Visions & Reflections

Did life begin in hot water?

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For a long time scientists interested in the problem of the origin of life have been mainly recruited among chemists, physicists and planetologists. Biologists were a priori more reluctant to speculate about the emergence of their favourite objects. This situation has changed during the last 2 decades following the discovery of ribozymes, leading to the theory of the RNA world [1], and with the discovery of microbial life at high temperature, from which some biologists inferred that life itself originated in a very hot environment [2, 3]. However, the RNA world and the hot origin of life theories strongly contradict each other, because RNA is a highly thermolabile molecule, making it difficult to imagine a primitive world of replicative RNA molecules paddling around in boiling water [4–8]. Up to now, this contradiction has been more or less ignored by both microbiologists—who are reluctant to let their imagination be constrained by chemical realities—and by evolutionary biochemists—who continue to perform RNA world experiments at room temperature. I am going to briefly discuss this problem here. What are the respective values of the RNA world and hot origin of life theories? Might it be possible to initiate new research programs to answer questions raised by their conflict? There are good arguments to support the idea that RNA was invented before DNA [1]. The strongest one is that cellular metabolism first produces RNA precursors which are then transformed into DNA precursors by an elaborated enzyme, the ribonucleotide reductase. DNA is thus most likely a late invention in the history of life (fig. 1). RNA is probably much more ancient than DNA, since it should have predated even the invention of modern proteins, which today are still

produced by the ribozyme activity of the large ribosomal RNA (rRNA) [9] (fig. 1). One can thus consider that, for some time, our planet has been exclusively populated by cells and viruses with RNA genomes and
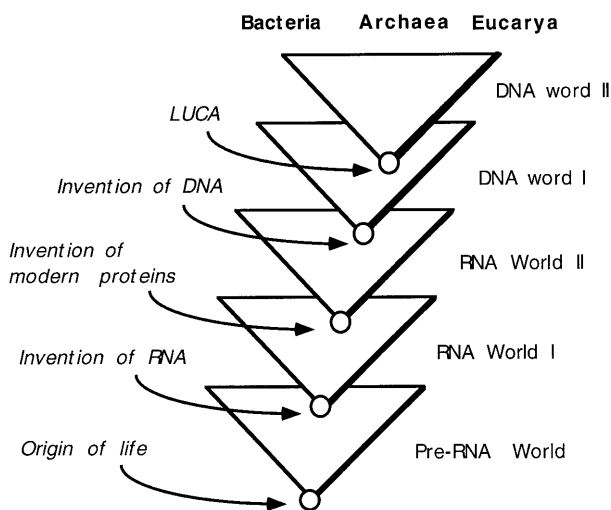

Figure 1. A schematic view of early life evolution via successive steps of diversification and extinction. At each step, a new invention allowed the successful diversification of organisms bearing it and extinction of their competitors which failed to use it. Modern proteins are those synthesised by RNA-based ribosomes (polypeptides synthesized by other mechanisms might well have existed in the first RNA world). The LUCA is not associated with the birth of the DNA world because it probably already contained many duplicated genes for DNA enzymes [12]. Cellularisation probably occurred early on, during the pre-RNA world, so all steps presented here involve cellular entities.
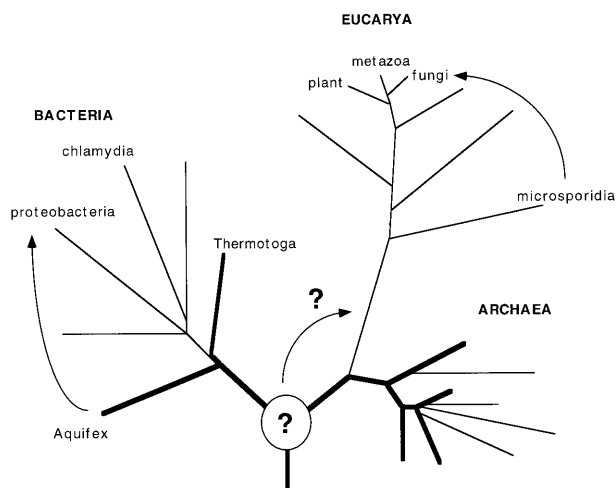
Figure 2. Problems with the universal tree of life. Schematic representation of the universal tree based on comparative analysis of rRNA and duplicated protein genes (adapted from [2, 11]) lineages of hyperthermophiles are in bold. The arrows indicate the proposed revised positions of microsporidiae [14] of *Aquifex* [15] and of the root of the tree [25].

RNA enzymes (the first age of the cellular RNA world in figure 1).

The hot origin of life hypothesis is more controversial. This idea has been promoted by the discovery of hyperthermophilic microbes that thrive in deep-sea hydrothermal vents and terrestrial hot springs [2, 3]. For some scientists, deep-sea vents appeared to be the ideal setting for the emergence of living forms because they provided a continuous flow of matter and energy, and because their ancestors in the Precambrian were protected against the deleterious effects of ultraviolet (UV) irradiation and early meteorite bombardment (review in [10]). However, the strongest support for a hot origin of life came from the work of molecular phylogeneticists who produced a universal tree of life based on comparative analyses of rRNA molecules [11]. In this tree, the lineages of hyperthermophiles (organisms growing optimally at temperatures above 80 °C and up to 110 °C) are characterised by short branches compared with their mesophilic counterparts (fig. 2). Furthermore, they are clustered around the root of the universal tree, which has been tentatively placed into the bacterial branch, based on the analysis of ancient duplicated proteins. Many authors concluded from this tree that hyperthermophiles have evolved at a slower rate than mesophiles, and thus have conserved more common traits with the last universal cellular ancestor (LUCA) [2, 3, 10, 11]. These ideas were challenged only by a few scientists who questioned the validity of the universal tree (re-

viewed in [12]), noticed that present-day hyperthermophiles are far from primitive (reviewed in [8]) and finally argued against a hot origin per se because of the chemical instability of biological molecules at high temperatures [3–8]. In particular, the hypothesis that the first steps of evolutionary life occurred at very high temperature seems at odds with the extreme instability of RNA [4, 6, 8, 13] (see fig. 1). RNA is indeed rapidly hydrolysed at temperatures near the boiling point of water, and the rate of this reaction is greatly enhanced by magnesium, which is a cofactor in most ribozyme reactions.

Despite these criticisms, the hot origin of life theory became more and more popular in the last years, in tandem with an increasing interest for hyperthermophiles, both as scientific curiosities and biotechnological gold mines. In particular, the idea of a hot subsurface origin is appealing for exobiologists since it suggests that life might have appeared (and still be present) even on planets with inhospitable surfaces.

However, new data from molecular phylogeny have recently started to reverse the previous trend in favour of a hot origin. Several well-documented protein phylogenies have suggested that many organisms [14] including hyperthermophiles [15] have been misplaced in the universal tree for methodological reasons (fig. 2). In particular, it has been suggested that the basal position of hyperthermophiles in the universal rRNA tree could be simply due to the high G + C content of their rRNA [16]. This high G + C content, which probably helps to stabilise critical secondary structures, could have slowed down the rate of evolution of rRNA molecules, artifactually grouping them near the root of the tree. Indeed, using a method that allows the original base composition of an ancestral sequence to be inferred from the base composition of modern sequences, Gouy and co-workers have recently concluded that the rRNA sequence from LUCA had a low G + C content, suggesting that it was not a hyperthermophile after all [17].

This new result is in agreement with the idea that present-day hyperthermophiles have been secondarily adapted to hyperthermophily. As a matter of fact, these fascinating microorganisms exhibit elaborated enzymes and chemicals which are likely secondary adaptation to high temperature, such as tetraether lipids made by the tail-to-tail condensation of diether lipids [18]. Also significant is the fact that all hyperthermophiles contain an elaborated adenosine triphosphate (ATP)-dependent enzyme, reverse gyrase, which should help to stabilise DNA at high temperature by increasing the number of topological links between the two DNA strands. Reverse gyrase is obviously a modern protein, since it was created by the fusion of a DNA topoisomerase and a DNA helicase [19]. It was thus suggested that hyper-

thermophilic life would not have been possible before the invention of reverse gyrase [13].

New data from comparative genomics and molecular phylogeny are also in line with the idea that modern hyperthermophiles are the products of a secondary adaptation to hot biotopes, at least in the case of bacteria. Adaptation to hyperthermophily occurred apparently more recently in bacteria than in archaea, since the hyperthermophilic bacterium *Aquifex eolicus* has acquired many genes from hyperthermophilic archaea by lateral gene transfer, possibly to help its adaptation to life at extremely high temperature (up to 95 °C) [20]. Adaptation to hyperthermophily might have even appeared several times independently in the bacterial domain, since some features of thermoadaptation in various bacterial lineages are analogues but not homologues [8].

Enough data need to be gathered to confirm (or not) these recent findings which, although convergent and impressive, are still based on the analysis of a few genes and enzymes. In particular, it would be worthwhile obtaining the complete genome sequence of some mesophilic archaea and mesophilic bacteria phylogenetically closely related to hyperthermophiles whose genome sequence is already available. Unfortunately, no such project is presently under way. Further comparative analysis of protein families using both structural and phylogenetic data to determine the direction of evolution (from cold to hot or from hot to cold) would also be extremely valuable [16]. A bottleneck in that case is that many evolutionists lack the skill to include structural analyses in their reflections, whilst structural biochemists are often unaware of the evolutionary problems raised by their studies. They have sometimes a naive faith in current phylogenetic trees and try to explain their observations based on these trees instead of proposing new evolutionary hypotheses based on their work.

The hypothesis of a hot origin for life is viable even if LUCA was not a hyperthermophile, since life might have appeared at high temperature and later on adapted to the cold [5]. However, in that case, it should be supported by arguments directly related to prebiotic chemistry. This is a highly controversial topic: some authors argue that high temperature should have prevented accumulation of prebiotic molecules, promoting instead their rapid degradation [4, 5], whereas others strongly support—based on theoretical arguments— the possibility of organic synthesis under high temperature and pressure in hydrothermal vents [21]. Chemists and biochemists need to experimentally test these ideas in reactors that mimic the deep-sea vent environment. Several authors have suggested that primordial molecules might have been stable at high temperature at solid-liquid interfaces [3]. For example, Wächtershauser

proposed that a complex chemiolitoautotrophic proto-cellular metabolism started at the surface of pyrite and argued that high temperature was required to compensate for the reduced mobility of molecules in this two-dimensional world [22]. Recently, several of the most simple chemical reactions predicted in Wächtershauser's theory have been successfully reproduced in the laboratory [23 and refs therein]. However, these putative early steps in the emergence of life are still far away from any primitive cellular RNA world [24]. A contrario, the idea that a cellular RNA world cannot exist at high temperature should itself be critically examined. The question is, what conditions could have stabilised primitive RNA molecules without inhibiting their potential ribozyme activities? Only if this turned out to be impossible would one have to conclude that life went through a mesophilic stage anyway, whatever the temperature at its origin.

In summary, we still do not know whether life originated in hot water, but this new hypothesis has already initiated exciting controversies and experimental work in the scientific community. More interdisciplinary programs, bringing together chemists and biochemists, especially RNA specialists and promoting the sequencing of genomes from mesophiles closely related to hyperthermophiles would be welcome.

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