHB content. These changes are due to increases in cell width but not in cell length [36]. It has been shown that changes in the volume and density of microbial cells are a function of the weight of the macromolecule forming the inclusion, as observed with glycogen in *E. coli*, PHB in *W. eutropha*, and sulfur accumulation in *Chromatium* spp. A certain degree of hydration of the polymer in the inclusion may explain the linear relationship between cell volume and the weight of the polymer [31]. Recent experiments suggest that PHB granules always contain about 15% water, which corresponds to about one H_2O molecule per repeat unit in the chain. This final water content has been proposed as the factor that delays nucleation in the polymer mass and thus prevents crystallization [19], which allows the cell to rapidly degrade this internal resource. Our ability to measure cell density and to identify those factors affecting it greatly expands our understanding of the ecology of planktonic microorganisms. The densities of particular microorganisms, or of physiologically diverse populations within one species, facilitate their separation by density gradient centrifugation [13].

"Messieurs, c'est les microbes qui auront le dernier mot"

Prokaryotes are neither structurally nor functionally as simple as was believed in Stanier's time. Until recently, they were considered to live rather isolate, asocial, reclusive lives. New research shows that, in fact, prokaryotes have elaborate chemical signaling systems that enable them to communicate within and between species. Prokaryotes in nature live and die in complex communities that in many ways resemble a multicellular organism. The release of pheromones induces bacteria in a population to respond in concert by changing patterns of gene expression, the phenomenon knwon as quorum sensing. The assembly of unicellular organisms into multicellular aggregates, mounds, and fruiting bodies is common in nature. The most ubiquitous environmental trigger appears to be nutrient stress, suggesting that the aggregation and formation of multicellular structures are adaptations that improve survival under unfavorable conditions. Prokaryotic communities develop as biofilms on all surfaces in aqueous environments. Differentiation into biofilms is controlled by the sequential expression of certain metabolic determinants, either as a pattern of adaptive responses to changing environmental parameters within the biofilm or as part of a "programmed" life cycle. Under nutrient limitation, it might be advantageous for a fraction of a bacterial population to lyse, providing nutrients for the remaining cells. The discovery that a subpopulation of cells in mature microcolonies of several bacterial populations undergoes autolysis, possibly as a prerequisite for the dispersal of the remaining viable cells, suggests a link between individual cells and multicellularity and cooperativity. From an ecological perspective, cell dispersal would be required for the continued exploration of habitats and food [53].

"Brief is the fruit of the youth, no more than the daily interval of sunlight on land; and when the spring of the life has been consumed, indeed it is better the death than to live, since many are the harms that invade the heart", recited the Greek poet Mimnermos, ca. 600 BC. Few things have captivated so powerfully the human imagination than the idea of prolonging life. Strange as it may seem, aging and death, which are the fates of humans, were not necessary at the beginnings of life, nor were they the final destiny of life over hundreds of millions of years. Prokaryotes are potentially immortal. The classical definition of life, i.e., birth, growth, reproduction, and death, cannot be applied in the same way to prokaryotes and eukaryotes. What is the limit of prokaryotic "immortality"? Spores represent the best example of cryptobiosis (from Gr. *cryptos*, hidden) or "latent life", in which a cell temporarily escapes from adverse conditions. Moreover, spores can be disseminated by the action of wind, water, or other organisms, to reach a favorable environment for germination. Thus, spores not only delay cellular death, but also allow new populations to develop in new places. A spore-forming bacterium cannot predict how long or in what environment it will remain in a lethargic state. By being prepared for the worst, bacteria indefinitely escape the temporal limitations of life, the ineluctable event of death.

Acknowledgements. This work was supported by grant CGL2005-04990/BOS (from the Spanish Ministry of Science and Technology). The authors are grateful for the suggestions and comments of Mercè Piqueras, Carmen Chica and Wendy Ran.

References

1. Adelberg EA (1998) The right place at the right time. Annu Rev Microbiol 52:1-40
2. Bennett JW, Phaff HJ (1993) Early biotechnology: the Delft connection. ASM News 59:401-404
3. Berlanga M, Montero MT, Hernández-Borrell J, Guerrero R (2006) Rapid spectrofluorometric screening of poly-hydroxyalkanoate-producing bacteria from microbial mats. Int Microbiol 9:95-102
4. Bhavsar AP, Brown ED (2006) Cell wall assembly in *Bacillus subtilis*: how spirals and spaces challenge paradigms. Mol Microbiol 60:1077-1090
5. Brock TD (1970) Biology of microorganisms. Prentice-Hall, Englewood Cliffs, NJ
6. Brune A, Frenzel P, Cypionka H (2000) Life at the oxic-anoxic interface: microbial activities and adaptations. FEMS Microbiol Rev 24:691-710
7. Buckley M, Roberts RJ (2007) Reconciling microbial systematics and genomics. Report Am Acad Microbiol, ASM Press, Washington, DC
8. de Wit R, Bouvier T (2006) 'Everything is everywhere, but, the environments selects'; what did Baas Becking and Beijerinck really say? Environ Microbiol 8:755-758
9. Doudoroff M, Stanier RY (1959) Role of poly-β-hydroxybutyrate in the assimilation of organic compounds by bacteria. Nature 183:1440-1442
10. Fuerst JA (2005) Intracellular compartmentation in planctomycetes. Annu Rev Microbiol 59:299-328
11. Gitai Z (2005) The new bacterial cell biology: moving parts and subcellular architecture. Cell 120:577-586
12. Gitai Z (2007) Diversification and specialization of the bacterial cytoskeleton. Curr Opin Cell Biol 19:5-12
13. Guerrero R, Pedrós-Alió C, Schmidt TM, Mas J (1985) A survey of buoyant density of microorganisms in pure culture and natural samples. Microbiología SEM 1:53-65
14. Guerrero R, Berlanga M (2006) "Life's unity and flexibility": the ecological link. Int Microbiol 9:225-235
15. Hermawan S, Jendrossek D (2007) Microscopical investigation of poly(3-hydroxybutyrate) granule formation in *Azotobacter vinelandii*. FEMS Microbiol Lett 266:60-64
16. James BW, Mauchline WS, Dennis PJ, Keevil CW, Wait R (1999) Poly-3-hydroxybutyrate in *Legionella pneumophila*, an energy source for survival in low-nutrient environments. Appl Environ Microbiol 65:822-827
17. Jendrossek D (2005) Fluorescence microscopical investigation of poly(3-hydroxybutyrate) granule formation in bacteria. Biomacromolecules 6:598-603
18. Jendrossek D, Selchow O, Hoppert M (2007) Poly(3-hydroxybutyrate) granules at the early stages of formation are localized close to the cytoplasmic membrane in *Caryophanon latum*. Appl Environ Microbiol 73:586-593
19. Jurasek L, Nobes GAR, Marchessault RH (2001) Computer simulation of in vitro formation of PHB granules: particulate polymerization. Macromol Biosci 6:258-265
20. Jurasek L, Marchessault RH (2004) Polyhydroxyalkanoate (PHA) granule formation in *Ralstonia eutropha* cells: a computer simulation. Appl Microbial Biotechnol 64:611-617
21. Kluyver AJ, van Niel CB (1956) The microbe's contribution to biology. Harvard University Press, Cambridge, MA
22. Kruse T, Gerdes K (2005) Bacterial DNA segregation by the actin-like MreB protein. Trends Cell Biol 15:343-345
23. Lake JA (2007) Disappearing act. Nature 446:983
24. Lederberg J (2006) *The Microbe's Contribution to Biology*–50 years after. Int Microbiol 9:155-156
25. Löwe J, van den Ent F, Amos LA (2004) Molecules of the bacterial cytoskeleton. Annu Rev Biophys Biomol Struct 33:177-198
26. Madison LL, Huisman GW (1999) Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. Microbiol Mol Biol Rev 63:21-53
27. Maloy S, Schaechter M (2006) The era of microbiology: a Golden Phoenix. Int Microbiol 9:1-8
28. Margulis L, Ashen JB, Solé M, Guerrero R (1993) Composite, large spirochetes from microbial mats: spirochete structure review. Proc Natl Acad Sci USA 90:6966-6970
29. Margulis L, Chapman M, Guerrero R, Hall J (2006) The last eukaryotic common ancestor (LECA): Acquisition of cytoskeletal motility from aerotolerant spirochetes in the Proterozoic Eon. Proc Natl Acad Sci USA 103:13080-13085
30. Malcom P (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755-805
31. Mas J, Pedrós-Alió C, Guerrero R (1985) Mathematical model for determining the effects of intracytoplasmatic inclusions on volume and density of microorganisms. J Bacteriol 164:749-756
32. Møller-Jensen J, Löwe J (2005) Increasing complexity of the bacterial cytoskeleton. Curr Opin Cell Biol 17:75-81
33. Müller S, Bley T, Babel W (1999) Adaptative responses of *Ralstonia eutropha* to feast and famine conditions analysed by flow cytometry. J Biotechnol 75:81-97
34. Osteryoung KW, Nunnari J (2003) The division of endosymbiotic organelles. Science 302:1698-1704
35. Pace NR (2006) Time for a change. Nature 441:289
36. Pedrós-Alió C, Mas J, Guerrero R (1985) The influence of poly-b-hydroxybutyrate accumulation on cell volume and buoyant density in *Alcaligenes eutrophus*. Arch Microbiol 143:178-184
37. Peters V, Rehm BHA (2005) In vivo monitoring of PHA granule formation using GFP-labeled PHA synthases. FEMS Microbiol Lett 248:93-100
38. Philip S, Keshavarz T, Roy I (2007) Polyhydroxyalkanoates: biodegradable polymers with a range of applications. J Chem Technol Biotechnol 82:233-247
39. Pötter M, Steinbüchel A (2005) Poly(3-hydroxybutyrate granule-associated proteins: impacts on poly(3-hydroxybutyrate) synthesis and degradation. Biomacromolecules 6:552-560
40. Quispel A (1998) Lourens G.M. Baas Becking (1895-1963), inspirator for many (micro)biologists. Int Microbiol 1:69-72
41. Schaechter M (2006) From growth physiology to systems biology. Int Microbiol 9:157-161
42. Schaechter M, Ingraham JL, Neidhardt FC (2006) Microbe. ASM Press, Washington, D.C.
43. Schaechter M, Ingraham JL, Neidhardt FC (2007) The road from *The Microbial World* to *Microbe*. Int Microbiol 10:153-156
44. Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D (2006) An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. Nature 440:110-114
45. Spath S (2004) van Niel's course in general microbiology. ASM News 70:359-363
46. Stanier RY, Doudoroff M, Adelberg EA (1957) The microbial world, 1st edn. Prentice-Hall, Englewood Cliffs, NJ
47. Stanier RY, van Niel CB (1962) The concept of a bacterium. Arch Mikrobiol 42:17-35
48. Stanier RY, Doudoroff M, Adelberg EA (1963) The microbial world, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ
49. Stanier RY (1980) The journey, not the arrival, matters. Annu Rev Microbiol 34:1-48
50. Stubbe J, Tian J (2003) Polyhydroxyalkanoate (PHA) homeostasis: the role of the PHA synthase. Nat Prod Rep 20:445-457
51. Tian J, Sinskey AJ, Stubbe J (2005) Kinetic studies of polyhydroxybutyrate granule formation in *Wautersia eutropha* H16 by transmission electron microscopy. J Bacteriol 187:3814-3824
52. Trachtenberg S, Gilad R (2001) A bacterial linear motor: cellular and molecular organization of the contractile cytoskeleton of the helical bacterium *Spiroplasma melliferum* BC3. Mol Microbiol 41:827-848
53. Webb JS, Givskov M, Kjelleberg S (2003) Bacterial biofilms: prokaryotic adventures in multicellularity. Curr Opin Microbiol 6:578-585
54. Woese CR, Fox GE (1977) Phylogenetic structure of prokaryotic domain: The primary kingdoms. Proc Natl Acad Sci USA 74:5088-509