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The Origin of Life

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"Generations of scientists may yet have to come and go before the question of the origin of life is finally solved. That it will be solved eventually is as certain as anything can ever be amid the uncertainties that surround us."

The Earl Nelson, *"There is Life on Mars"*,
The Citadel Press, New York, 1956

1. Introduction

How, where and when did life appear on Earth? Although Charles Darwin was reluctant to address these issues in his books, in a letter sent on February 1st, 1871 to his friend Joseph Dalton Hooker he wrote in a now famous paragraph that "it is often said that all the conditions for the first production of a living being are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sort of ammonia and phosphoric salts, --light, heat, electricity present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed..." (Darwin, 1871).

Darwin's letter summarizes in a nutshell not only his ideas on the emergence of life, but also provides considerable insights on the views on the chemical nature of the basic biological processes that were prevalent at the time in many scientific circles. Although Friedrich Miescher had discovered nucleic acids (he called them nuclein) in 1869 (Dahm, 2005), the deciphering of their central role in genetic processes would remain unknown for almost another a century. In contrast, the roles played by proteins in manifold biological processes had been established. Equally significant, by the time Darwin wrote his letter major advances had been made in the understanding of the material basis of life, which for a long time had been considered to be fundamentally different from inorganic compounds. The experiments of Friedrich Wöhler, Adolph Strecker and Aleksandr Butlerov, who had demonstrated independently the feasibility of the laboratory synthesis of urea, alanine, and sugars, respectively, from simple

starting materials were recognized as a demonstration that the chemical gap separating organisms from the non-living was not insurmountable.

But how had this gap first been bridged? The idea that life was an emergent feature of nature has been widespread since the nineteenth century. The major breakthrough that transformed the origin of life from pure speculation into workable and testable research models were proposals, suggested independently, in the 1920s by Aleksandr I. Oparin and John B. S. Haldane, as well as others (see Bada and Lazcano, 2003). They suggested that the first life forms were the outcome of a slow, multi-step process that began with the abiotic synthesis of organic compounds and the formation of a 'primordial soup'. Here their proposals diverged. While Haldane argued that viruses represented a primordial stage that had appeared prior to cells, Oparin argued colloidal gel-like systems had formed from the soup, leading to anaerobic heterotrophs that could take up surrounding organic compounds and use them directly for growth and reproduction. Although the details of these proposals have been superseded, the Oparin-Haldane hypothesis of chemical evolution provided a conceptual framework for the experimental development of the study of the origin of life. Laboratory experiments have shown how easy it is to produce a variety of organic compounds, including biochemically important monomers, under plausible cosmic and geochemical conditions. The robust nature of these reactions has been demonstrated by the finding that organic compounds are ubiquitous in the Universe, as shown by their presence in carbon-rich meteorites, cometary spectra, and interstellar clouds where star and planetary formation is taking place.

2. A timescale for the emergence of life

Although traditionally it had been assumed that the origin and early evolution of life involved several billions of years (Lazcano and Miller, 1994), such views are no longer tenable. While it is true it is not possible to assign a precise chronology to the appearance of life, in the last few years estimates of the available time for

this to occur have been considerably reduced. Determination of the biological origin of what have been considered the earliest traces of life is now rather contentious issue. This is not surprising. The geological record of the early Archean is sparse, and there are very few rocks older than 3.5 billion-years-ago (Gya). The rocks with ages > 3.5 Gya that are preserved have been extensively altered by metamorphic processes (van Zuilen et al., 2002) and thus any direct life evidence of ancient life have apparently been largely obliterated.

Although the biological nature of the microstructures present in the 3.5 Gya Apex cherts of the Australian Warrawoona formation (Schopf, 1993) has been disputed (Brasier et al, 2002; García -Ruiz et al., 2003), there is evidence that life emerged on Earth very early in its history. The proposed timing of the onset of microbial methanogenesis, based on the low ^{13}C values in methane inclusions, has been found in hydrothermally precipitated quartz in the 3.5 Gya Dresser Formation in Australia (Ueno et al. 2006), although this finding has also been challenged (Lollar and McCollom, 2006). However, sulfur isotope investigations of the same site indicate biological sulfate-reducing activity (Shen et al. 2001), and analyses of 3.4 Gya South African cherts suggest that they formed in a marine environment inhabited by anaerobic photosynthetic prokaryotes (Tice and Lowe 2004). These results, combined with reports on 3.43 Gya stromatolites from Western Australia (Allwood et al., 2007) support the idea that the early Archean Earth was teeming with prokaryotes, and that the origin of life must have taken place as soon as the conditions were suitable to permit the survival of these types of organisms.

The early Archean fossil record speaks for the relatively short timescale required for the origin and early evolution of life on Earth, and suggests that the critical factor may have been the presence of liquid water, which became possible as soon as the planet's surface finally cooled below the boiling point of water. Unfortunately, there is no geological evidence of the environmental conditions on the early Earth at the time of the origin of life, nor are any molecular or physical remnants preserved that provide information about the evolutionary processes that preceded the appearance of the first cellular organisms found in

the early fossil record. Direct information is generally lacking not only on the composition of the terrestrial atmosphere during the period of the origin of life, but also on the temperature, ocean pH, and other general and local environmental conditions which may or may not have been important for the emergence of living systems.

3.The primitive Earth environment

Considerable progress has been made in our understanding of environmental conditions of the early Earth and in how the transition from abiotic to biotic chemistry may have occurred (for example see Bada, 2004). Nevertheless, there are still enormous gaps in our description of how the simple organic compounds associated with life as we know it reacted to generate the first living entities and how these in turn evolved into organisms that left behind actual evidence of their existence in the rock record.

To evaluate how life may have began on Earth, we must assess what the planet was like during its early history and under what conditions the processes thought to be involved in the origin of life took place.-Life as we know it depends on the presence of liquid water and organic polymers such as nucleic acids and proteins. The available evidence suggest that during an early stage biological systems lacked proteins and depended largely on catalytic and replicative polyribonucleotides, but water was likely essential from the very beginning, as it provides the medium for chemical reactions to take place. Without these basic components, as far as we know, life is impossible.

It is unlikely that water made its first appearance on Earth as a liquid. During the Hadean period, the volatile components that were trapped inside the accreting planet were released (degassed) from the interior of the juvenile Earth to form a secondary atmosphere. Any primary atmosphere (if one existed at all) must have been lost, as evidenced by the depletion of rare gases in Earth's atmosphere compared to cosmic abundances (Kasting, 1993a). As a consequence of the

nearly simultaneous formation of Earth's core with accretion, the metallic iron was removed from the upper mantle, which would have allowed the volcanic gases to remain relatively reduced and produce a very early atmosphere that contained compounds such as CH₄, NH₃ and H₂. Since the temperature at the surface was high enough to prevent any water from condensing, the atmosphere would have consisted mainly of superheated steam along with these other gases (Kasting, 1993b). Even though the secondary atmosphere may have been lost several times during large impact events, especially the one that formed the moon, it would have been regenerated by further outgassing from the interior and as well as re-supplied from later impactors.

It is generally accepted that the impactors during the latter stages of the accretion process have originated from outer the Solar System and would have been comparable in composition to comets. As suggested in 1961 by the late Spanish chemist Joan (John) Oró, cometary volatile compounds, which appear to be the most pristine materials surviving from the formation of the solar system, may have supplied a substantial fraction of the volatiles on the terrestrial planets, perhaps including organic compounds that may have played a role in the origin of life on Earth (Oró, 1961). It has been suggested that the water on the Earth was provided entirely from this source. However, measurements of the deuterium content of water in comets Halley, Hyakutake and Hale-Bopp indicate that comets likely delivered only a fraction of the Earth's water, while the largest fraction was trapped during the earlier accretionary phase and released via degassing (Robert, 2001).

It is reasonable to assume that the atmosphere that developed on the Earth over the period 4.4 to 3.8 Gya (perhaps several times if was eroded by large impact events) was basically a mix of volatiles delivered by volatile rich impactors such as comets and outgassing from the interior of an already differentiated planet. This atmosphere was probably dominated by water steam until the surface temperatures dropped to ~100°C (depending on the pressure), at which point water condensed out to form early oceans (Wilde et al., 2001). The reduced species, which were mainly supplied by volcanic outgassing, are very sensitive to

UV radiation that penetrated through the atmosphere due to the lack of a protective ozone layer. These molecules were probably destroyed by photodissociation, although there might have been a steady-state equilibrium between these two processes that allowed a significant amount of these reduced species (especially H₂) to be present in the atmosphere (for example, see Tian, et al., 2005). Eventually as H₂ escaped from the Earth into space, reduced species in the atmosphere would have been depleted. Thus, in general the overall consensus at present is that the early the atmosphere was dominated by oxidized molecules such as CO₂, CO and N₂. A similar atmosphere is present on Venus today, although it is much more dense than the atmosphere of the early Earth.

The climate on the early Earth at this stage depended mainly on two factors: the luminosity of the Sun and the radiative properties of the atmosphere. Standard theoretical solar evolution models predict that the Sun was about 30 % less luminous than today (Gilliland, 1989). If the atmosphere of the early Earth were the same as it is now, the entire surface of the planet would have been frozen. However, as discussed extensively by Kasting (1993 a, b), a CO₂ rich atmosphere may have been present throughout the Hadean and Early Archean period, resulting in a significant greenhouse effect that would have prevented the oceans on the early Earth from freezing. The basic argument is that during this period of early Earth history, there were probably no major continents, and thus no extensive silicate weathering. Because this process is nowadays the long-term loss process for CO₂ removal and storage, the conclusion is that CO₂ would have been primarily contained in the atmosphere and ocean. With the assumption of a solar luminosity of ~70% of the present value, a steady-state atmosphere containing ~ 10 bars of CO₂ could have been required in order to maintain a mean surface temperature greater above the freezing point of water.

In summary, the current models for the early terrestrial atmosphere suggest that it consisted of a weakly reducing mixture of CO₂, N₂, CO, and H₂O, with lesser amounts of H₂, SO₂, and H₂S. Reduced gases such as CH₄ and NH₃ are considered to be nearly absent or present only in localized regions near volcanoes or hydrothermal vents.

During such early times volcanic islands may have been prevalent, and large continents had not yet formed (Zahnle et al., 2007). Recent considerations of the early carbon cycle suggest that before extensive tectonic recycling of crustal sediments became common, most of the carbon on the Earth's surface would have remained buried in the crust and mantle as calcium carbonate (Sleep and Zahnle, 2001). There is thus the possibility that the CO₂ concentrations in the early atmosphere were not high enough to prevent the formation of a global ice-covered ocean (Bada et al, 1994). If this were indeed the case, the thickness of the global ice sheet has been estimated to be on the order of 300 m, which would have been thin enough to allow melting by an impactor of ~100 km in diameter. The frequency of impacts of such ice-melting bolides has been estimated to be one event every 10⁵-10⁷ years between about 3.6 to 4.5 Gya, suggesting periodic thaw-freeze cycles associated with the ice-melting impacts. The precursor compounds imported by the impactor or synthesized during the impacts, such as HCN, would have been washed into the ocean during the thaw periods, which have been termed "Impact Summers" (Zahnle et al., 2007). In addition, CH₄, NH₃, H₂, and CO derived from hydrothermal vents would have been stored in the unfrozen ocean below the ice layer, which would have protected these gases from the ultraviolet radiation. Following a large impact, the trapped gases would have been expelled into the atmosphere where they could have persisted for some time before they were destroyed by photochemical reactions. During these episodes, highly reducing conditions may have prevailed.

4. Organic compounds on the primitive Earth

Today, organic compounds are so pervasive on the Earth's surface that it is hard to image the Earth devoid of organic material. However, during the period immediately after the Earth first formed some 4.5 Gya, there would have been no organic compounds present on its surface. This was because soon after accretion, the decay of radioactive elements heated the interior of the young Earth to the melting point of rocks (Wetherill 1990). Volcanic eruptions expelled molten rock and hot scorching gases out of the juvenile Earth's interior creating a global

inferno. In addition, the early Earth was also being peppered by mountain-sized planetesimals, the debris left over after the accretion of the planets. Massive volcanic convulsions, coupled with the intense bombardment from space, generated surface temperatures so hot that the Earth at this point could very well have had an "ocean" of molten rock, i. e., a "magma ocean".

Although temperatures would have slowly decreased as the infall of objects from space and the intensity of volcanic eruptions declined, elevated temperatures likely persisted for perhaps a hundred million years or longer after the formation of the Earth. During this period, temperatures would have probably been too hot for organic compounds to survive. Without organic compounds, life as we know could not have existed. However, based on data from ancient zircons, by approximately 4 Gya (or perhaps even earlier) the Earth's surface must have cooled down to the point that liquid water could exist and global oceans began to form (Wilde et al., 2001). It was during this period that organic compounds would have first started to accumulate on the Earth's surface, as long as there were abiotic processes by which they could synthesize and accumulate. What was the nature of these processes?

5. Synthesis of organic compounds on the primitive Earth?

The hypothesis that the first organisms were anaerobic heterotrophs is based on the assumption that abiotic organic compounds were a necessary precursor for the appearance of life. The laboratory synthesis of organic compounds from inorganic starting material was first achieved in the 1820's, when Friedrich Wöhler demonstrated that urea could be formed in high yield by the reaction of cyanogen and liquid ammonia and by the heating ammonium cyanate. Although it was not immediately recognized as such, a new era in chemical research had been begun. In 1850 Adolph Strecker achieved the laboratory synthesis of alanine from a mixture of acetaldehyde, ammonia and hydrogen cyanide. This was followed in 1861 by the experiments of Aleksandr Butlerov who showed that the treatment of formaldehyde with alkaline catalysts, such as calcium hydroxide, leads to the synthesis of a variety of sugars. The laboratory synthesis of

biochemical compounds was soon extended to include more complex experimental settings, and by the end of the 19th century a large amount of research on organic synthesis had been performed (Bada and Lazcano, 2003).

However, these 19th century organic syntheses were not conceived as laboratory simulations of the “warm little pond” that Darwin mentioned to Hooker, but rather as attempts to understand the autotrophic mechanisms of nitrogen assimilation and CO₂ fixation in green plants (see Bada and Lazcano, 2002a).

The first convincing demonstration of the possible synthesis of organic compounds under prebiotic conditions was accomplished 1953 by Stanley L. Miller who investigated the action of electric discharges acting for a week on a mixture of CH₄, NH₃, H₂, and H₂O; racemic mixtures of several protein amino acids were produced, as well as hydroxy acids, urea, and other organic molecules (Miller, 1953, 1955)

Miller achieved his results by means of an apparatus designed to simulate the interaction between an atmosphere and an ocean (see **Figure 1**). As an energy source, Miller chose a spark discharge, considered to be the second largest energy source, in the form of lightning and coronal discharges, on the early Earth after UV radiation (Miller and Urey, 1959). The apparatus was filled with various mixtures of methane, ammonia and hydrogen as well as water, the later which was then heated during the experiment. A spark discharge between the tungsten electrodes, which simulated lightning and corona discharges in the early atmosphere, was produced using a high frequency Tesla coil with a voltage of 60,000 V. The reaction time was usually a week or so and the maximum pressure 1.5 bars. With this experimental setup, Miller was able to transform almost 50% of the original carbon (in the form of methane) into organic compounds. Although almost of the synthesized organic material was an insoluble tar-like solid, he was able to isolate amino acids and other simple organic compounds from the reaction mixture. Glycine, the simplest amino acid, was produced in 2% yield (based on the original amount of methane carbon), whereas alanine, the simplest amino acid with a chiral center, showed a yield of 1%. Miller was able to

Urey-experiment, but in much smaller yields than the amino acids. In addition, it has been found that guanine can be produced in a direct "one-pot" synthesis from the polymerization of aqueous solutions of ammonium cyanide (Yuasa et al., 1984).

Although these results are extremely encouraging, the atmospheric composition that formed the basis of the Miller-Urey experiment is not considered today to be plausible by many researchers. Although it is generally agreed that free oxygen was absent from the primitive Earth, there is no general agreement on the composition of the primitive atmosphere; opinions vary from strongly reducing ($\text{CH}_4 + \text{N}_2$, $\text{NH}_3 + \text{H}_2\text{O}$, or $\text{CO}_2 + \text{H}_2 + \text{N}_2$) to neutral ($\text{CO}_2 + \text{N}_2 + \text{H}_2\text{O}$). In general, those working on prebiotic chemistry lean towards more reducing conditions, under which the abiotic syntheses of amino acids, purines, pyrimidines, and other compounds are very efficient, while non-reducing atmospheric models are favored by planetologists. A weakly reducing or neutral atmosphere appears to be more in agreement with the current model for the early Earth. Although Miller and Urey (1959) originally rejected the idea of non-reducing conditions for the primitive atmosphere, a number of experiments were later on carried out in Miller's laboratory using CO and CO_2 model atmospheres (Schlesinger and Miller, 1983). It was found that not only were the yields of the amino acids reduced, but also that glycine was basically the only amino acid synthesized under these conditions. A general trend showed that as the atmosphere became less reducing and more neutral, the yields of synthesized organic compounds decreased drastically although they were never zero. The presence of methane and ammonia appeared to be especially important for the formation of a diverse mixture of amino acids. The main problem in the synthesis of amino acids and other biologically relevant organic compounds with non-reducing atmospheres is the apparent lack of formation of hydrogen cyanide, which is an intermediate in the Strecker pathway and an important precursor compound for the synthesis of nucleobases (Ferris et al., 1978). However, as mentioned earlier, localized high concentrations of reduced gases may have existed around volcanic eruptions and in these localized environments reagents such as HCN, aldehydes and ketones

may have been produced, which after washing into the oceans could have become involved in the prebiotic synthesis of organic molecules.

Whether the primitive atmosphere was reducing or neutral may be a moot issue, however, with respect to the synthesis of organic compounds on the early Earth. It has recently been shown that contrary to previous findings, significant amounts of amino acids are produced in spark discharge experiments using CO₂/N₂/liquid water mixtures (Cleaves et al., 2008). The low yields of amino acids found previously were apparently the result of oxidation during hydrolytic workup by nitrite and nitrate produced in the reactions, as well as by the inhibition of synthesis caused by the low pH conditions generated during the experiment. Addition of calcium carbonate to the system to buffer the system near neutral pH, along with oxidation inhibitors such as ascorbic acid or Fe²⁺ prior to hydrolysis, results in the recovery of several hundred times more amino acids than reported previously.

In the recent neutral atmosphere experiments, the amounts of free cyanide, aldehydes and ammonia are low, suggesting that the intermediates may be bound as nitriles. The observed amino acids may have formed by several mechanisms, including the Strecker synthesis or the related Bucherer-Bergs pathway. Although the amino acids observed and their relative abundances are similar to those found when oligomers formed by the self-condensation of HCN in aqueous solution are hydrolyzed (Ferris et al., 1978), the absence of polymeric material in the experiments appears to discard this mechanism. Experiments with slightly reducing model atmospheres (Schlesinger and Miller, 1983) suggest that the addition of traces of CH₄ and/or H₂ in neutral atmosphere simulations would likely further enhance the production of amino acids.

Because of supposed problems associated with the direct Miller-Urey type syntheses on the early Earth, a different hypothesis for the abiotic synthesis of organic compounds has been proposed. This suggestion resulted from the discovery of deep-ocean hydrothermal vents. A group of researchers have argued that the remarkable properties of the hydrothermal vent environments,

particularly their protection from the harsh conditions caused by large impact events, might have played an important role in the origin of life. Since it is thought by some that the last common ancestral organism of all extant life on Earth was a thermophile, several researchers have proposed the hypothesis that the organic compounds necessary for the origin of life were actually synthesized under vent conditions (Holm and Andersson, 1995). A major proponent of this hypothesis is Everett Shock and coworkers who have calculated that thermodynamic-based equilibria favor the formation of compounds such as amino acids at hydrothermal vent temperatures (Shock, 1990; Shock and Schulte, 1998). Vent-based synthesis is considered to be especially important in vents associated with off-axis systems (Kelley et al., 2001; Holm and Charlou, 2001). However, at the high temperatures (>350°C) associated with most vent discharges, amino acids and other biomolecules have been found to rapidly decompose (Bernhardt et al., 1984; Miller and Bada, 1988; Bada et al., 1995). For example, amino acids are destroyed in time scales of minutes at temperatures >300°C. The rate of hydrolysis for RNA at pH 7 extrapolated to elevated temperatures gives a half-life of 2 min at 250 °C for the hydrolysis of every phosphodiester bond; at 350 °C the half-life is 4 s. For DNA, the half-lives for depurination of each nucleotide at pH 7 are nearly the same as the hydrolysis rates for RNA (Miller and Lazcano, 1995). It has been pointed out by Lazcano (1997) that if the origin of life took place over a sufficiently long period of time, all the complex organic compounds in the ocean, derived from whatever sources, would be destroyed by passage through the hydrothermal vents. It is thus possible that hydrothermal vents are much more effective in regulating the concentration of critical organic molecules in the oceans rather than playing a significant role in their direct synthesis.

Despite the problems with stability at high temperatures, the vent-induced synthesis theory has been championed by Huber and Wächtershäuser (2006) who have described experiments claimed to “narrow the gap between biochemistry and volcanic geochemistry.” Using high concentrations of HCN (0.2 M) and high pressures of CO (75 bars) in the presence of Ni/Fe catalysts, a variety of amino and hydroxyl acids are produced at 100°C. However, there are

concerns about the relevance of this type of experiment to the natural geochemical environments that would be expected to have existed on the early Earth, especially the high concentrations and pressures of starting reagents (Bada et al., 2007).

6. Did prebiotic organic compounds come from space?

The difficulties supposedly involved with the endogenous, Earth-based synthesis of amino acids and nucleobases led to the development of alternatives. In the early 1990s, Chyba and Sagan (1992) reanalyzed Oró's 1961 proposal on the role of cometary nuclei as sources of volatiles to the primitive Earth, and proposed that the exogenous delivery of organic matter by asteroids, comets and interplanetary dust particles (IDPs) could have played a significant role in seeding the early Earth with the compounds necessary for the origin of life. This conclusion was based from the knowledge about the organic composition of meteorites. It is important to note that, if this concept is valid, impacts on the early Earth not only created devastating conditions that made it difficult for life to originate, but also at the same time perhaps delivered the raw material necessary for setting the stage for the origin of life. In an even wider view, this hypothesis could have profound implications on the abundance of life in the universe. The source of the essential organic compounds required for the origin of life is not constrained by the conditions on a particular planet, but rather organic compound synthesis is a ubiquitous process that takes place on primitive solar system bodies such as asteroids and comets. The possibility for the origin of life is thus considerably increased, provided the essential organic compounds are delivered intact to a planet that is suitable for further chemical evolution. This is yet to be fully demonstrated.

Carbonaceous chondrites, a class of stony meteorites, are considered to be the most primitive objects in the solar system in terms of their elemental composition, yet they have a high abundance of organic carbon, more than 3 weight-% in some cases. The meteorites most extensively analyzed for organic compounds include the CMs Murchison (which fell in 1969 in Victoria, Australia)

and Murray (1950, Kentucky, USA), and the CI class Orgueil meteorite (1864, France). The carbon phase is dominated by an insoluble fraction. The majority (up to 80 %) of the soluble organic matter is made up by polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons, carboxylic acids, fullerenes and amino acids (Botta and Bada, 2002). The purines adenine, guanine, xanthine and hypoxanthine have also been detected, as well as the pyrimidine uracil in concentrations of 200 to 500 parts per billion (ppb) in the CM chondrites Murchison and Murray and in the CI chondrite Orgueil (Stoks and Schwartz, 1977, 1981). In addition, a variety of other nitrogen-heterocyclic compounds including pyridines, quinolines and isoquinolines were also identified in the Murchison meteorite (Stoks and Schwartz, 1982), as well as sugar acids (polyols) (Cooper et al, 2001) and membrane-forming lipidic compounds (Deamer and Pashley, 1989).

It has been recently found that the CI type meteorites such as Orgueil contain a distinct amino acid composition in comparison to the CMs (Ehrenfreund et al., 2001). The simple amino acid mixture, consisting of just glycine and racemic alanine, found in CI carbonaceous chondrites is interesting in the sense that it has been generally thought that a wide variety of amino acids were required for the origin of life. As discussed below, however, among the candidates for the first genetic material is peptide nucleic acid (PNA), a nucleic acid analogue in which the backbone does not contain sugar or phosphate moieties (Egholm et al., 1992; Nelson et al., 2000). For the PNA backbone, achiral amino acids such as glycine, possibly delivered by CI type carbonaceous chondrites to the early Earth, may have been the only amino acid needed for the origin of life.

7. Prebiotic polymerization processes

The organic material on the early Earth before life existed, regardless of its source, likely consisted of a wide array of different types of simple monomeric compounds, including many that play a major role in biochemistry today. How these simple abiotic organic constituents were assembled into polymers and then

into the first living entities is presently the most challenging area of research in the origin of life.

Simple monomers on the primitive Earth would need to undergo polymerization, a thermodynamically unfavorable process. It is generally assumed that polymers composed of at least 20-100 monomeric units (20-100 mers) are required in order to have any catalytic and replication functions (Joyce, 2002; de Duve, 2003). Thus, early polymerization processes must have been capable of producing polymers of at least this minimum size.

There is no evidence of abiotically produced oligopeptides or oligonucleotides in meteorites, so condensation reactions clearly must have taken place directly on the primitive Earth. Synonymous terms like 'primordial broth' or 'Darwin's warm little pond' have led in some cases to major misunderstandings, including the simplistic image of a worldwide ocean, rich in self-replicating molecules and accompanied by all sorts of biochemical monomers. The term 'warm little pond', which has long been used for convenience, refers not necessarily to the entire ocean, but to parts of the hydrosphere where the accumulation and interaction of the products of prebiotic synthesis may have taken place. These include oceanic particles and sediments, intertidal zones, fresh water shallow ponds and lakes, lagoons undergoing wet-and-dry cycles and eutectic environments associated with glacial ponds.

Simple organic compounds dissolved in the primitive oceans or other bodies of water would need to be concentrated by some mechanism in order to enhance polymerization processes. Concentration involving the selective adsorption of molecules onto mineral surfaces has been suggested as one means of promoting polymerization and this process that has been demonstrated in the laboratory using a variety of simple compounds and activated monomers (Hill et al., 2001; Ferris, 2002). The potential importance of mineral assisted catalysis is demonstrated by the montmorillonite promoted polymerization of activated adenosine and uridine derivatives producing 25-50 -mer oligonucleotides (Ferris,

2002), the general length range considered necessary for primeval biochemical functions.

Because absorption onto surfaces involves weak non-covalent van der Waals interactions, the mineral based concentration process and subsequent polymerization would be most efficient at cool temperatures (Sowerby et al., 2001; Liu and Orgel, 1998). This, however, presents somewhat of a problem because as the length of polymers formed on mineral surfaces increases they tend to be more firmly bound to the mineral (Orgel, 1998). In order for these polymers to be involved in subsequent interactions with other polymers or monomers they would need to be released. This could be accomplished by warming the mineral although this would also tend to hydrolyze the absorbed polymers. A way around this problem would be release the polymers by concentrated salt solutions (Hill et al., 2001), a process that could take place in tidal regions during evaporation or freezing of seawater.

The direct concentration of dilute solutions of monomers could also be accomplished by evaporation and by eutectic freezing of dilute aqueous solutions, which coupled with other physicochemical mechanisms such as the adherence of biochemical monomers to active surfaces, could have raised local concentrations and promoted polymerization. The evaporation of tidal regions and the subsequent concentration of their organic constituents have been proposed in the synthesis of a variety simple organic molecules (Nelson et al., 2001). Eutectic freezing of dilute reagent solutions has also been found to promote the synthesis of key biomolecules (Levy et al., 2000). These same concentration processes also could have played a key role in the synthesis of polymers. It has been shown that the freezing of dilute solutions of activated amino acids at -20°C yields peptides at higher yields than in experiments with highly concentrated solutions at 0° and 25°C (Liu and Orgel, 1997). In addition, recent studies have shown that eutectic freezing is especially effective in the non-enzymatic synthesis of oligonucleotides (Kanavarioti et al., 2001).

Salty brines may have also been important in the formation of peptides and perhaps other important biopolymers as well. According to the Rode and co-workers (Rode, 1999), "salt-induced peptide formation reaction" (SIPF) provides a pathway for the efficient synthesis of peptides and possibly proteins directly from simple amino acids in concentrated NaCl solutions containing Cu (II). Yields of di- and tri-peptides in the 0.4 to 4 % range have been reported using starting amino acid concentrations in the 40-50 mM range. Clay minerals such as montmorillonite apparently promote the reaction. Again, the evaporation of tidally flushed lagoons or the freezing of the primitive oceans could have produced the concentrated salt-rich brines needed to promote this salt-induced polymerization process.

8. Did pyrite play a role in the origins of life on Earth?

For some time one of the most serious rivals to the "primordial soup" heterotrophic theory was derived from the ideas of Wächtershäuser (1988). According to his "pioneer metabolic theory", life began with the appearance of an autocatalytic two-dimensional chemolithotrophic metabolic system that took place on the surface of the highly insoluble mineral pyrite in the vicinity of hydrothermal vents. Replication followed the appearance of this non-organismal iron sulfide-based two-dimensional "life", in which chemoautotrophic carbon fixation took place by a reductive citric acid cycle, or reverse Krebs cycle, of the type originally described for the photosynthetic green sulfur bacterium *Chlorobium limicola*. Molecular phylogenetic trees show that this mode of carbon fixation and its modifications (such as the reductive acetyl-CoA or the reductive malonyl-CoA pathways) are found in anaerobic archaea and the most deeply divergent eubacteria, which has been interpreted as evidence of its primitive character (Maden 1995). But is the reverse Krebs cycle truly primordial?

The reaction $\text{FeS} + \text{H}_2\text{S} = \text{FeS}_2 + \text{H}_2$ is a very energetically favorable. It has an irreversible, highly exergonic character with a standard free energy change of $G_o = -9.23 \text{ kcal/mol}$, which corresponds to a reduction potential $E_o = -620 \text{ mV}$. Thus,

the FeS/H₂S combination is a strong reducing agent, and has been shown to provide an efficient source of electrons for the reduction of organic compounds under mild conditions. Pyrite formation can produce molecular hydrogen, and reduce nitrate to ammonia, acetylene to ethylene, thioacetic acid to acetic acid, as well as promote more complex syntheses (Maden 1995), including peptide-bond formation that results from the activation of amino acids with carbon monoxide and nickel/iron sulfides (Huber and Wächtershäuser 1998). Although pyrite-mediated CO₂ reduction to organic compounds has not been achieved, the fixation under plausible prebiotic conditions of carbon monoxide into activated acetic acid by a mixture of co-precipitated NiS/FeS/S has been reported (Huber and Wächtershäuser 1998). However, in these experiments the reactions occur in an aqueous environment to which powdered pyrite has been added; they do not form a dense monolayer of ionically bound molecules or take place on the surface of pyrite. In addition, a careful examination of the Huber and Wächtershäuser amino acid-peptide synthesis scheme has found that it is highly unlikely to achieve formation of oligomers that had the ability to promote effective autocatalysis processes associated with the “pioneer metabolic theory” (Ross 2008).

None of the above experiments proves by itself that both enzymes and nucleic acids are the evolutionary outcome of surface-bounded metabolism. In fact, the results are also compatible with a more general, modified model of the primordial soup in which pyrite formation is recognized as an important source of electrons for the reduction of organic compounds. It is thus possible that under certain geological conditions the FeS/H₂S combination could have reduced not only CO but also CO₂ released from molten magna in deep-sea vents, leading to biochemical monomers. Peptide synthesis, for instance, could have taken place in an iron and nickel sulfide system (Huber and Wächtershäuser 1998) involving amino acids formed by electric discharges via a Miller-type synthesis. If the compounds synthesized by this process do not remain bound to the pyrite surface, but drift away into the surrounding aqueous environment, then they would become part of the prebiotic soup, not of a two-dimensional autocatalytic reaction scheme.

The essential question in deciding between these two different theories is not whether pyrite-mediated organic synthesis can occur, but whether direct CO₂ reduction and synthesis of organic compounds can be achieved by a hypothetical two-dimensional living system that lacks genetic information. Proof of Wächtershäuser's hypothesis requires demonstration of not only the tight coupling of the reactions necessary to drive autocatalytic CO₂ assimilation via a reductive citric acid cycle, but also the interweaving of a network of homologous cycles which, it is assumed, led to all the anabolic pathways as well as replication (Maden 1995). This has not been achieved. In fact, experimental results achieved so far with the FeS/H₂S combination in reducing N₂ and CO are consistent with a heterotrophic origin of life that acknowledges the role of sulfur-rich minerals and other catalysts in the synthesis and accumulation of organic compounds.

9. How did replication first originate?

The primordial broth must have been a bewildering organic chemical wonderland in which a wide array of different molecules were constantly synthesized, destroyed, or incorporated into cycles of chemical transformations. Regardless of the complexity of the prebiotic environment, life as we know it could not have evolved in the absence of a genetic replicating mechanism to guarantee the maintenance, stability, and diversification of its basic components under the action of natural selection. The appearance of the first molecular entities capable of replication, catalysis and multiplication would have marked the origin of both life and evolution. What were the fundamental characteristics of these first molecular living entities that distinguished them from non-living chemistry?

The leap from biochemical monomers and small oligomers to living entities is an enormous leap indeed! How the ubiquitous nucleic acid-based genetic system of extant life originated is one of the major unsolved problems in contemporary biology. The discovery of catalytically active RNA molecules gave considerable credibility to prior suggestions that the first living entities were largely based on

catalytic RNA molecules (ribozymes), in a hypothetical stage in the early evolution of life called the RNA world (Gilbert, 1986; Joyce, 2002). This possibility is now widely accepted, but the limited stability of the components of RNA implies that this molecule was probably not a direct product of prebiotic evolution.

The ribose component of RNA is very unstable which makes its presence in the prebiotic milieu unlikely (Larralde et al. 1995). Both lead hydroxide (Zubay, 1998) and borate minerals apparently stabilize ribose (Ricardo et al., 2004), and cyanamide is known to react with ribose to form a stable bicyclic adduct (Springsteen and Joyce, 2004). However, in order to be involved in the polymerization reactions leading to RNA, ribose would likely have to be present in solution, where it would be prone to decomposition. Moreover, the huge number of possible random combinations of derivatives of nucleobases, sugars, and phosphate that may have been present in the prebiotic soup, make it unlikely that a RNA molecule capable of catalyzing its own self-replication arose spontaneously (Joyce, 2002). These difficulties have led to proposals of pre-RNA worlds, in which informational macromolecules with backbones different from those of extant nucleic acids may have also been endowed with catalytic activity, i.e., with phenotype and genotype also residing in the same molecule. Thus, a simpler self-replicator must have come first and several possible contenders have been suggested. The RNA precursor would have had the capacity to catalyze reactions and to store information, although the component nucleobases and the backbone that held the polymer together were not necessarily the same as those in modern RNA and DNA. The nature of the genetic polymers and the catalytic agents that may have preceded RNA is presently unknown.

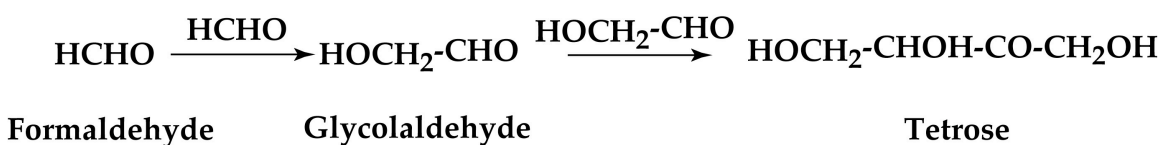
There are now several known examples of molecular genetic systems that have been studied in the laboratory and these provide examples of the types of molecular systems that could have given rise to early self-replicating entities (see Bada 2004 and references therein). Possible candidates include nucleic acid analogues (Miller 1997; Egholm et al. 1992; Nelson et al. 2000; Eschenmoser 1999, 2004) such as peptide nucleic acid (PNA), where the backbone consists of linked

amino acid derivatives such as N-(2-aminoethyl)glycine or AEG (the nucleobases are attached by an acetic acid linkage to the amino group of glycine) and threose nucleic acid (TNA), where the backbone is made up of L-threose connected by 3', 2' phosphodiester bonds. Both of these candidates form double helical structures through Watson-Crick base pairing with complementary strands of themselves, RNA and DNA. With PNA-PNA helices both left- and right-handed structures are produced in equal amounts, while the PNA-RNA and PNA-DNA helices are both right-handed. Although appealing as possible candidates for the first self-replicating molecular living entities, both PNA and TNA have positive and negative aspects, with the main negative factors being the lack of any demonstrated oligomerization process producing these nucleic acid analogues under plausible prebiotic conditions and the lack of any demonstrated catalytic properties.

PNA is attractive because its backbone is achiral (lacking handedness) which eliminates the need for the selection of chirality before the time of the origin of life. Its components, AEG and nucleobases linked to acetic acid, have been produced under simulated prebiotic conditions (Nelson et al., 2000). However, PNA is susceptible to an N-acyl migration reaction producing a rearranged PNA. This problem could be minimized, however, by blocking the N-terminal position by acetylation for example.

Based on an extensive study of sugar-based nucleic acids, TNA appears to be superior with respect to its base-pairing attributes especially with RNA compared to other possible sugar-based nucleic acids (Eschenmoser, 1999, 2004; Schöning et al., 2000). The tetrose sugar in TNA could have been synthesized during the reaction cascade that takes place during the formose reaction first described by Butlerov in 1861. The 4-carbon sugars threose and erythrose could have been readily synthesized by the dimerization of glycolaldehyde (see reaction II), which in turn could have been produced from the dimerization of formaldehyde. Even though the presence of a 4-carbon sugar in TNA reduces this problem to 2 sugars and 4 stereoisomers, it represents a formidable challenge

to demonstrate how oligonucleotides composed of only L-threose could be preferentially synthesized under prebiotic conditions.



Reaction II: The formation of tetrose sugars from the dimerization of formaldehyde and glycolaldehyde. This is only part of the overall formose series of reactions and both hexoses and pentoses would have been produced by subsequent reactions.

It is possible that PNA preceded TNA and in fact assisted in the transition to first TNA molecules. As stated above, the selection of the chiral sugar component of TNA would have required some sort of selection process to be in operation. Orgel and co-workers have suggested that the incorporation of chiral sugar dinucleotides at the end of a PNA chain, that could have occurred simply by chance, can induce chirality into a nucleic acid produced by PNA induced oligomerization (Kozlov et al., 2000). PNA could have thus provided a means of conveying the critically important biological property of chirality into polymers near the time of the origin of life. This possibility potentially solves an ongoing dilemma about whether the origin of chirality occurred before the origin of life, or whether its origin occurred during the evolution of early living entities.

Regardless of the type of nucleic acid-like analogue, or other type of replicator system, that was used by the first self-replicating entities, polymer stability and survival would have been of critical importance. Nucleic acids in general have very short survival times at elevated temperatures. RNA is very unstable because of the presence of the phosphodiester bond involving the 2'-hydroxyl group of ribose. The stability of TNA is unknown at present, but it is probably somewhat similar to the stability of DNA. Although also no known, it is possible that other

tetrose sugar-based nucleic acids were less stable than TNA because of their tendency to form less stable helical structures (Eschenmoser, 1999) and that the selection of TNA was the result of this factor. The stability of PNA been partly investigated and provided the N-acyl migration reaction can be minimized, the amide linkage in PNA would be expected to have a stability at neutral pH similar to that of peptide bonds in proteins (Wang, 1998). This suggests that in environments with temperatures of around 25°C, its survival time would be in the range of 10⁴-10⁵ years. Salty brines may have played a role in early nucleic acid survival because high salt concentrations have been found to enhance nucleic acid stability. For example, the stabilities of several tRNAs were significantly increased in 1-2 M NaCl solution in comparison to that in pure water (Tehei et al., 2000). The stability of DNA also increases with increasing salt concentration (for example, see Bada, 2004). If these results were applicable to other nucleic acid analogues such as PNA and TNA, then salt solutions would have provided a protective environment that could have enhanced the survival of early self-replicating molecular entities.

Were membranes required for the emergence of replicating systems? The emergence of life may be best understood in terms of the dynamics and evolution of sets of chemical replicating entities. Whether such entities were enclosed within membranes is not yet clear, but given the prebiotic availability of amphiphilic compounds this may have well been the case. Membrane-forming lipidic compounds may have been provided to the primitive Earth from extraterrestrial sources (Deamer and Pashley, 1989). This source could potentially supply a wide variety of aliphatic and aromatic hydrocarbons, alcohols, and branched and straight fatty acids, including some which are membrane-forming compounds. Prebiotic lipidic molecules may have also resulted from abiotic synthesis, as shown by the formation of normal fatty acids, glycerol, glycerol phosphate and other lipids (Oró et al., 1977). Also experiments have shown that simple mixtures of fatty acids and glycerol form from mixtures of mono-, di- and triglycerides under mild conditions (Hargreaves et al, 1970). It is very attractive to assume that compartmentalization within liposomes formed by amphiphilic molecules of prebiotic origin was essential for the emergence of

life. RNA molecules adsorbed onto clays such as montmorillonite, which can catalyze the formation of RNA oligomers, can be encapsulated into fatty acid vesicles whose formation in turn is accelerated by the clay. By incorporating additional fatty acid micelles, these vesicles can grow and divide while still retaining a portion of their contents needed to support RNA replication. In this manner, some of the basic machinery needed for RNA self-replication could have been compartmentalized into proto-type cells (Hanczyc et al., 2003; Chen 2006).

Despite the success of the RNA encapsulation experiments, there are problems with this “within the vesicle” scenario (Griffiths 2007). For example, in order to maintain a steady supply of molecules needed for survival of a replicating system inside a vesicle, molecules would need to cross a lipid-like bilayer barrier. This may have been a major obstacle for charged species such as amino acids. As a result perhaps the first replicating system evolved “outside the vesicle” (Griffiths 2007). In this case, vesicles attached on mineral surfaces could have been in close proximity to simple self-replicating molecules that used the catalytic properties of the mineral to enhance reactions involved in polymerization and replication. As these the two systems evolved in complexity, synergistic interactions could have developed that allowed some of the components involved in the replicating system to become freely transported into the vesicle. At this point the two systems could have merged and formed a single compartmentalized self-replication system.

10. The transition towards a DNA/RNA/protein world

The evolution of the first living molecular living entities into ones based entirely on RNA, i.e., the RNA World, would have been a major step in the evolution towards modern biochemistry. RNA has been found to be an all-in-one molecule that can not only store information but also catalyze reactions. Laboratory based "test tube evolution" experiments have demonstrated that ribozymes have the capacity to carry out a wide range of important biochemical reactions including the joining together of RNA fragments (ligation) and peptide bond formation (Bartel and Unrau, 1999). The list of demonstrated catalytic reactions is extensive

and suggests that the RNA World could have had a large repertoire of catalytic RNA molecules, perhaps functioning in concert with one another (Doudna and Cech, 2002). The complex series of reactions needed to permit multiplication, genetic transfer and variation required in the RNA World has so far not been demonstrated in the laboratory, but optimism remains because of the relative immaturity of this area of research (Bartel and Unrau, 1999; Doudna and Cech, 2002; Joyce, 2002).

The invention of protein synthesis and the encapsulation of reaction machinery needed for replication may have taken place during the RNA World. Four of the basic reactions involved in protein biosynthesis are catalyzed by ribozymes and it has been noted the complimentary nature of these reactions is not likely accidental but rather suggestive that they had a common origin most likely in the RNA World (Kumar and Yarus, 2001). If this was the case, then the origin of a primitive nucleobase code used for protein biosynthesis had its origin in the RNA World, although the bases used in the early code could have been different from the ones used today (Kolb et al., 1994).

It is possible that by the time RNA-based life appeared on Earth, the supplies of simple abiotic organic compounds derived from the sources discussed above would have been greatly diminished. Many of the components of the primordial soup had likely been extensively converted into polymers, including those associated with living entities, and thus the raw materials needed to sustain life had become largely exhausted. This implies that the origin of simple metabolic-like pathways must have been in place in order to ensure a supply in the ingredients needed to sustain the existence of the primitive living entities. In this case, some metabolic pathways needed to produce essential components required by primitive living entities were perhaps originally non-enzymatic or semi-enzymatic autocatalytic processes that later became fine tuned as ribozymatic and protein-based enzymatic processing became dominate.

More so than with earlier living molecular entities, the main limitation in the RNA World, would have been the extreme instability of RNA. This in turn

implies that RNA molecules must have been very efficient in carrying out self-replication reactions in order to maintain an adequate inventory of molecules needed for survival. The instability of RNA could have been the primary reason for the transition to the DNA/protein World where, because of the increased stability of the genetic molecules, survival would have been less dependent on polymer stability. According to Joyce (2002), it is possible that in the RNA World ribozymes arose that could catalyze the polymerization of DNA and in this manner information stored in RNA could be transferred to the more stable DNA. Another reason for DNA takeover could have been that because of increased stability much longer oligomers could have accumulated. This would have provided an enhanced storage capacity for information that could be passed on to the next generation of living entities. Before long, RNA which once played the singular role of replication and catalysis was replaced by the more efficient and robust DNA/Protein World, wherein RNA was demoted to a role of messenger/transcriber of DNA-stored information needed for protein biosynthesis.

11. Did life arise in a high-temperature environment?

Even though DNA is more stable than RNA, it is still rapidly degraded at elevated temperatures. In addition, protein enzymes denature rapidly at elevated temperatures. This must have at least initially limited the environments where DNA/Protein based life could survive for any significant period of time, and as was the case for all other earlier nucleic acid-based living entities, survival would have been the most favorable under cool conditions (Bada and Lazcano, 2002). Nevertheless, several researchers have advocated high temperatures, especially those associated with hydrothermal vent systems, as the environment where DNA/Protein based entities first arose. Proponents for a high temperature origin cite the fact that the universal tree of extant life appears to be rooted in hyperthermophilic organisms. Thus, if the last common ancestor (LCA) to all modern biology was a hyperthermophile, then it is concluded that the first DNA/protein based life must have arisen in a similar type of environment.

At first glance, both the molecular and the paleontological fossil records appear to support a hyperthermophilic origin of life. Life on Earth arose early. Large-scale analysis suggests that soon after its formation the surface of primitive Earth was extremely hot. The planet is generally thought to have remained molten for some time after its formation 4.6 Gya (Wetherill, 1990), but mineralogical evidence of a 4.3 to 4.4 Gya hydrosphere implies that its surface rapidly cooled down (Wilde et al., 2001). However, there is theoretical and empirical evidence that the planet underwent late accretion impacts (Byerly et al., 2002; Schoenberg et al., 2002) that may have boiled the oceans as late as 3.8 Gya (Sleep et al., 1989).

Could modern biochemistry have arisen under these high temperature conditions? The proposals of a high temperature origin of life face major problems, including the chemical decomposition of presumed essential biochemical compounds such as amino acids, nucleobases, RNA, and other thermolabile molecules, whose half-lives for decomposition at temperatures between 250-350° C are at the most a few minutes (White, 1984; Miller and Bada, 1988).

It is true, of course, that high temperatures allow chemical reactions to go faster. Primitive enzymes, once they appeared, could have been inefficient and thus the rate enhancement associated with higher temperatures would be one way to overcome this limitation (Harvey, 1924). However, as summarized elsewhere (Islas et al, 2007), high-temperature regimes would lead to:

- (a) reduced concentrations of volatile intermediates, such as HCN, H₂CO and NH₃;
- (b) lower steady-state concentrations of prebiotic precursors like HCN, which at temperatures a little above 100 °C undergoes hydrolysis to formamide and formic acid and, in the presence of ammonia, to NH₄HCO₃;

- (c) unstability of reactive chemical intermediates like amino nitriles ($\text{RCHO}(\text{NH}_2)\text{CN}$), which play a central role in the Strecker synthesis of amino acids; and
- (d) loss of organic compounds by thermal decomposition and diminished stability of genetic polymers.

The recognition that the deepest branches in rooted universal phylogenies are occupied by hyperthermophiles is controversial and does not provide by itself conclusive proof of a high-temperature origin of DNA/protein based life (Brochier and Phillippe, 2002). Given the huge gap existing in current descriptions of the evolutionary transition between the prebiotic synthesis of biochemical compounds and the LCA of all extant living beings, it is probably naive to attempt to describe the origin of life and the nature of the first living systems from molecular phylogenies (Islas et al., 2007). In addition, lateral gene transfer of thermoadaptative traits has apparently greatly compromised the genetic record present in modern organisms, which makes any conclusions about the environment where the DNA/Protein World originated questionable (Doolittle, 1999, 2000; Becerra et al., 2007a). An analysis of protein sequences has found only one enzyme, reverse gyrase, that is specific to hyperthermophiles; other proteins are apparently not ancestral to these organisms and are likely simply heated-adapted versions of those present in cooler temperature organisms (Forterre, 2002). Even if the LCA was a hyperthermophile (Gaucher et al., 2008; Gouy and Chaussidon, 2008), there are alternative explanations for their basal distribution, including the possibility that they are (a) a relic from early Archean high-temperature regimes that may have resulted from a severe impact regime (Sleep et al., 1989; Gogarten-Boekels et al., 1995); (b) adaptation of Bacteria to extreme environments by lateral transfer of reverse gyrase (Forterre et al., 2000) and other thermoadaptative traits from heat-loving Archaea; and (c) outcompetition of older mesophiles by hyperthermophiles originally adapted to stress-inducing conditions others than high temperatures (Miller and Lazcano, 1995).

12. From the origin of protein synthesis to the last common ancestor of life

Once DNA/Protein-based life, and probably RNA-based life as well, became dominant and evolved in sophistication, the way carbon was produced and sequestered on the Earth's surface was forever changed, especially after photosynthesis appeared. The amount of organic carbon produced by early life via the autotrophic fixation of CO₂, CH₄ and simple organic compounds such as formic and acetic acids would have far exceeded the amounts of organic compounds remaining, or still being synthesized, from either home-grown processes or extraterrestrial sources under the best conditions. The reservoir of organic material present on the Earth then shifted from one initially characterized by compounds of abiotic origin to one made up entirely of biologically derived components.

It is likely that only in the DNA/Protein World that compartmentalized of the biochemical machinery by cell-like membrane structures comparable to those used in modern biology became widespread. This is thus the first time that any direct evidence of life's existence might have been preserved in the form of physical fossils in the rock record. Earlier stages would have only left behind molecular remnants and these have not survived the ravages of geochemical abuse over billions of years of geologic time. Although traces of hydrocarbon biomarkers have been detected in 2.7 Gya sedimentary rocks, the oldest unambiguous molecular fossils found to date (Brocks et al., 1999), diagenetic processes, biological assimilation and post-depositional metamorphism have long since eradicated the earlier record of abiotic organic chemistry and the components associated with primitive molecular-based life.

All known organisms share the same essential features of genome replication, gene expression, basic anabolic reactions, and membrane-associated ATPase-mediated energy production. The molecular details of these universal processes not only provide direct evidence of the monophyletic origin of all extant forms of life, but also imply that the sets of genes encoding the components of these complex traits were frozen a long time ago, i. e., major changes in them are very

strongly selected against and are lethal. It is true that no ancient incipient stages or evolutionary intermediates of these molecular structures have been detected, but the existence of graded intermediates can be deduced, rendering unnecessary the supernatural origin advocated both by old-fashioned and contemporary creationists.

For instance, the fact that RNA molecules by themselves are capable of performing all the reactions involved in peptide-bond formation suggests, as stated earlier, that protein biosynthesis evolved in an RNA world (Zhang and Cech, 1998), i.e., that the first ribosome lacked proteins and was produced only by RNA. This possibility is supported by the crystallographic data that has shown that ribosome catalytic site, where peptide bond formation takes place, is composed of RNA (Ban, et al., 2000; Nissen, et al., 2000). Clues to the genetic organization of primitive forms of translation are also provided by paralogous genes, which are sequences that diverge not through speciation, but after a duplication event. For instance, the presence in all known cells of pairs of homologous genes encoding two elongation factors, which are GTP-dependent enzymes that assist in protein biosynthesis, provides evidence of the existence of a more primitive, less-regulated version of protein synthesis that took place with only one elongation factor. In fact, the experimental evidence of *in vitro* translation systems with modified cationic concentrations lacking both elongation factors and other protein components (Gavrilova et al., 1976; Spirin, 1986) strongly supports the possibility of an older ancestral protein synthesis apparatus prior to the emergence of elongation factors.

Analysis of the increasingly large database of completely sequenced cellular genomes from the three major domains in order to define the set of the most conserved protein-encoding sequences to characterize the gene complement of the LCA of extant life. The resulting set is dominated by different putative ATPases, and by molecules involved in gene expression and RNA metabolism. DEAD-type RNA helicase and enolase genes, which are known to be part of the RNA degradosome, are as conserved as many transcription and translation genes. This suggests the early evolution of a control mechanism for gene

expression at the RNA level, providing additional support to the hypothesis that during early cellular evolution RNA molecules played a more prominent role. Conserved sequences related to biosynthetic pathways include those encoding putative phosphoribosyl pyrophosphate synthase and thioredoxin, which participate in nucleotide metabolism. Although the information contained in the available databases corresponds only to a minor portion of biological diversity, the sequences reported here are likely to be part of an essential and highly conserved pool of proteins domains common to all organisms (Becerra et al., 2007b).

The high levels of genetic redundancy detected in all sequenced genomes imply not only that duplication has played a major role in the accretion of the complex genomes found in extant cells, but also that prior to the early duplication events revealed by the large protein families, simpler living systems existed which lacked the large sets of enzymes and the sophisticated regulatory abilities of contemporary organisms. The variations of traits common to extant species can be easily explained as the outcome of divergent processes from an ancestral life form that existed prior to the separation of the three major biological domains, i.e., the LCA or cenancestor. No paleontological remnants likely remain that bear testimony of its existence, so the search for a fossil of the cenancestor is bound to prove fruitless.

Analysis of an increasingly large number of completely sequenced cellular genomes has revealed major discrepancies with the topology of rRNA trees. Very often these differences have been interpreted as evidence of horizontal gene transfer (HGT) events between different species, questioning the feasibility of the reconstruction and proper understanding of early biological history (Doolittle, 1999, 2000). There is clear evidence that genomes have a mosaic-like nature whose components come from a wide variety of sources (Ochman et al., 2000). Depending on their different advocates, a wide spectrum of mix-and-match recombination processes have been described, ranging from the lateral transfer of few genes via conjugation, transduction or transformation, to cell fusion events involving organisms from different domains.

Universal gene-based phylogenies ultimately reach a single universal entity, but the bacterial-like LCA (Gogarten et al., 1989) that we favor was not alone. It would have been in the company of its siblings, a population of entities similar to it that existed throughout the same period. They may have not survived, but some of their genes did if they became integrated via lateral transfer into the LCA genome. The cenancestor is thus one of the last evolutionary outcomes of a series of ancestral events including lateral gene transfer, gene losses, and paralogous duplications that took place before the separation of Bacteria, Archaea, and Eucarya (Lazcano et al., 1992; Glansdorff, 2000; Castresana, 2001; Delaye et al., 2004; Becerra et al., 2007b).

13. Conclusions and perspectives

Although there have been considerable advances in the understanding of chemical processes that may have taken place before the emergence of the first living entities, life's beginnings are still shrouded in mystery. Like vegetation in a mangrove swamp, the roots of universal phylogenetic trees are submerged in the muddy waters of the prebiotic broth, and how the transition from the non-living to the living took place is still unknown. Given the huge gap existing in current descriptions of the evolutionary transition between the prebiotic synthesis of biochemical compounds and the last common ancestor of all extant living beings, it is probably naive to attempt to completely describe the origin of life and the nature of the first living systems from molecular phylogenies.

Our current understanding of genetics, biochemistry, cell biology, and the basic molecular processes of living organisms have challenged many original assumptions of the heterotrophic theory. The view advocated here assumes that, even if the first living entities were endowed with minimum synthetic abilities, their maintenance and replication depended primarily on organic compounds synthesized by prebiotic processes. An updated heterotrophic hypothesis assumes that the raw material for assembling the first self-maintaining,

replicative chemical systems was the outcome of abiotic synthesis, while the energy required to drive the chemical reactions involved in growth and reproduction may have been provided by cyanamide, thioesters, glycine nitrile, or other high energy compounds (de Duve 1995; Lazcano and Miller 1996).

The basic tenet of the heterotrophic theory of the origin of life is that the maintenance and reproduction of the first living entities depended primarily on prebiotically synthesized organic molecules. As summarized here, there has been no shortage of discussion about how the formation of the primordial soup took place. But, have too many cooks spoiled the soup? Not really. It is very unlikely that any single mechanism can account for the wide range of organic compounds that may have accumulated on the primitive Earth, and that the prebiotic soup was formed by contributions from endogenous syntheses in both a reducing or a neutral atmosphere, metal sulphide-mediated synthesis in hydrothermal vents, and exogenous sources such as comets, meteorites and interplanetary dust. This eclectic view does not beg the issue of the relative significance of the different sources of organic compounds --it simply recognizes the wide variety of potential sources of organic compounds, the raw material required for the emergence of life.

As discussed here, the existence of different abiotic mechanisms by which biochemical monomers can be synthesized under plausible prebiotic conditions is well established. Of course, not all prebiotic pathways are equally efficient, but the wide range of experimental conditions under which organic compounds can be synthesized demonstrates that prebiotic syntheses of the building blocks of life are robust, i.e., the abiotic reactions leading to them do not take place under a narrow range defined by highly selective reaction conditions, but rather under a wide variety of experimental settings.

The synthesis of chemical constituents of contemporary organisms by non-enzymatic processes under laboratory conditions does not necessarily imply that they were either essential for the origin of life or available in the primitive environment. Nonetheless, the remarkable coincidence between the molecular

constituents of living organisms and those synthesized in prebiotic experiments is too striking to be fortuitous, and the robustness of this type of chemistry is supported by the occurrence of most of these biochemical compounds in the 4.5 Gya carbonaceous meteorites (Ehrenfreund et al., 2002). So it becomes plausible, but not proven, that similar synthesis took place on the primitive Earth. For all the uncertainties surrounding the emergence of life, it appears to us that the formation of the prebiotic soup is one of the most firmly established events that took place on the primitive Earth.

Mainstream scientific hypothesis on the origin of life, which have been developed within the framework of an evolutionary analysis, have led to a wealth of experimental results and the development of a coherent historical narrative linking many different disciplines and raising major philosophical issues. It is true that there are large gaps in the current descriptions of the evolutionary transition between the prebiotic synthesis of biochemical compounds and the LCA of all extant living organisms, but attempts to reduce it have allowed a more precise description of the beginning of life. Now days a central issue in origin of life research is to understand the abiotic synthesis of an ancestral genetic polymer endowed with catalytic activity, its further evolution to an RNA world, and ultimately into the DNA/RNA/Protein World characteristic of all life on Earth. We face major unsolved problems, but they are not completely shrouded in mystery, unsolvable or unknowable.

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Figure 1: The apparatus used in the 1953 Miller experiment. The 500-cc flask was used to represent the oceans and the 5-liter flask the atmosphere. A spark discharge generated across the electrodes with a Tesla coil, invented by Nikola Tesla in 1891, was used to mimic lightning and corona discharges in the atmosphere.

