Prokaryotic Basis of Eukaryotic Eco-Evo Development

M. Berlanga, M. Viñas, and R. Guerrero

1 Introduction: Evolution, 160 Years After Darwin

The biosphere has been described by E. O. Wilson as a seamless membrane of organisms wrapped around the Earth's surface where the totality of life exists. It is this biosphere, more than any other feature of Earth that makes our planet unique in the known universe (thus far). The Earth is a planet with liquid water. This is fundamental. There is plenty of water elsewhere in the universe, but as far as we know, nowhere else has water filled with life. Even a single drop of water may contain a thousand variations of microscopic organisms. The ecological life support system (the biosphere) consists of an extraordinary net of interrelationships, energy and nutrient flows, and a variety of cyclic events. According to the Gaia hypothesis, on Earth, the atmosphere-hydrosphere, surface sediments, and all living beings together (the biota) behave as a single integrated system with properties more analogous to systems of physiology than those of physics. Gaia is the global life

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and environment. From this perspective, the planetary surface can be seen as a body rather than a place (Margulis 1998; Doolittle 2017; Lovelock 2019).

More than 30 billion kinds of life, placed unambiguously into five vast groups (bacteria, protists, fungi, animals, and plants), have evolved over the past 3500 million years from our small common ancestors. Through an uninterrupted sequence, our genes come from the same molecules that were present in the first primitive cells that formed on the Earth's surface in a range of different possible environments: the shores of the first warm and shallow bodies of water, or systems similar to the "deep-sea vents" of today's oceans, or the hot (but permissive) shores of volcanic lava, or several other possible locations still to be described.

All the great innovations in the evolution of cell metabolism took place in the "prokaryotic world," before the emergence of the first eukaryotic cells (being those protists, plants, fungi, or animals). Prokaryotes were the sole inhabitants of the planet until approximately 1800 million years ago (Guerrero 1998; López-García and Moreira 2015), where different prokaryotic chimeras were "transformed" into different nucleated cells, the eukaryotes.

Modern biology has been built upon two key ideas. The first, conceived in the nineteenth century, is that all life evolved from single-celled organisms through a process of natural selection. The second, which was fine-tuned in the twentieth century, is that organisms are governed by the laws of physics and chemistry, rather than a mystical "vital force." The two ideas draw strength from each other: the concept of natural selection is more convincing once organisms are understood as physicochemical entities.

In the time of Charles Darwin, although many kinds of microorganisms (especially protists and some bacteria) had already been discovered over 200 years previously, the role of prokaryotes in biology and their importance as the basis of all life on Earth were completely unknown. Thus, when describing the "tangled bank" of life in the last paragraph of his landmark book *The Origin of Species* (1859), Darwin can only refer to what we might call 'macroorganisms', a name without any phylogenetic or taxonomic meaning. It is ironic that in his book, addressed to scientists and the lay public alike, Darwin presented overwhelming evidence for the theory of natural selection, establishing a major idea still entirely valid today, without actually explaining where new species come from.

The importance of microbiology (sensu *lato*, the study of both bacteria and their protists descendants) to our understanding of evolution has only recently begun to be appreciated. The cells of microorganisms, as the units of life, are now recognized as key to the evolution of larger life forms, which are bound to the basic mechanisms of microbial evolution, metabolism, and genetics.

When Louis Pasteur and Robert Koch and their coworkers discovered the essential role played by microorganisms in infectious diseases in the last quarter of the nineteenth century, microbiology became a science with practical implications for the well-being of humankind, but with scarce importance for the core of biological science. However, the study of bacteria has made an essential contribution to general biological knowledge (Guerrero and Berlanga 2006, 2007). Albert J. Kluyver (1888–1956) and Cornelis B. van Niel (1897–1985) postulated the metabolic unity

of life and proposed the use of microorganisms to elucidate the biochemical pathways and energy transformations occurring in all living beings (Kluyver and van Niel 1956; Lederberg 2006; Schaechter 2006). All cells use electrochemical ion gradients (usually proton gradients) across membranes for the synthesis of adenosine triphosphate (ATP). Proton gradient is as universal as the genetic code, but why does all life depend on membrane bioenergetics or how has energy influenced evolution? Influenced by Martinus W. Beijerinck (1851–1931), who established the scientific principles of prokaryotic physiology and ecology, Lourens G.M. Baas Becking (1895–1963) set down 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 the Gaia hypothesis (Quispel 1998) and his ideas, which he summarized as "everything is everywhere, but the environment selects," have played an important role in modern studies of the biogeography of microorganisms and the assembly and development of natural communities.

Prokaryotes possess remarkable characteristics and are endowed with a functional potential unknown in the rest of the living world. Miniscule and ubiquitous, they exhibit metabolic variability and flexibility as well as genetic plasticity (horizontal transfer of DNA). Together, these properties allow microorganisms to rapidly adapt to unfavorable and/or changing environmental conditions. We have come to realize that humans are—and always have been—completely dependent on microbial life (Lederberg and McCray 2001; Guerrero et al. 2013). Life itself not only began with prokaryote microorganisms, but also its continuity on Earth depends on them.

2 The Prokaryotic Metabolic World

Living organism "selectively" picks up from the environment some compounds to "create its own world." On the other hand, living organisms excrete products that modify the surrounding habitat. An autopoietic unit is a system capable of self-maintenance by sensing the environment and adapting to changing circumstances. However, autopoiesis alone, while necessary, is not a sufficient condition for sustaining life. Organisms constantly interact with their habitats, not only selectively taking up compounds for their particular needs but also excreting metabolic products and thus changing their environment (Guerrero and Berlanga 2016). The development of ecosystems, or ecopoiesis, has prevented the depletion of the biogenic elements of the planet's surface, something that otherwise would have taken place after a maximum of 200 or 300 million years. Thus, without the emergence of ecopoiesis, which established and maintained the first trophic chains, early life would have been extinguished. Recycling, a feature of the Earth since the beginning of cellular life, is the condition sine qua non by which nutrients are not depleted and requires the collaboration of many different types of cells and metabolisms (Guerrero and Berlanga 2006; Falkowski et al. 2008). Recycling is an essential feature of the Earth and is present on the planet surface since the first steps of cellular life. The

emergence of recycling is thus the consequence of cooperation between species (Guerrero et al. 2002; Sachs and Hollowed 2012).

The members of prokaryotic ecosystems coevolve through their interactions, which constitute the basis of collective metabolic functionality. Prokaryotic cells unavoidably produce resources that benefit other members (i.e., cells or populations) in the habitat. The beneficiaries of these by-products may evolve metabolic dependency on the "donor" cells and dispense with their own costly metabolic pathways. They are "donors." Those other "receivers" of such by-products will tend to delete their own costly pathways for those products. Thus, such metabolic dependency can favor the spread of more obligate coevolved partnerships. This suggests the existence of interdependent cooperative interactions in communities and that bacterial cooperation should leave a clear genomic signature via complementary loss of shared diffusible functions (Sachs and Hollowed 2012). In an anaerobic community, there is nutritional interdependence among the microbial populations. The degradation of organic matter begins with the activity of hydrolytic and fermenting primary anaerobes, followed by syntrophic bacteria and then homoacetogenic, methanogenic, or sulfidogenic secondary anaerobes.

This type of symbiosis is especially relevant, sometimes obligatory, in low energy environments lacking strong electron acceptors, and where many endergonic reactions can become exergonic only when one partner acts as an electron sink for the other (Sieber et al. 2012). For a long time, the enrichment or detection of organisms capable of anaerobic growth on methane and ammonium compounds was unsuccessful, a hindrance eventually overcome by the use of more than one type of prokaryote. For instance, anaerobic methane oxidation can be observed when a syntrophic consortium of an archaeon and a sulfate-reducing bacterium is involved (Timmers et al. 2015). Also, it was shown that the bacteria *Candidatus* Methylomirabilis oxyfera and *Candidatus* Methanoperedens nitroreducens can couple the anaerobic oxidation of methane with denitrification (Welte et al. 2016). It is now well-known that anaerobic ammonium oxidation (or "anammox," as it has been named) contributes significantly to the biological nitrogen cycle in the world's oceans, being responsible for up to 50% of marine N_2 production. The essential process of anammox is mediated by a unique monophyletic group of bacteria that branches deeply in the Planctomycetales (Oshiki et al. 2016).

The most highly evolved interspecific association between prokaryotes is found in phototrophic consortia. For instance, 'Chlorochromatium aggregatum' is a motile, barrel-shaped aggregate formed by a single cell of '*Candidatus* Symbiobacter mobilis' (a polarly flagellated, nonpigmented, heterotrophic bacterium), which is surrounded by approximately 15 epibiont cells of *Chlorobium chlorochromatii* (a nonmotile photolithoautotrophic green sulfur bacterium) (Liu et al. 2013). Most known prokaryote-prokaryote symbioses involve cell-cell contact but not direct endosymbiosis. A rare example of prokaryotic endosymbiosis is provided by the Nanoarchaeota, first described in 2002 as an obligate endosymbiont group, with the marine hyperthermophilic crenarchaeon *Ignicoccus hospitalis* acting as host (Munson-McGee et al. 2015). Ribosomal sequence studies have since shown that Nanoarchaeota is a widespread and diverse group of *Archaea* capable of inhabiting a

broad spectrum of temperatures and geochemical environments that may be associated with a wide diversity of host organisms (Jarett et al. 2018).

3 Prokaryotes at the Dawn of Eukaryotic Life

The history of life on the planet is mostly the history of single-celled prokaryotes. The earliest division of life separated *Archaea* from *Bacteria*, whereas *Eukarya* is a more recent, "secondary" group (Williams et al. 2013; McInerney et al. 2014). The organism from which bacteria and archaea emerged is termed the last universal common ancestor (LUCA). It is important to remember that LUCA was not the first form of life but a "prokaryote" organism that existed when *Bacteria* and *Archaea* first diverged (Sousa et al. 2016; Cornish-Bowden and Cárdenas 2017). Various approaches exist to detect sets of orthologous sequences in bacteria and archaea to reconstruct the microbial ecology and physiology of LUCA, but a clear explanation remains a current challenge because of incomplete genome annotation, inaccurate function annotation, and imperfect understanding of the cellular environments where proteins function. Today, only limited information can be gained about LUCA, and physiological details are largely speculative (Berkermer and McGlynn 2020). In any case, life arose in a world without oxygen, or with only a minimal concentration of the element, so the first organisms were anaerobes (Weiss et al. 2016). Oxygen was present in the atmosphere roughly 2.5 billion years ago and accumulated in the oceans roughly 600 million years ago (Holland 2006; Lyons et al. 2014; Ward et al. 2016). Although the Earth has changed drastically, with oxic habitats now dominant, anaerobes have not disappeared; surrounded by oxic environments, they are still present in a range of ecosystems, such as microbial mats, sediments, or the intestinal gut.

Carl Woese and colleagues showed that all cellular life could be divided into three major evolutionary lines or domains: *Archaea* and *Bacteria* (both prokaryotes) and *Eukarya* (or eukaryotes) (Woese et al. 1990). In subsequent years, molecular phylogenetic analyses strongly suggested that eukaryotes and the archaea represent sister groups in the tree of life. Studies in the recent genomic era have shown that eukaryotic cells possess a mixture of archaeal and bacterial features in addition to those specific to eukaryotes (Eme et al. 2017). Eukaryotic genomes have two fractions, each with different functions and evolutionary origins: the "informational genes," with a role in translation, transcription, and replication, which are closely related to archaeal homologs, and the "operational genes," involved in energy, intermediary metabolism, and synthesis of cell components, which are more closely related to their bacterial homologs (Rivera et al. 1998; Thiergart et al. 2012).

All step in evolution depends on many former steps. It is now well-established that the bacterial ancestors of the membrane-bound organelles, mitochondria and chloroplasts, evolved from Alphaproteobacteria and Cyanobacteria, respectively. Accepted in mainstream science, the serial endosymbiotic theory (SET) describes the symbiogenetic origin of mitochondria and plastids in eukaryotic cells, as

originally proposed by Lynn Margulis. Symbiogenesis refers to the appearance of new morphologies, tissues, metabolic pathways, behaviors, or other recognizable evolutionary novelties in living organisms (Guerrero et al. 2013). Margulis' seminal article "On the origin of mitosing cells" (published under the name of Lynn Sagan) (Sagan 1967) has been hugely influential on evolutionary thinking, and even today, more than 50 years later, her visionary ideas about the role of symbiosis in eukaryogenesis and evolution remain remarkable. However, the origin of her eukaryotic "nucleocytoplasm" is still controversial (López-García et al. 2017). For the eukaryotic cytoskeleton and the mitotic apparatus, Margulis proposed that, similar to mitochondria and chloroplasts (many of whose genes are found in the cell nucleus), a remnant genome of a spirochete would be found in connection with the (9+2) microtubular basal bodies associated with eukaryotic flagella (Margulis et al. 2000). However, such remnant genomes have not been detected, and phylogenomic analyses have not revealed any particular similarity between eukaryote and spirochete genomes.

The first detailed electron micrographs of the bacterium *Planctomyces* were highly revelatory because an internal membrane could be clearly observed in the cytoplasm. It had previously been reported that organisms belonging to the phylum Planctomycetes possess several features typical of eukaryotes, such as cytosolic compartmentalization and endocytosis-like macromolecule uptake (Boedeker et al. 2017), which prompted discussion that the phylum could be a proto-eukaryotic group or somehow a forerunner of the eukaryotic nuclear membrane. However, detailed structural-biochemical studies showed that Planctomycetes possess a peptidoglycan cell wall and a cell plan comparable to other Gram-negative bacteria. Furthermore, the nucleus-like structure resembles an invagination of the cytoplasmic membrane rather than a separate compartment. Thus, while the phylum is distinct from other bacteria, its bacteria-like features challenge a eukaryotic ancestry (McInerney et al. 2011; Wiegand et al. 2018).

Today, there is no doubt that contemporary eukaryotes are chimeras integrated by at least two or three types of cells: (a) an ancient alphaproteobacterium that developed into mitochondria, (b) an ancient cyanobacterium that evolved into chloroplasts, and (c) the host cells of (a) and (b), which were probably from an archaeal lineage, recently named 'Asgard' archaea (Imachi et al. 2020). As prokaryotic symbioses essentially involve syntrophy, eukaryogenesis by symbiogenesis is most likely based on metabolic cooperation (López-García et al. 2017; Imachi et al. 2020).

Eukaryogenesis comprises the evolutionary events that occurred between the existence of the first eukaryotic common ancestor (FECA) and the last eukaryotic common ancestor (LECA). FECA represents the beginning of the coevolution of the archaea-bacteria consortium, and its only living descendants are eukaryotes (Eme et al. 2017). Phylogenomic and comparative genomic analyses have led to the hypothesis that LECA lived ~1000–1800 Ma ago (Eme et al. 2014), although the timing and influence of mitochondrial endosymbiosis in the origin of eukaryotic cells are still controversial. One model proposes that endosymbiosis occurred in a proto-eukaryotic host that already had many eukaryotic cellular features (such as the

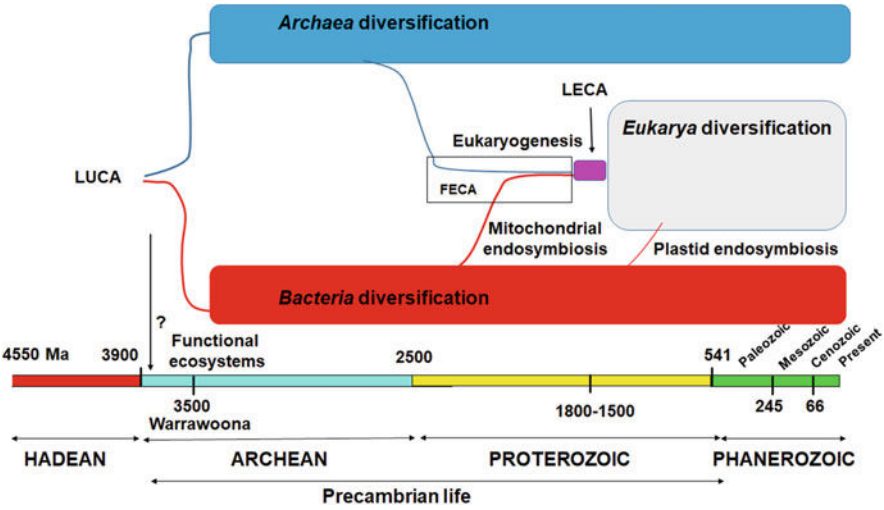

Fig. 1 Earth's timescale, showing the deepest division of life on the planet into *Archaea* and *Bacteria*, and the more recent separation of a secondary cellular group, the eukaryotes. The last universal common ancestor (LUCA) would be the "prokaryote" that existed when *Bacteria* and *Archaea* diverged. Eukaryogenesis comprises the evolutionary sequence of events between the appearance of the first eukaryotic common ancestor (FECA) and the existence of the last eukaryotic common ancestor (LECA)

nucleus and a sophisticated endomembrane system). In contrast, another model postulates that mitochondrial endosymbiosis took place very early during eukaryogenesis (~1.700 Ma) and has been the main driving force behind the evolutionary innovations between FECA and LECA (Eme et al. 2017; López-García et al. 2017). The primary plastid endosymbiosis occurred after LECA and before the last common ancestor of Archaeplastida between 900 and 1300 Ma (Eme et al. 2014) (Fig. 1).

Mitochondria not only provided an efficient energy metabolism of oxygen respiration, but also genes of mitochondrial origin seem to be involved in a variety of cell activities in eukaryotes, such as DNA repair, and complex cell signaling events, such as those involved in regulating cell pluripotency, division, differentiation, senescence, and death (Friedman and Nunnari 2014; Lin et al. 2007; Giacomello et al. 2020). Mitochondria probably played a role in important changes in the host cell at various functional levels. In some anaerobic eukaryotic organisms without mitochondria, certain mitochondrial activities can be maintained thanks to the genes transferred to the nucleus and the maintenance of derived organelles such as hydrogenosomes with possible energy-related activities. Therefore, mitochondria may be intimately linked to the origin of eukaryotes.

Current data suggest that eukaryotes might have emerged from an archaeal lineage known as the Asgard archaeal superphylum. The name refers to the realm of the gods in Norse mythology, as do the names of related lineages or phyla, Lokiarchaeota, Thorarchaeota, Heimdallarchaeota, and Odinarchaeota (Spang et al.

2015; Zaremba-Niedzwiedzka et al. 2017). Members of the phylum Lokiarchaeota were isolated from underwater rift valley sediments on the Knipovich ridge in the Arctic Ocean, near the site of Loki's Castle hydrothermal vents, for which the phylum is named. The superphylum Asgard shares more similar genes with eukaryotes than the rest of the known archaea. These genes encode "eukaryotic signature proteins," including those involved in membrane trafficking, vesicle formation and/or transportation, and ubiquitin and cytoskeleton formation (Spang et al. 2015; Eme et al. 2017; Imachi et al. 2020).

Metagenomic studies have suggested that Asgard archaea are capable of syntrophic degradation of amino acids to short-chain fatty acids and H_2, possibly by interacting with H_2-scavenging (and indirectly O_2-scavenging) sulfate-reducing bacteria. They are dependent on symbiotic interactions for anabolism (biosynthetic pathways in nearly all extant forms) and have fermentative metabolic features (Imachi et al. 2020). Unlike eukaryotes, which use ester-type lipids, the archaea possess ether-type lipids, which raises the question, if the eukaryotic host lineage originated from within the archaea, how did the bacterial-like eukaryotic phospholipids evolve? It has been reported that different lipid types can mix together without losing membrane integrity, suggesting that host ether-type lipids could have been replaced with ester-type lipids (Caforio et al. 2018). A more complete picture of the origin of eukaryotes and their early evolution from prokaryotic ancestors will surely emerge in the near future, given the new research opportunities being created by the technology-driven revolution in genomic and metagenomic analysis.

4 Several Traits of Eukaryotic Cells Are Also Present in Prokaryotes

Two characteristics usually attributed in exclusivity to eukaryotes are multicellularity and epigenetics. The classical study of bacteria as planktonic unicellular life forms is recognized in the axenic ("pure") culture paradigm. However, in nature, bacteria predominantly exist as communities of sessile cells that develop as biofilms. Biofilm formation is a nearly universal bacterial trait. The term biofilm refers to heterogeneous structures comprising different populations of microorganisms surrounded by a matrix (mostly of exopolysaccharides) that allows their attachment to inert (e.g., rocks, glass, and plastic) or organic (e.g., skin, cuticle, and mucosa) surfaces (Berlanga and Guerrero 2016a). Biofilm development presents three distinct phases: attachment (from planktonic cells to sessile cells), maturation (active sessile cells), and release (from sessile cells to planktonic cells). Altogether, bacterial biofilms have a complex structure and a coordinated and cooperative physiology that can be considered analogous to multicellular organisms (Nadell et al. 2008).

About epigenetics, the differences in gene expression between planktonic cells and biofilm communities include the upregulation and downregulation of distinct

Table 1 Several examples of conserved or homologous bacterial molecules in eukaryotic cells

	Bacterial function	Eukaryotic homolog
FtsZ	Cell division (septum formation)	Tubulin
FtsA	Stabilization of Z-ring; recruitment of proteins to the division zone	Actin
MreB/Mbl	Cell shape in rods	Actin
Crescentin	Cell shape in *Caulobacter*	Intermediate filaments
Cellulose	Extracellular polysaccharides, which form protective envelopes around the cells, e.g., *Acetobacter*	Basic structural material of plant cells
Peptidoglycan (*N*-acetylglucosamine and *N*-acetylmuramic acid)	Cell wall	*N*-acetylglucosamine in chitin (exoskeleton of insects and fungal wall cells)
Sterols (tetracyclic triterpenoid lipids)[a]	For maintaining membrane fluidity and stability: Lanosterol (Myxobacteria) and cycloartenol (Bacteroidetes and Alphaproteobacteria)	Stigmasterol (plants) Ergosterol (fungi) Colesterol (animals)

[a]See "Sterol biosynthetic pathways and their function in bacteria" by J.D. Franke, this book

sets of genes (Nakamura et al. 2016). Furthermore, in many bacterial species, DNA methylation controls reversible switching (phase variation) of gene expression, a phenomenon that generates phenotypic cell variants. Therefore, bacterial epigenetics enables the adaptation of bacterial populations to changing environments (Sánchez-Romero and Casadesús 2020).

Another bacterial trait is their metabolism. The enormous metabolic diversity of bacteria allows them to occupy the most diverse ecological niches imaginable and to be at the base of every trophic net in the biosphere (Guerrero and Berlanga 2006). However, a simple observation of a bacterium under the photonic microscope is not very revealing. Most bacteria appear as plain rods or small spheres, without any visible characteristics. Due to this apparent morphological simplicity and their miniscule size, prokaryotes were initially believed to lack any cytoplasmic organization or other traits typical of eukaryotes (Guerrero and Berlanga 2007).

We now know that the bacterial cytoplasm is incredibly complex in terms of structure and its ability to synthesize highly specialized molecules (Pilhofer and Jensen 2013). Like their eukaryotic counterparts, bacteria employ a full complement of cytoskeletal proteins. The bacterial cytoskeleton localizes proteins and DNA to specific subcellular addresses at specific times and is indirectly responsible for the maintenance of the cellular form, DNA segregation and cell division, and the formation of inclusion bodies such as polyhydroxyalkanoates or magnetosomes; it also controls some types of surface cell movement (Cabeen and Jacobs-Wagner 2010) (Table 1).

At a molecular level, bacterial cells are highly organized (Govindarajan and Amster-Choder 2016). An attribute of living systems is a nonuniform spatial and temporal cellular distribution of macromolecules. Bacterial proteins are localized by

the sensing of "geometric cues," and subtle differences in membrane curvature initiate spore formation in several Firmicutes (Updegrove and Ramamurthi 2017).

The dynamic nature of the bacterial wall is a recent discovery. The production of membrane vesicles (MVs) is a universal mechanism for intercellular communication in both eukaryotes and bacteria. In terms of structure and biological activities, Gram-negative bacteria-released outer-membrane vesicles (OMVs) and Gram-positive bacteria-released MVs share significant similarities with mammalian cell-derived MVs (e.g., microvesicles and exosomes) (Schwechheimer and Kuehn 2015; Brown et al. 2015; Yu et al. 2018). Bacterial OMVs/MVs have been shown to play diverse roles in nutrient uptake, antimicrobial defense, horizontal gene transfer, biofilm nucleation, toxin release, and immune system modulation that can facilitate infection and pathogenicity (Pathirana and Kaparakis-Liaskos 2016; Dean et al. 2020). OMVs are produced by wall evaginations during bacterial growth and are not products of cell lysis (Schwechheimer and Kuehn 2015). Ranging from 20 to 250 nm in diameter, they contain pieces of outer membranes, outer membrane proteins, components of the periplasmic space, and sometimes also DNA and RNA and pieces of cytoplasmic membranes. Notably, the protein profiles of OMVs are similar but not identical to those of the outer membrane. Several proteins are in greater representation and others are absent, suggesting that the formation and release of OMVs do not occur randomly, but they are an active and regulated process (Schwechheimer and Kuehn 2015).

Programmed cell death, or apoptosis, is a fundamental process in eukaryotes, highly evolutionarily conserved, and crucial for proper embryogenesis, maintenance of the immune system, and elimination of damaged cells. Apoptosis is different from the random process of necrotic cell death, since it eliminates individual cells without inducing an inflammatory response. As well as existing in several protists (unicellular eukaryotes), apoptotic processes also seem important in bacteria, such as cell lysis in bacillus sporulation, vegetative cell lysis in the formation of fruiting bodies in myxobacteria, and competence for DNA transformation in streptococcal cells undergoing autolysis (Lewis 2000; Koonin and Zhang 2016). One of the most studied systems of apoptosis in bacteria involves a genetic unit composed of two genes, one encoding a labile antitoxin that interferes with the lethal action of the toxin encoded by the other. This toxin-antitoxin genetic system (for example, the *maz*EF system) has been found in *Escherichia coli* and other bacteria, including several pathogens (Nikolic 2019). Nevertheless, although genes and their products have been identified as involved in bacterial apoptotic mechanisms, any similarity to mammalian apoptosis may be only very slight (Häcker 2013).

The day-night cycle, resulting from the Earth's rotation, and natural selection have favored the formation of genetic circuits that act as "timekeepers" and maintain the internal and environmental clocks in synchrony. Over the past 4 billion years, the length of Earth's rotation has significantly changed. Initially, during the early Archean Eon (3900–3500 Myr ago) the Earth completed one rotation cycle in a period of 4 h, which grew to approximately 20 h during the first Cambrian period (about 542 Myr ago), before eventually reaching the 24 h of the present day (Bhadra et al. 2017). Circadian rhythms are ubiquitous among eukaryotes, but evidence of

biological clocks that drive these rhythms has also been described in prokaryotes, for example, in cyanobacteria and gut bacteria. In cyanobacteria, a cluster of three genes (*kaiA*, *kaiB*, and *kaiC*) encodes central oscillators with a periodicity of ~24 h, which is essential for the regulation of nitrogen fixation, photosynthesis, and cell division (Cohen and Golden 2015). In the human intestine, *Enterobacter aerogenes* responds to the circadian hormone melatonin and exhibits an endogenous daily rhythm (Paulose et al. 2016).

Recent blooming of research on the intestinal microbiome has revealed that the myriad of microorganisms residing in the animal gut play an essential role in the health of the host. The gut microbiota is reported to exhibit circadian oscillation and is synchronized with the host circadian clock (Liang and FitzGerald 2017). Gut bacteria pathways involved in energy, carbohydrate, and amino acid metabolism are enriched in the active phase. Furthermore, such daily oscillation leads to changes in metabolites excreted by the microbiota, such as butyrate and propionate, which increase at the beginning of the active phase and remain lower throughout the rest of the day (Liang and FitzGerald 2017). Adaptation between the host and its bacteria collectively depends on a circadian clock that communicates signals to the gut microbiota, leading to synchronization and functional coordination (Fig. 2).

5 The Concept of the Holobiont as a Unit of Functionality

Evolutionary biologists have traditionally described genetic changes as the main source of phenotypic variation. Mutations lead to adaptation through natural selection and finally generate diversity among species. However, another mechanism of evolutionary innovation could be symbiogenesis and the concept of the holobiont, an entity better adapted to the environment than its individual constituents. Joshua Lederberg (1925–2008) defined the holobiont as "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space" (Lederberg and McCray 2001). As such, the holobiome, i.e., the total genetic information provided by an animal or plant and its associated microbiota, plays a crucial role in the complex and coordinated coevolution of holobiont organisms. Symbiogenesis could provide support for the theory of punctuated equilibrium proposed by Niles Eldredge and Stephen J. Gould (Eldredge and Gould 1977), which describes evolution as largely static, interrupted by abrupt and often dramatic intervals, brief on a geologic timescale, as evidenced by the periodic major changes in fossil records.

Many prokaryotes are known to live in association with eukaryotes, which can acquire their symbionts by maternal inheritance (transovarial or acquisition in utero) or by transmission from the surrounding habitat, which involves a new colonization with each generation) (Berlanga and Guerrero 2016b). Although embryogenesis in animals often takes place without direct contact with bacteria, tissues formed in subsequent development interact with coevolved microbial species, leading to the development of healthy and stable microbial communities (McFall-Ngai 2002).

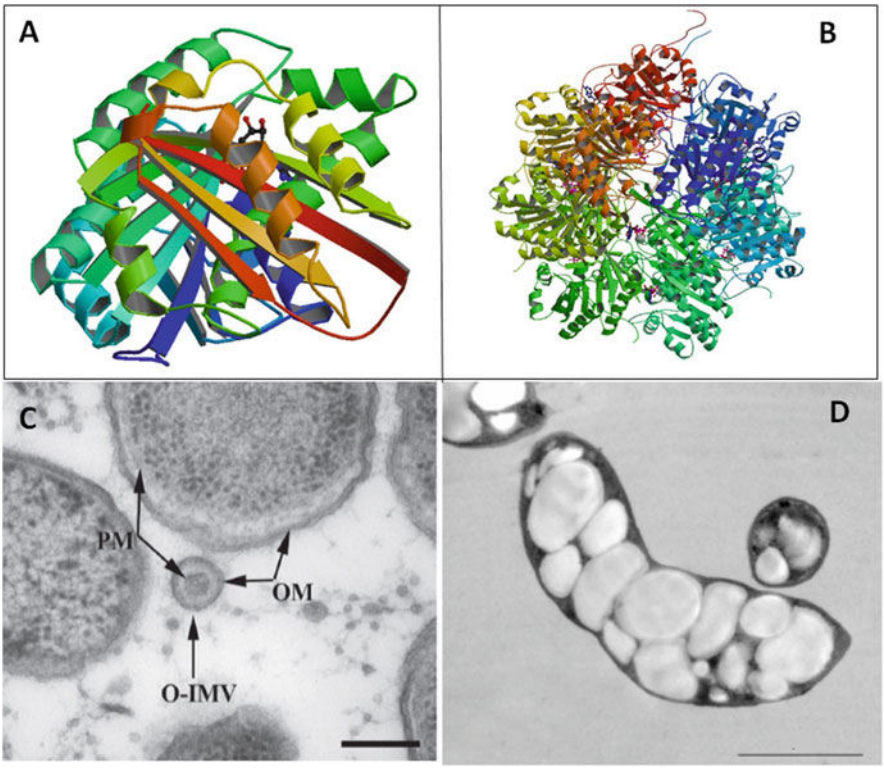

Fig. 2 (**a**) Cytoskeletal protein FtsZ from *Pseudomonas aeruginosa* GDP. (Protein Data Bank: https://doi.org/10.2210/pdb2VAW/pdb.). (**b**) Circadian clock protein KaiC crystal structure from *Synechococcus elongatus* PCC 7942. (Protein Data Bank: https://doi.org/10.2210/pdb2GBL/pdb.) (**c**) O-IMVs (outer-inner membrane vesicles, marked with arrows) released by *Pseudomonas* PAO1 (Pérez-Cruz et al. 2015). (**d**) Transmission electron micrograph of strain MAT-28 from Ebro Delta microbial mats showing granules of PHB (polyhydroxybutyrate). Accumulation of intracellular storage polymers, such as PHB, serves as an endogenous source of carbon and energy during starvation (Guerrero and Berlanga 2007)

Indigenous microbiota normally colonize areas of the animal body that are exposed to the external environment, such as the skin, eyes, oral cavity, and the respiratory, urinary, reproductive and gastrointestinal tracts.

Despite the abundance of microorganisms in the environment of an animal, only certain populations are able to permanently inhabit the available body sites, even though the composition of specific host-associated microbial communities may vary as a function of time and place. These associations must be maintained with fidelity during the life history of a given organism, over generations of species and through evolutionary time. In the evolution of beneficial symbiosis, species interaction is therefore maintained by natural selection (Wein et al. 2019).

The holobiont is an essential life-changing force that has resulted in a complex coordinated coevolution of life forms. As part of animal/plant systems, microbial

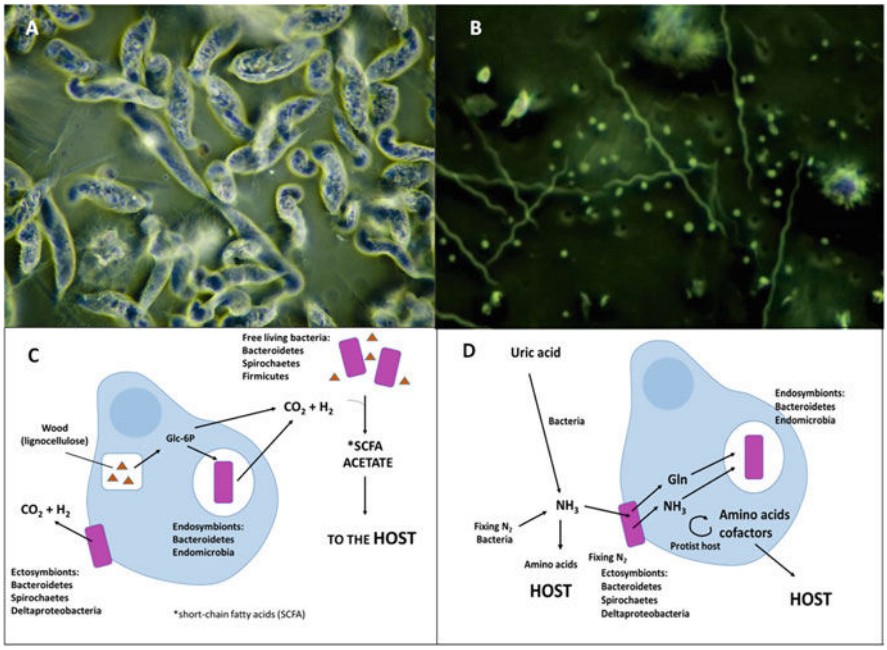

Fig. 3 (**a**) Protist microbiota from the hindgut of the lower termite *Reticulitermes grassei* (photograph by R. Duro). (**b**) Spirochetes from *R. grassei* (photograph by R. Duro) (Guerrero et al. 2013). (**c**) The illustration shows the fermentative breakdown of wood polysaccharides. (**d**) Nitrogen supplied to the host by bacterial symbionts (Berlanga 2015)

communities may have differing net impacts on host fitness according to the actual environmental circumstances (Gould et al. 2018). They are fundamental in a range of physiological processes, including tissue development, nutrient absorption, immunity, and circadian regulation (Flint et al. 2012; Parkar et al. 2019).

The gut microbial structure is shaped by the host diet and local environment (Yun et al. 2014). Different diets may change the gut environment, affecting factors such as the C/N ratio, the O_2–H_2 gradient, and intermediate metabolites, so shifts in the bacterial community are also expected (Berlanga et al. 2009). A random event (such as a dietary change or antibiotic treatment) may alter the environment with possible repercussions on the community structure (Berlanga et al. 2018) (Fig. 3).

The microbial community may maintain stability by responding to disturbance with resistance, remaining unchanged in composition, or resilience, and recovering the state prior to the disturbance in terms of species (composition) or function (genes) (Faust et al. 2015). During an antibiotic treatment, within this dynamic state of composition and function, the remaining microbiota can compensate for the loss of other community members. However, sometimes the disturbance of the community or environment produces such an abrupt change in microbial composition that a turning point is reached, preventing a return to the previous "stable" state. In the case of the gut microbiota, this new situation could be associated with a

"healthy" or "diseased" state. The latter, known as dysbiosis, leads to reduced fitness and can eventually generate disease in the host.

6 Coda

From our anthropocentric vision, the evolutionary pathway leading from the Cambrian explosion to humans may seem a milestone in the history of life. However, the major evolutionary step was in fact the development of nucleated cells, and since then, evolution has only produced variations of the same essential type of organism, i.e., the eukaryotes.

Although many kinds of microorganisms (especially protists and some bacteria) have been known since the end of the seventeenth century, their importance as the basis of all life has been understood only quite recently. Thus, in the last paragraph of *The Origin of Species*, where Darwin describes life's interconnectedness as a "tangled bank," he refers only to animals and plants. As we have discussed, prokaryotes were believed to lack any internal organization or other characteristics typical of eukaryotes. However, the last few decades have demonstrated that the structure and function of the prokaryotic cell are much more 'intricate' than initially thought. Prokaryotes can not only sense their environment and respond as individual cells to specific external challenges but can act cooperatively and perform communal activities. In many microbial ecosystems, the functionally active unit is not a single species or population (clonal progeny of the same prokaryote) but a consortium of several (usually more than two) types of cells living in close symbiotic associations. Prokaryotes live and die in complex communities that in many ways resemble multicellular organisms. Life on Earth has changed drastically from when it first emerged, with the main difference regarding microbes being the rise of oxic habitats and the evolution of a complex diversity of populations that integrate communities. Although biological (including microbiological) diversity may vary considerably across ecosystems, metabolic interactions remain similar, showing a "taxo-ecological homeostasis" (Berlanga et al. 2009), in which, to borrow an analogy from Evelyn Hutchinson, the actors of the play may change, but the plot remains the same.

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