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## Evolution of the genomic systems of prokaryotes and its momentous consequences

Received: 15 February 2001 / Accepted: 30 April 2001 / Published online: 11 September 2001  
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**Abstract** The earliest self-reproducing cell on Earth, our common ancestor, was probably as small as present-day bacteria. It gave rise to a very large and durable clone whose descendants must have been the only living occupants of the oceans for about one thousand million years. They reached astronomical numbers of separate, disjunct cells, and synthesized many new genes. Their small volume could not accommodate ever larger genomes and useful new genes replaced resident, less successful sequences, thus increasing diversity and the number of strains with highly specialized, distinct, bioenergetic potentialities. Also, selective pressure favored strains able to participate successfully in division of labor and in the sharing of diverse abilities in mixed communities, counterbalancing the limited capacities of individual genomes. Lateral gene transfer mechanisms appeared and were progressively improved, furthering the development of diversity. The prokaryotes' constructive evolution resulted in the formation of a worldwide web of genetic information, and a global bacterial superbiosystem (superorganism). By contrast, eukaryotic evolution of organisms has been typically Darwinian. Diversification of eukaryotic organisms was, however, considerably enriched and accelerated by symbioses with prokaryotes. The more broadly diversified bioenergetic potential of prokaryotes considerably increased the diversity of eukaryotes. Without their participation, our biosphere would have remained much less diverse and less dynamic. Environmental homeostasis has been maintained all along by guided bacterial evolution.

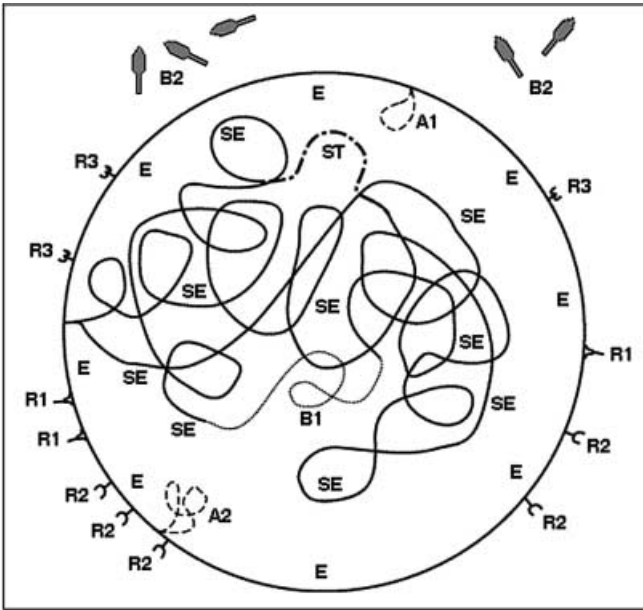
**Keywords** Prokaryotes · Evolution · Genomic systems

### Introduction

Prokaryote evolution is emerging as a most significant subject in biology, modifying and replacing the former eukaryote-centered approach. However, some essential differences between all bacteria (prokaryotes, including Archaeobacteria) and the eukaryotes are still ignored by many [17, 20, 28]. The following main features of bacteria have to be kept in mind to understand their place and fundamental role in nature, now as well as during their long and dynamic evolution:

1. The volume of most bacterial cells is 1,000 times smaller than that of eukaryotic cells and it contains fewer genes. This has probably been so since the origin of life. Microfossils convincingly show that small size was already a feature of bacterial cells 3,000 million years ago [2]. Small size allows for rapid multiplication and high concentrations of cells (as many as  $10^9$  ml<sup>-1</sup> in a favorable habitat). Small size has acted as a positive factor in cellular diversification of prokaryotes, each strain maintaining a minimal genome with specialized bioenergetic abilities. Local communities of complementary strains where division of labor is practiced compensate in part for the limited potential of individual genomes.
2. Prokaryote genes are not organized in chromosomes; rather, they are assembled in circular, self-replicating DNA molecules. The genes coding for the division of the cell and for its essential bioenergetic activities are located in the largest of the DNA molecules, the large replicon – also called the genophore (Fig. 1). Genes carrying the information for non-essential functions are usually present in much smaller, self-replicating, DNA structures called small replicons (plasmids and prophages). They are present in all prokaryotic cells and their number varies from one to, in some cases, more than 15 [7]. Small replicons do not belong exclusively to their host cell. Their copies may engage in lateral (horizontal) gene transfer (LGT) and “visit” different types of strains to which they bring new,

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**Fig. 1** The different types of DNA molecules of a bacterial cell. Stable and permanent constituents of the large replicon (genophore): *SE* genes encoding basic, essential bioenergetic functions, *ST* genes coding for lateral gene transfer (LGT) by transformation. Visiting genes, exchangeable between different types of strains (small replicons): *A1* non self-transmissible plasmid, *B1* prophage – stable, intracellular phase of a temperate bacteriophage integrated in the length of the large replicon, *B2* extracellular genes of a prophage inside the head of its temperate forms “liberated” from an identical cell of the same strain and able, among other properties, to fetch favorable genes for their host strains, *E* “equator” of the cell’s membrane where the self-replicating DNA molecules start their replication, *R1*, *R2*, *R3* receptors participating in the transfer of self-replicating small replicons

useful genes. Small replicons had their own long evolution, in part outside their present type of host cell. Recent work [4] brings further support to the belief that bacteriophages (like bacteria) are the result of both vertical and horizontal evolution. They are capable of exchanging multigenic elements (modules). Small replicons are an essential part of the global prokaryotic entity; a concrete proof of the latter’s unity of system [30]. Other genes also contribute to LGT but reside in the large replicon of their host cell. The genes that encode the information for the transformation mechanism are distributed among several of the best known prokaryotic families, and bacterial gene transfer by natural genetic transformation occurs in the environment [15]. We infer that after thousands of millions of years of evolution, transformation remains a generalized phenomenon with its main features preserved. It takes place in many natural habitats [21] and has been observed among widely different taxonomic and trophic groups, including Archaeobacteria [8]. In addition to general LGT mechanisms, within most prokaryotic strains transposons can easily move genes from one type of replicon to another or to different points on

the large replicon, thus increasing the likelihood of transfer to other cells. The observation made by Japanese workers four decades ago [31] that infectious bacteria received (probably from soil bacteria) resistance genes to antibiotics was a major landmark in the understanding of the role and importance of small replicons in the biology of bacteria. Subsequently, and as early as the 1960s and 1970s, some microbiologists realized that LGT plays a fundamental role in the life of most bacteria and in their evolution [1, 13, 24, 29]. More recently, studies with protein or ribosomal RNA nucleotide sequences have proved that LGT has been surprisingly frequent in prokaryotes since the beginning of life on Earth, a major and continuing evolutionary force transcending the borders of higher taxa or groups including that of Eubacteria–Archaeobacteria [6, 14, 33, 34].

3. The large majority of bacteria live in typical, “social” prokaryotic entities: communities of metabolically complementary strains [26]. These communities can adjust easily and quickly to changes in their surroundings as they respond to selective pressures by accepting “outside” strains that are better equipped to face a specific challenge. In such circumstances, less adapted strains are gradually displaced, with adaptive modifications in populations taking place and representing episodes of constructive evolution. Similarly, at the cellular level, the option to accept a more favorable gene brought in by transformation or by visiting small replicons (via LGT) exists for all prokaryotes studied. Recent studies show clearly that collaboration among strains of Eubacteria on the one hand, and between Eubacteria and Archaeobacteria, on the other, is frequent and often essential to their survival. Large numbers of prokaryotes (many as yet undescribed) live in the soils of forests, of cultivated fields and of grasslands, in aquatic habitats [32], in plankton [5], in the rumen of wild and domesticated ruminants [12], and at the surface of the leaves of all plants (phyllosphere) [11], where they form exceedingly complex consortia or communities. Simpler, but well-structured archaeal–eubacterial symbiotic aggregates (e.g. apparently one eubacterial strain and one archaeobacterial strain) have been found in large numbers in some ocean sediments [3]. In complex consortia, incessant selective pressures select the best mixture of phenotypes available for the prevailing conditions. Since very large numbers of both cells and genes are involved, numerous options to solve problems in a computer-like fashion are made available [25]. The entire prokaryotic genetic patrimony is thus available to most cells, resulting in a global biological communication system [27]: a world-wide web of genetic information at the disposal of prokaryotic cells [28]. Often, the modifications are only temporary, but some represent permanent constructive evolutionary changes. The replaced genes or strains are not eliminated all at once; rather, they slowly fade out, and identical copies are maintained in other

- niches. We infer that there have been few extinctions of genes or types of cells in bacteria, in contrast to the successive elimination of eukaryotic species.
4. Adaptive biochemical complementarity between cells of communities and LGT result in global solidarity among prokaryotes. The forces of selective pressure favor the best solution for local cells and groups, and for the global bacterial superorganism resulting in the maintenance of its homeostasis and, as a consequence, that of the biosphere. Nearly three decades ago, the Gaia hypothesis, based on studies of the stability of reactive atmospheric gases, had predicted that the biosphere is maintained in a form favorable to life by its living elements [16]. Bacterial activities have been the most constant positive factor of global homeostasis and they directly influence the basic chemistry of the Earth's surface.
  5. Bacteria associate not only with themselves but, to a very large extent, with eukaryotes too. Examples are numerous and can be found everywhere. The very close symbiotic relationship between the highly specialized bacterial communities that inhabit the rumen of many herbivorous animals allows the latter to use complex carbohydrates like cellulose as a source of energy [12]. Cellulolytic and lignin-degrading termites benefit from similar symbiotic communities, and many insects and marine animals also ultimately depend on the metabolic versatility (cellulolysis, nitrogen-fixation, methanogenesis) of symbiotic prokaryotes. Following a series of publications by Margulis, two major revolutionary notions were introduced into contemporary biology: eukaryotes originated from the endosymbiosis between three bacterial strains with complementary bioenergetic capacities [18], and the evolution of eukaryotes has later been complemented by a large variety of endo- and/or ectosymbioses with prokaryotes, resulting in the extreme richness in form and diversity of life around us, and homeostasis for our biosphere [19]. Bacteria are the living beings with the longest evolution on Earth, the direct descendants of the last common ancestor. The prokaryotic world has adjusted to, and collaborated with, the eukaryotes while maintaining its own evolution in parallel. Some notions about prokaryotes (including the contents of some biology textbook chapters) should be reappraised and modified to take into consideration the special genetic and metabolic systems of bacteria, their fundamental roles in the biosphere, and, particularly, their evolution [28].

been a bacterium (prokaryote). Within a few centuries, it probably gave rise to a giant clone which progressively invaded the watery habitats, in particular the oceans [28]. Those expanses of water may have covered, as they do now, about two thirds of our planet's surface and had a similar, fairly stable, chemical composition. It represented a giant culture medium in which the bacteria multiplied, eventually reaching astronomical total numbers. In these enormously vast and populated niches, a large number of different genes were produced as a consequence of serial random mutations. These new genes, when favorable for the cells that carried them, were eventually replicated, increased in number and disseminated in the oceans. The global bacterial clone was thus progressively enriched not only numerically, but with cells which had acquired new bioenergetic potentialities and, thereby, diversity. These rapidly dividing cells remained small and therefore a stringent limit was imposed on the total number of new genes that they could accept and maintain. In the evolution of bacteria, individual cells did not tend to become larger with ever more complex and larger genomes. Selective pressure worked in two directions: efficient division of labor by complementarity between cells of the same community, and refinement of mechanisms of LGT that opened the global stock of hereditary information to other strains. Among the LGT mechanisms, the temperate phages reached the highest capacity for gene exchanges [28]. Prokaryotes probably had no serious enemies for one thousand million years. They did not need special protective conditions to permit their evolution. A contrast can be made here with the evolution of social animals' communities (bees, ants, termites, some rodent species, etc.), which early in their collective development built their own protected and well-isolated shelters (e.g. hives) to take advantage of reasonably safe conditions for a guided evolution based on division of labor and a secure, buffered mini-environment.

Prokaryotes that contributed to stabilize or even improve the general living conditions in their niches were preferably selected. This ensured not only their own survival but helped improve, as already mentioned, the homeostasis of the biosphere. Sharing of complementary bioenergetic capabilities, and genes from different strains, among bacteria favored multiplication and survival of individual cells with a minimal genome. A global communication system – a free market of genetic material for bacteria – was opened [17, 25]. Instead of “opting” for the Mendelian laws of heredity, bacteria created a global, potentially common genome. Consequently, we conclude that there are no species in prokaryotes; they lack reproductive isolation. Table 1 summarizes some of the strong evolutionary contrasts and innovative bonds between prokaryotes and eukaryotes.

Naturally, not all the cells in populations adapted to local niches remain there permanently. Many are dispersed by wind, the migration of animals, the flow of ships and water, of commercial goods, of human

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### **Guided, constructive evolution of prokaryotes**

The earliest organism on earth (the hypothetical last common ancestor) that had a sufficient level of cellular organization to divide into two offspring cells, and from which all life on our planet descends, is believed to have

**Table 1** Strong evolutionary contrasts and innovative bonds between prokaryotes and eukaryotes

Prokaryotes (Bacteria)	Eukaryotes
<p>Appeared about 3,500 million years ago in the oceans as a single clone of disjunct cells; were the only living entity for the next one thousand million years</p> <p>Cells multiplied, reached astronomical numbers, synthesized a large quantity and variety of new genes, giving rise to enormous bioenergetic diversification among offspring. Selective pressure favored metabolic complementarity and division of labor among small cells with minimal genomes, and nassociation in local communities – an essential feature of the prokaryotic world</p> <p>Early in prokaryotic evolution, mechanisms for lateral gene transfer (LGT) appeared, which had a surprisingly similar evolution in most bacteria. LGT by genetic transformation and exchange of “visiting” genes (plasmids and prophages, Fig. 1) greatly facilitated efficient communication among the cells, gave them access to a global genome and prevented massive extinctions of genes. Exchange of cells between communities and LGT between cells are the basis of a global prokaryotic super bio-system or superorganism</p> <p>About 2,500 million years ago predator bacteria appeared and fed on other strains without major disruption of the main structure and functions of the superorganism</p> <p>A very large number of prokaryotic cells were engaged in symbioses with eukaryotes and established successful, permanent collaborations with them. They multiplied abundantly in their new conditions, generally adopting eukaryotic means of gene transmission and evolution</p>	<p>Originated about 1,500 million years ago from endosymbiosis of different types of bacteria, one of the latter being a predator. The descendants kept the ancestral genes and added new ones by synthesis and random serial mutations</p> <p>All eukaryotes stopped exchanging genes via LGT. Their evolution has been, and still is, typically Darwinian, based on the “struggle for life”</p> <p>Eukaryotic genes were organized in chromosomes, with species representing typical, genetically isolated, constitutive elements. However, they engaged with metabolically complementary prokaryotes in ecto- and endosymbioses that resulted in the most fateful evolutionary events, among them the addition of photosynthesizing Cyanobacteria to the ancestors of vascular plants</p>

populations, and other means. For example, on the east coast of the United States, Chesapeake Bay alone has been estimated to receive some  $10^{10}$  l of foreign ballast water each year. Each liter typically contains about  $10^9$  bacteria and  $7 \times 10^9$  virus-like particles [23], many of them probably temperate phages.

Today’s global prokaryotic superorganism is the result of a guided, constructive evolution strikingly different from the typical Darwinian evolution of the eukaryotes. The latter may have been initiated with the appearance, about 2.5 billion years ago, of a new type of bacteria: the predators [10]. They attacked and digested other types of bacteria, in this way inaugurating the fierce competitive Darwinian aspect of evolution. However, they did not seriously menace or replace the other types of bacteria existing at the time. The latter survived and developed into the global superorganism that we observe today. Nevertheless, the predators may have been very important in the evolution of life on our planet. One of the predator strains is thought to have associated with two other types of bacteria to form the first (and possibly unique) endosymbiosis between prokaryotic cells, and to be at the origin of the first eukaryotic cell [9, 18]. If, as conjectured, one of these endosymbionts was an Archaeobacterium, this would explain in good part the evident similarities between some gene complexes (e.g. transcription, translation) of Archaeobacteria and those of eukaryotes and vice versa.

Like the predatory bacteria before them, the eukaryotes had a typically Darwinian evolution. It allowed for the loss of many genes through successive extinctions

and, as far as is known, their genetic information was almost exclusively transmitted to and maintained in the offspring, without resorting to LGT. A major, fateful type of genetic innovation occurred progressively: the symbioses between eukaryotes and prokaryotes that had complementary bioenergetic properties. The bacteria involved in these associations as endosymbionts lost their capacity to engage in LGT with other members of the prokaryotic superorganism, and the new eukaryotic species born of the association followed the typical Darwinian evolutionary paths of the eukaryotic ancestors. By contrast, the bacteria participating in ectosymbioses retained their ability to engage in LGT activities with other prokaryotic cells as evidenced, for example, by the bacteria of the alimentary tract microbiota that exchange genetic resistance to antibiotics probably obtained originally from soil strains. They continued to evolve by benefitting from both vertical and horizontal gene transfer. Many authors consider that the most pivotal symbiotic innovation has been the acceptance of cyanobacteria by protoctists, marine animals and, in particular, the associations that led to the origin of algae, lichens and vascular plants. Originally, life was dominant in, if not limited to, the aquatic niches, since continental lands did not offer favorable conditions. But, due mostly to vascular plants, the continents became covered with vegetation, a biomass that made possible the appearance, survival and evolution of terrestrial animals. Later, the association between some plants and nitrogen-fixing bacteria led to the development of new species capable of growing on

nitrogen-poor soils. The body of each higher animal accommodates physically and physiologically thousands of millions of bacteria for the mutual benefit of both host and microbiota. Practically no animals, and few fungi, live without the partnership of bacterial ecto- or endosymbionts. In the typical Darwinian evolution of eukaryotes, and in the absence of artificial, man-made genetic engineering, little outside information penetrates the genetic message contrasting with LGT in prokaryotes. In the latter, LGT produces highly dynamic genomes with the introduction and deletion of substantial amounts of DNA [22]. However, symbiogenesis with thousands of different bacterial genes has decisively enriched the limited metabolic potential of eukaryotic organisms, accelerating and facilitating their adaptation much more than would have been achieved by random mutation alone. The multicellular organisms have probably been among the most important contributions of this mixed eukaryotic-prokaryotic symbiosis, whereas the bacterial superorganism retained its unicellular structure.

These facts throw a different light on the evolution of life on our planet. The classic concept of struggle for life has much more meaning in eukaryotes than in prokaryotes, which collaborate both metabolically and via LGT. For eukaryotes, the high price attached to reproductive isolation has been a loss of genes and species which became extinct. In contrast, the prokaryotes' guided and constructive evolution saved and dispersed successful genes and, hence, resulted in a much broader bioenergetic diversity. The elements of the prokaryotic empire have survived and progressed since the origin of life. They have created a powerful and highly efficient global superorganism or biosystem which is also responsible for the maintenance of our biosphere's homeostasis. Eukaryotes, since their origin, have benefitted from the collaboration extended to them by prokaryotes.

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