PHYLOGENY OF EXTREMOPHILES

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Contents

Summary

The phylogeny of extremophiles and the origin of life are closely related and highly controversial topics. In this essay, we critically review the "classical" model, which presents the universal tree of life rooted in the bacterial branch and emerging from a prokaryote-like, hyperthermophilic, last common ancestor (LCA). Our conclusion is that there is no firm bedrock on which to build such a vision and that another model can account for the emergence of life and of extremophiles. We present the arguments supporting the concept of a protoeukaryotic, genetically redundant, promiscuous, and non hyperthermophilic LCA, endowed with *sn*-1, 2 glycerol ester lipids, from which the primordial and hyperthermophilic Archaea emerged by a reductive process implying the advent of *sn*- 2, 3 glycerol ether lipids. Bacteria emerged independently from the LCA by reductive evolution; the presence of different types of membrane lipids in independent lines of thermophilic bacteria suggests they adapted to high temperatures by convergent processes; some bacterial hyperthermophilic traitsmay have been acquired from Archaea.. The cardinal characteristics of piezophiles, psychrophiles, and extreme halophiles are briefly reviewed. In our present state of knowledge, adaptations to low temperature (psychrophily) appears secondary in Archaea; extreme halophily also seems to be a characteristic acquired by a branch of Archaea. Thorough analyses of proteins from abyssal organisms will be necessary to determine whether primordial forms of life were piezophilic (adapted to high pressure). A large number of extreme

biotopes (some of them difficult of access) remain to be investigated to make the tree of life more representative of biodiversity.

1. Introduction

The notion of "extremophile" covers a large variety of organisms which all share one common property: they are adapted to at least one of the conditions, which seem to stretch the adaptive capacity of life to a limit. The anthropomorphic undertone of this concept becomes clear when one realizes that any extremophile is actually restricted to an environment characterized by an "extreme" parameter, whether the temperature, an elevated hydrostatic pressure, an extreme pH, or high salinity. Adaptations to these various types of extremes raise questions of very different scopes. Earth is a cold planet; the portion of the biosphere above the surface of the crust is mainly oceanic, 62% of it below 1000 m, and thus under 5 °C, barring a few exceptions. Psychrophily (adaptation to cold) is therefore "extreme" only in the sense that it characterizes organisms thriving just above the freezing point of water, a natural temperature limit for growing cells (we are of course considering only organisms devoid of thermoregulation). Psychrophily is, however, not restrictive in terms of biodiversity since psychrophiles comprise a large variety of prokaryotes and eukaryotes. The same is true for piezophily (adaptation to high pressure) which also characterizes the largest part of the biosphere.

By contrast with low temperature and high pressure, elevated temperature (i.e., close to or higher than the boiling point of water), and high or low pH, as well as high salinity, not only mark the end of a range compatible with life but also correspond to a drastic decrease in biodiversity, with a marked preponderance of prokaryotes. Nevertheless, except for high temperature, which above 62 °C selects only for prokaryotes, extremes of pH may accommodate eukaryotes (even small invertebrates in the case of high pH) and some environments of high salinity are host to algae and protozoa. We will however limit ourselves to prokaryotes. The main reasons for this choice are the preponderance of prokaryotes among extremophiles and the close connections between three topics: the emergence of hyperthermophily, the origin of life and the nature of the last ancestor common to all inhabitants of this planet

Treating the phylogeny of extremophiles as a topic implies there are reasons to think these organisms occupy singular positions in the genealogy of life. From the evolutionary point of view, we must, however, distinguish pervasive environmental factors—which select for concerted adaptation of all cellular functions and are the only ones we will consider here—from those involving a more limited register. Temperature and pressure obviously belong to the first category, in contrast to high or low pH, since most alkaliphiles and acidophiles maintain an intracellular pH not far from neutrality. Only in the extreme case of the thermoacidophilic archaeon *Picrophilus oshimae*, which grows fastest at pH 0.7, does the intracellular pH fall to 4.6, whereas it can reach 9.5 in some extreme alkaliphilic Archaea; these values are probably close to the limits compatible with function and stability of cellular macromolecules. Very high salinity also selects for global adaptation of cellular functions because of the extensive intracellular accumulation of inorganic ions (mostly K^+) necessary to achieve osmotic balance between the cell and the environment; as a result, enzymes from extreme halophiles often require high ionic strength for function in contrast to the homologous proteins from less demanding halophiles such as marine bacteria.

Until the beginning of the twenty-first century, much more attention has been devoted to the origin of thermophily than psychrophily, piezophily, or halophily. This is mainly for historical reasons. When the comparative analysis of small subunit (SSU) ribosomal (r) RNA revolutionized microbiology by bringing to light a third domain of organisms—the Archaea—it also revealed that the most ancient lines of descent in both prokaryotic divisions (Archaea and Bacteria) consisted of hyperthermophilic organisms. On the other hand, no eukaryotes were found able to multiply above 62 °C; a statement that remains valid in spite of extensive screening. When a few years later the tree of life was rooted in the bacterial branch by computer analysis of two sets of paralogous proteins (see Section 2.2), many biologists assumed that the last common ancestor of the three domains (the LCA) had been a prokaryote-like, hyperthermophilic organism and that life itself originated at high temperature. It follows that key issues about the phylogeny of extremophiles are the origin of extreme thermophily and to what extent, if at all, the thermal regime of the LCA and the temperature of the cradle of life are related questions.

2. The Structure of the Tree of Life

2.1. The Last Common Ancestor (LCA) and the Three Domains

Central to all discussions on the phylogeny of living organisms is the concept of a "last universal common ancestor" (LUCA or LCA) for Archaea, Bacteria, and Eukarya. The existence of this mythical entity, the very concept of which can be traced back to Darwin himself, seems legitimized by the occurrence of families of homologous proteins across the three domains, the general unity of biochemistry in terms of basic metabolic and energetic processes, the near-universality of the genetic code and the argument that, even if terrestrial life knew several attempts, all but one is expected to have survived natural selection so as to dominate the whole planet. None of these arguments is compelling, however; it was argued that catalytic and autoreplicative polymers not identical to contemporary nucleic acids or even totally unrelated may have preceded them and provided a molecular scaffold for their assembly; the most extreme form of this hypothesis, formulated by Cairns-Smith, presented clays as "the first organisms." In this view, the advent of nucleic acids as depository for genetic information is not the only possible outcome. Other forms of life may thus exist which would not be detected by probing with standard oligonucleotides. Moreover, within the framework of nucleic acids themselves, it is not known whether the genetic code as we know it is the only possible one or a "frozen accident." In terms of mechanisms of enzymatic catalysis, we will probably not know what can be ascribed to chance rather than necessity before having discovered at least one other example of life based on carbon chemistry. However, these reservations concern more the origin of life itself than the origin of extant biodiversity. We will, therefore, keep the advent and the diversification of a LCA as the best working hypothesis to discuss the issues raised in the Introduction.

C.Woese chose the small subunit (SSU) of ribosomal RNA (rRNA), usually designated by its sedimentation coefficient in Svedberg (S) units (16SrRNA in prokaryotes, 18SrRNA in eukaryotes), to probe deep evolutionary kinships between extant organisms; indeed, this component is universal and exerts such a central and basic function that it is not exposed to the relentless and highly variable selective pressure operating at the level of more peripheral functions. It was thus thought to provide the best possible molecular clock. Moreover, it was argued that the functional connections between SSUrRNA and other non dispensable ribosomal macromolecules should confer it a relative immunity to lateral gene transfer (LGT), a phenomenon prone to blur phylogenetic relationships. This concept was extended towards those cellular functions which can be qualified as "informational" rather than "operational" (the "complexity" hypothesis of Jain, Rivera, and Lake). As a matter of fact, recent studies suggested that rRNA may have been exchanged in nature between closely related organisms and an experimental analysis published by T. Asai, D. Zaporojets, C.Squires and C.L. Squires in 1999 even showed that such an exchange was possible between *E. coli* and *Salmonella typhimurium* without noticeable loss of viability or efficiency in protein synthesis whereas chimeras consisting of *E.coli* and yeast rRNA were much less efficient than either of the parental molecules. Keeping these facts in mind, genealogies based on the comparative analysis of SSUrRNA molecules nevertheless remain today our best reference to discuss the "anomalies" that were found to abound in phylogenetic trees based on comparisons of amino acid sequences.

The concept of a neat distinction between three domains indeed came under fire when it was realized that protein sequence trees often did not match the tree derived from SSUrRNA sequences. Such protein trees can not be represented by vertical lines of descent because they are polyphyletic: some bacterial genes look like archaeal or eukaryotic ones or vice versa. The most popular hypothesis to explain such discrepancies between SSUrRNA and protein trees has been and remains lateral (i.e., horizontal) gene transfer (LGT) between distantly related organisms. Despite the fact that most of these analyses were purely statistical and thus exposed to a variety of artefacts, this concept of wide-ranging, interdomain, LGT was used by Martin and Doolittle to propose that the genealogy of life is not a tree but a net within which a clean branching out of separate domains can not be defined. At the end of the year 2000, we reviewed the evidence interpreted in this way and had to conclude that, in most cases of alleged LGT, even among prokaryotes, alternative interpretations had been neglected. Of course, the transfer of some bacterial genes to eukaryotic genomes is in keeping with the symbiotic origin of mitochondria and chloroplasts.

Besides statistical artefacts stemming from annotation errors, from differences in guanosine-cytosine genome contents or from variations in evolutionary rates between organisms and between genes, one explanation stands out as an alternative to LGT because it gives a less *ad hoc* account of apparent polyphyly. To have been capable of evolving towards an advanced cellular stage, the LCA must have been a genetically redundant organism. This redundancy would have been dynamic if frequent exchanges or fusion events took place between LCA cells as Woese himself proposed; such events would have been facilitated if those primordial cells had not yet developed structured cell walls. Furthermore, to have engendered the metabolic diversity displayed by microbial life this LCA population was probably highly heterogeneous. One could therefore predict that the ancestors of each of the three domains possessed *at least two copies* of many, perhaps most of their genes. Later on, in the course of the progressive differentiation of each domain into different branches, haphazard loss of one of the paralogous copies would have generated a totally unpredictable polyphyletic pattern. A striking example of this—but by no means an isolated one—is the phylogenetic analysis by Labedan and his colleagues of the carbamoyltransferases involved in arginine and pyrimidine biosynthesis.

This type of explanation avoids the difficulties arising at each of the steps involved in putative gene transfer between domains; indeed, for most of the phylogenetic discrepancies where LGT was invoked, no other selective drive could be proposed for the acquisition of the putative foreign gene than the complementation of defective mutants (except, maybe, for the emergence of thermophilic bacteria as discussed in Sections 4 and 5).Yet, a gene damaged by mutation is much more likely to be replaced by an intact exemplar from cells of the same species or from a closely related one sharing the same ecology. Moreover, to produce lasting results, LGT requires replication, maintenance, and efficient expression of the transferred gene, which in the case of interdomain transfer means cumulating all possible difficulties. We are certainly not denying evidence for LGT within the same species (this has been amply demonstrated for *Escherichia coli* by Radman and his colleagues), or among closely related organisms (such as *Thermococcus* and *Pyrococcus*) or even between relatively distant members of a large group such as the Proteobacteria (alias Gram-negative bacteria) where arguments independent from statistics (presence of transposons, integrons, gene islands, presence or absence of whole sets of genes in different strains of the same species, especially pathogens) actually highlight the importance of LGT in speciation, but we consider that the case for interdomain transfer is rather weak; this holds also for tRNA synthetases in spite of the reputation of promiscuity they have gained from pure statistics. Moreover, inferring the incidence of interdomain transfer from the probable frequency of foreign (but nevertheless mostly bacterial-like) genes hosted by *E. coli* is highly misleading. Besides, it should be noted that comparative analysis of whole proteomes and phylogenetic analysis based on gene sharing rather than sequence identity did not suggest that extensive LGT ever took place between domains. Finally, incorrect assumptions were occasionally made to reject differential loss of paralogues as an explanation for lack of monophyly. For example, when paralogous copies of a gene were found in different but connected radiations of a particular group, it was inferred that an organism appearing ancestral in the genealogy could still possess both copies; this reasoning does not take into account that the only organisms we can look at are contemporary and may have suffered gene losses as well. Therefore, not finding the two copies in an "earlier" branch is unconclusive.

Others, such as Eisen and Sicheritz-Ponten, also criticized recently the systematic application of the LGT concept among microorganisms. As far as eukaryotes are concerned, the symbiotic origin of mitochondria and chloroplasts is of course compatible with the notion of gene transfer from the prokaryotic ancestors of these organelles towards the nucleus; it was even proposed by Doolittle that the phagocytic nutrition of animal cells may have led to the incorporation of bacterial genes into the nucleus of the feeding cell. However, Salzberg recently questioned the identification of numerous genes of putative bacterial origin in the human genome and stressed the purely statistical nature of most of these identifications to the detriment of other explanations.

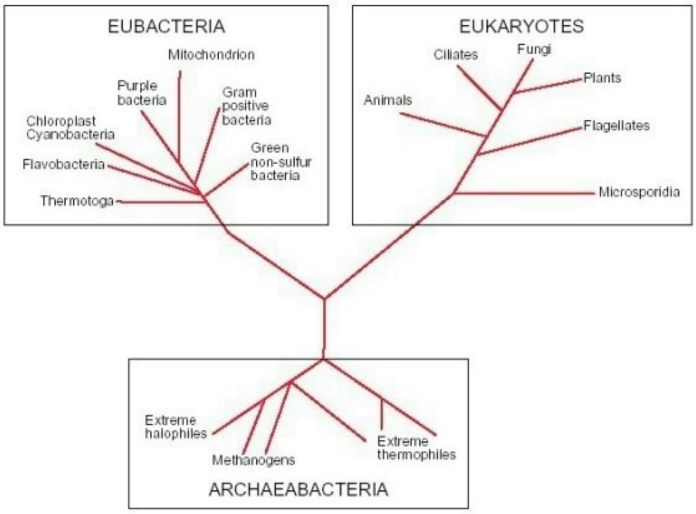

Figure 1. An early, still-unrooted version of the SSUrRNA distance tree

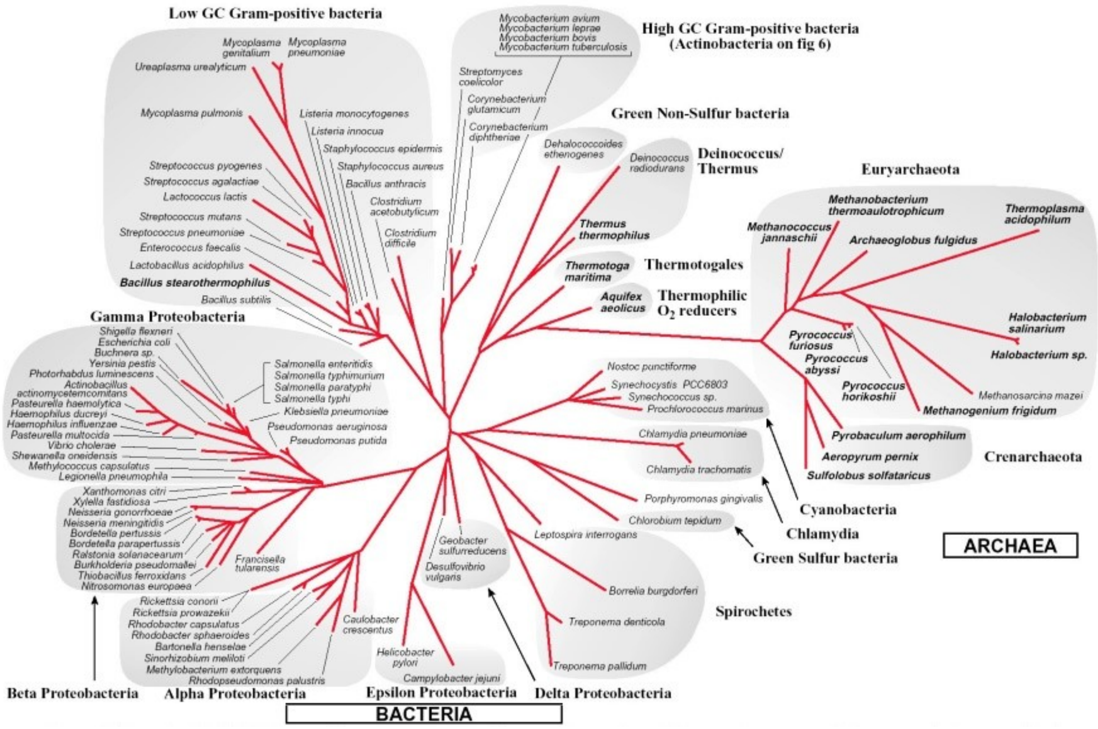

Figure 2. Unrooted SSUrRNA tree of the prokaryotes based on organisms whose full genomic sequence is known or being completed Extremophiles (thermophiles, psychrophiles, halophiles) are in bold type. Bacteria contrast with Archaea by their long, deep, and poorly resolved branches. A more radical version of bacterial phylogeny is given in Figure 6.

Adapted from Nelson K.E., Paulsen I.T., Heidelberg J.F., and Fraser C.M. 2000. Status of genome projects for nonpathogenic bacteria and archaea. *Nature Biotechnology* 18, 1049–1054.

At this stage, we therefore conclude that the existence of the three domains of life (Archaea, Bacteria, Eukarya) appears legitimized by the systematic differences brought to light in their respective SSUsRNA sequences and that this discovery will remain a milestone in the history of biology. Even though phylogenies based on rRNA are not immune to statistical artefacts and leaves some basic branchings unresolved particularly among Bacteria and early Eukarya, the coherence of this new systematics is supported by the host of correlations that confers to each of the three domains a distinctive label. In its unrooted form (Figures 1 and 2) the three-domain tree emphasizes this distinctness but also draws attention to the relative dwarfness of the archaeal bush; short branches indicate slow evolutionary rates. In particular, the relatively high GC content necessary to stabilize rRNA in hyperthermophiles (which constitute all ancient archaeal lines of descent) reduces the number of possible nucleotide substitutions in these molecules and therefore slows down their evolutionary rate.

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Biographical Sketches

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