ELSEVIER

Prebiological evolution and the physics of the origin of life

Luis Delaye, Antonio Lazcano [*]

Facultad de Ciencias, UNAM, Apdo. Postal 70-407, Cd. Universitaria, 04510 Mexico, Mexico

Abstract

The basic tenet of the heterotrophic theory of the origin of life is that the maintenance and reproduction of the first living systems depended primarily on prebiotically synthesized organic molecules. It is unlikely that any single mechanism can account for the wide range of organic compounds that may have accumulated on the primitive Earth, suggesting that the prebiotic soup was formed by contributions from endogenous syntheses in reducing environments, metal sulphide-mediated synthesis in deep-sea vents, and exogenous sources such as comets, meteorites and interplanetary dust. The wide range of experimental conditions under which amino acids and nucleobases can be synthesized suggests that the abiotic syntheses of these monomers did not take place under a narrow range defined by highly selective reaction conditions, but rather under a wide variety of settings. The robustness of this type of chemistry is supported by the occurrence of most of these biochemical compounds in the Murchison meteorite. These results lend strong credence to the hypothesis that the emergence of life was the outcome of a long, but not necessarily slow, evolutionary processes. The origin 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. This scheme is not at odds with the theoretical models of self-organized emerging systems, but what is known of biology suggest that the essential traits of living systems could have not emerged in the absence of genetic material able to store, express and, upon replication, transmit to its progeny information capable of undergoing evolutionary change. How such genetic polymer first evolved is a central issue in origin-of-life studies.
© 2005 Elsevier B.V. All rights reserved.

---

[*] Corresponding author.
E-mail address: alar@correo.unam.mx (A. Lazcano).

Contents

1. Introduction

During a memorable 1939 lecture at the Royal Institution in London, wrote Max Perutz, the famous John D. Bernal stated that "all protein that we know now have been made by other proteins, and these in turn by others". How did such process got started? When Bernal repeated the same argument in a later discussion, Perutz [67] adds, "the physicist W. H. Bragg asked him where the first protein had come form. Instead of replying 'I do not know', Bernal skillfully sidestepped Bragg's awkward question".

Perutz does not writes how Bernal avoided the issue raised by Bragg, but the story reveals the strong scientific appeal that issues related to the nature of life and the origin of biological systems that has been brewing among physicists since the pre-DNA double helix times. Such trend, which was highlighted by Schrödinger's 1945 seminal book *What is Life?*, continues to this day, as shown by the manifold attempts to describe the emergence of life in terms of non-linear interactions and non-equilibrium constraints, the thermodynamics of irreversible processes, pattern formation, chaos, attractors, fractals and, more recently, complexity theory. Such approaches should be seen as open invitations to develop multi-disciplinary research programs but, as noted by Fenchel [16], in some cases invocations to spontaneous generation appear to be lurking behind appeals to undefined "emergent properties" or "self-organizing principles" that are used as the basis for what many life scientists see as grand, sweeping generalizations with little relationship to actual biological phenomena.

The proposal of an heterotrophic origin of life is strongly supported by a number of rather successful prebiotic simulation experiments, as well as by the characterization of organic molecules of biochemical significance in meteorites and other extraterrestrial minor bodies rich in organic material. These results lend strong credence to the hypothesis that the emergence of life was the outcome of a long, but not necessarily slow, evolutionary processes. This conclusion is not at odds with the theoretical models of highly complex functionally organized systems favored nowadays by some physicists, but as of today none of these have provided manageable descriptions of the origin of life. Mainstream evolutionary

biologists and prebiotic chemists tend to be wary of explanations that assume that the emergence of life was the outcome of timeless mathematical or physical principles in which replication, selection, and adaptation play no role. Such lack of interest does not implies, of course, a belief that the natural processes that led to the first life forms were exempt from the constraints imposed by physics, or that explanations on the appearance of life should reduce themselves to the issue of the emergence of nucleic acids or their precursors. However, in spite of a number of mesmerizing theoretical and experimental analogs [39,94] what is known of biology suggest that the essential traits of living systems could have not emerged in the absence of genetic material able to store, express and, upon replication, transmit to its progeny information capable of undergoing evolutionary change. How such genetic polymer first evolved is one of the most basic questions in origin-of-life studies. Those involved in this field know they have plenty to be modest about, and they tend to be. For most life scientists, research on the origin of life should be addressed conjecturally, in an attempt to construct a coherent, non-teleological historical narrative with and inquiring and explanatory character [36]. How the current information on the distribution of abiotically synthesized organic compounds both in extraterrestrial environments and under simulated laboratory conditions can be combined with the idea of an RNA world is discussed in this review.

2. The physical setting of the origin of life

It is unlikely that the paleontological record will provide direct data on how life first appeared. There is no geological evidence of the environmental conditions on the Earth at the time of the origin of life, nor any fossil register of the evolutionary processes that preceded the appearance of the first cells. Direct information is 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 values, and other general and local environmental conditions which may or may not have been important for the emergence of life. Moreover, the attributes of the first living organisms are unknown. They were probably simpler than any cell now alive, and may have lacked not only protein-based catalysis, but perhaps even the familiar genetic macromolecules, with their ribose-phosphate backbones. It is possible that the only property they shared with extant organisms was the structural complementarity between monomeric subunits of replicative genetic polymers able to transmit to its progeny information capable of undergoing evolutionary change. Hence, caution must be exercised in extrapolating molecular phylogenies back into primordial times. Comparative genomics is a blooming field that has an extraordinary potential for our understanding early cellular evolution, but it cannot be applied to events prior to the evolution of protein biosynthesis. Older stages are not yet amenable to this type of analysis, and the organisms at the base of universal phylogenies are ancient species, not primitive unmodified microbes.

However, the traits shared by all known living beings are far to numerous and complex to assume that they evolved independently. Minor differences in the basic molecular processes of the three main cell lines can be distinguished, but all known organisms share the same genetic code and 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 provide direct evidence of the monophyletic origin of all known forms of life, while their variations can be easily explained as the outcome of divergent processes from an ancestral lifeform, *fons et origo* of all contemporary organisms. When and how did such ancestral form arise?

It is not possible to assign a precise chronology to the appearance of life. However, in the past few years estimates of the available time for this to occur have been considerable reduced. As shown by recent debates, determination of the biological origin of what have been considered the earliest traces of life is a rather contentious issue, an outcome of a scarce Archaean geological record with very few rocks older than 3.5 billion years. Those that remain have been so extensively altered by metamorphic processes that any direct life evidence of life predating this limit has apparently been largely obliterated, and most of the rocks which have been preserved have been metamorphosed to a considerable extent [89].

Nevertheless, there is evidence that life emerged on Earth as soon as it was possible to do so. It has been argued that the microstructures interpreted as cyanobacterial remnants in the 3.5 billion years-old Apex sediments of the Australian Warrawoona formation [78] could be the outcome of abiotic hydrothermal processes [6,24]. However, recent analysis of 3.4 billion years-old South African cherts indicates the existence of photosynthetic microbial mats in ancient marine environments [87]. Such rapid development 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 down.

Water provides the medium for chemical reactions to take place, and the polymers required to carry out the central biological functions of replication and catalysis. How did it accumulate on the primitive Earth? The depletion of rare gases in the Earth's atmosphere compared to cosmic abundances shows that any primary atmosphere, if the planet ever had one, was rapidly lost [38]. Moreover, it is unlikely that water made its first appearance on Earth as a liquid. Soon after the Earth was formed, the release of the volatiles trapped within the accreting planetesimals very likely lead to a secondary atmosphere. Since current evidence suggests that the Earth's core formed when accretion was taking place, removal of metallic iron from the upper mantle must have lead to a highly reduced atmosphere of volcanic origin containing chemical species such as CH_4, NH_3 and H_2. Due to the high surface temperature, however, the bulk of the atmosphere would have consisted of superheated steam [38]. However, large impact events such as the one that lead to the Moon's formation would have eroded this primitive atmosphere, which would have been replaced by further outgassing events.

Moon-forming impacts must have been relatively rare, but it is generally accepted that during the latter stages of the accretion process the influx of comet-like bodies that originated from further out in the Solar System impacting the primitive Earth must have been considerable and could have led to the accumulation of significant amounts of water and other volatiles [64]. Cometary nuclei, which appear to be the most pristine materials surviving from the formation of the Solar System, may have supplied organic compounds that could played a role in the origin of life on Earth [1,7,8,64].

One reason for proposing an extraterrestrial origin of the components of the prebiotic soup is the CO_2-rich model of the primitive Earth's atmosphere [38]. Of course, the presence of an extraordinarily complex array of organic molecules in meteorites, comets, interplanetary dust and interstellar molecules argues for the robustness of organic chemistry in the Universe, but also raises the issue of their possible role in the origin of life. As noted below, it is likely that exogenous sources of organic compounds contributed to the synthesis of the primitive soup. The major sources of exogenous compounds would appear to be comets and dust, with asteroids and meteorites being minor contributors. Asteroids would have impacted the Earth frequently during the Hadean and early Archean, but the amount of organic material brought in would seem to be small, even if the asteroids are assumed to be Murchison meteorite-type object. Carbonaceous chondrites, a class of stony meteorites, are among the most primitive objects in the Solar System in terms of their elemental composition. The most extensively analyzed meteorites

for organic compounds include the Murchison and Murray meteorites, as well as the CI class Orgueil meteorite. The Murchison meteorite contains approximately 1.8% organic carbon, but most of this is a polymer, and there are only about 100 parts per million of amino acids (which represents, assuming a void volume of 10% and a density of approximately 2.0, 0.10 gm/kg meteorite, or 2.0×10^{-2} M of amino acids). The majority (up to 80%) of the soluble organic matter in meteorites is made up by polycyclic aromatic hydrocarbons (PAHs), followed by the carboxylic acids, the fullerenes and amino acids, which are about an order of magnitude less abundant [5]. The purines adenine, guanine, xanthine and hypoxanthine have also been detected, as well as the pyrimidine uracil in concentrations of 200–500 parts per billion in the CM chondrites Murchison and Murray and in the CI chondrite Orgueil [84,85, 90]. In addition, a variety of other nitrogen-heterocyclic compounds including pyridines, quinolines and isoquinolines were also identified in the Murchison meteorite [86], as well as sugar acids (polyols) [10] and membrane-forming lipidic compounds [12].

Comets are the most promising source of exogenous compounds [66]. At summarized elsewhere [3], it is reasonable to assume that the atmosphere that developed on the Earth over the period 4.4–3.8 billion years ago was essentially a mix of volatiles delivered by bodies such as cometary nuclei, combined with the products of outgassing processes from the interior of an already differentiated planet. This atmosphere was probably dominated by water steam until the surface temperatures dropped to $\sim$100 °C (depending on the pressure), at which point water condensed out to form early oceans [93]. As the Earth had cooled down and the influx of myriads of comets and asteroids had settled down, the reduced chemical species, which were mainly supplied by volcanic outgassing and are very sensitive to UV radiation that penetrated through the atmosphere due to the lack of a protective ozone layer, were probably destroyed by photodissociation, although there might have been steady state equilibrium between these two processes that allowed a significant amount of these reduced species to be present in the atmosphere.

3. Primordial heterotrophy and the emergence of life

It is generally believed that after Louis Pasteur had disproved the spontaneous generation of microbes using his famous swam-necked flasks experiments, the discussion of life beginning's had been vanished to the realm of useless speculation. However, scientific literature of the first part of the 20th century shows the many attempts by major scientists to solve this problem. The list covers a rather wide range of explanations that go from the ideas of Pflüger on the role of hydrogen cyanide on the origin of life, to those of Svante Arrhenius on panspermia, and includes Leonard Troland's hypothesis of a primordial enzyme formed by chance events in the primitive ocean, Alfonso L. Herrera's sulfocyanic theory on the origin of cells, Harvey's 1924 suggestion of an heterotrophic origin in a high-temperature environment, and the provocative 1926 paper that Hermann J. Muller wrote on the abrupt, random formation of a single, mutable gene endowed with catalytic and autoreplicative properties [43].

In spite of their diversity, most of these explanations went unnoticed, in part because they were incomplete, speculative schemes largely devoid of direct evidence and not subject to fruitful experimental testing. Although some of these hypotheses considered life as an emergent feature of nature and attempted to understand its origin by introducing principles of historical explanation, the dominant view was that the first forms of life had been photosynthetic microbes endowed with the ability fix atmospheric CO_2 and to use it with water to synthesize organic compounds. A major scientific breakthrough occurred, however,

when Oparin [60] suggested a hetrototrophic origin of life that assumed that prior to the emergence of the first cells a prebiotic synthesis of organic compounds led to the accumulation of the primitive broth.

Such ideas were supported not only by the evidence of organic compounds in meteorites, but also by the striking 19th experimental demonstrations that biochemical compounds such as urea, alanine, and sugars could be formed under laboratory conditions, as had been demonstrated by Wöhler, Strecker and Butlerow, respectively. Oparin's proposal, which was based on his Darwinian credence in a gradual, slow evolution from the simple to the complex, stood in stood in sharp contrast with the then prevalent idea of an autotrophic origin of life. Since a heterotrophic anaerobe is metabolically simpler than an autotrophic one, the former would necessarily have evolved first. Thus, based on the simplicity and ubiquity of fermentative reactions, Oparin [60] suggested in a small booklet that the first organisms must have been heterotrophic bacteria that could not make their own food but obtained organic material present in the primitive milieu.

Careful reading of Oparin's [60] pamphlet shows that, in contrast to common belief, at first he did not assume an anoxic primitive atmosphere. In his original scenario he argued that while some carbides, i.e., carbon-metal compounds, extruded from the young Earth's interior would react with water vapor leading to hydrocarbons, others would be oxidized to form aldehydes, alcohols, and ketones (such as acetone). These molecules would then react among themselves and with NH_3 originating from the hydrolysis of nitrides (nitrogen-metals), to form "very complicated compounds", as Oparin wrote, from which proteins and carbohidrates would form. These ideas were further elaborated and refined in a more extensive book whose English translation was published in 1938 [61]. In this book Oparin's original proposal was revised, leading to the assumption of a highly reducing milieu in which iron carbides of geological origin would react with steam to form hydrocarbons. Their oxidation would yield alcohols, ketones, aldehydes, etc., that would then react with ammonia to form amines, amides and ammonium salts. The resulting protein-like compounds and other molecules would form a hot dilute soup, in which would aggregate to form colloidal systems such as coacervates, from which the first heterotrophic microbes evolved. Like many others at the time, Oparin did not address in his 1938 book the origin of nucleic acids, because their role in genetic processes was not even suspected. Because of this, inheritance of primordial genetic information was assumed by Oparin to be the result of growth and division in the coacervate drops he advocated as models of precellular systems.

4. Pyrite and the origin of life

Although by the late 19th century an autotrophic origin of life was part of mainstream biological thought, currently the best known alternative to the heterotrophic theory stems from the work of Wächtershäuser [92]. According to this hypothesis, life began with the appearance of an autocatalytic two-dimensional chemolithotrophic metabolic system based on the formation of the highly insoluble mineral pyrite. The synthesis in activated form of organic compounds such as amino acid derivatives, thioesters and keto acids is assumed to have taken place on the surface of FeS and FeS_2 in environments that resemble those of deep-sea hydrothermal vents. Replication followed the appearance of 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 sulphur 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 [51]. This assumes, however, that the root of molecular phylogenetic trees can be extrapolated down to the very origin of life which, as argued below, is a rather contentious issue.

The reaction $FeS + H_2S = FeS_2 + H_2$ is a very favourable one. It has an irreversible, highly exergonic character with a standard free energy change $\Delta G^0 = -9.23$ kcal/mol, which corresponds to a reduction potential $E^0 = -620$ mV. Thus, the FeS/H_2S 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. Although pyrite-mediated CO_2 reduction to amino acids, purines and pyrimidines is yet to be achieved, the FeS/H_2S combination is a strong reducing agent that has been shown to reduce nitrate and acetylene, as well as to induce peptide-bonds that result from the activation of amino acids with carbon monoxide and (Ni, Fe)S [34,51]. Acetic acid and pyruvic acid have been synthesized from CO under simulated hydrothermal conditions in the presence of sulfide minerals [9,33]. However, the empirical support for Wächtershäuser's central tenets is meager. Life does not consist solely of metabolic cycles, and none of these experiments proves that enzymes and nucleic acids are the evolutionary outcome of multistep autocatalytic metabolic cycles surface-bounded to FeS/FeS_2 or some other mineral. As argued elsewhere [3], experiments using the FeS/H_2S combination are also compatible with a more general, modified model of the primitive soup in which pyrite formation is recognized as an important source of electrons for the reduction of organic compounds.

5. Prebiotic syntheses of amino acids and nucleobases: an optimistic assessment

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. Experimental evidence in support of Oparin's proposal of chemical evolution came first from Harold C. Urey's laboratory, whom had been involved with the study of the origin of the Solar System and the chemical events associated with this process. Urey had also considered the origin of life in the context of his proposal of a highly reducing terrestrial atmosphere [88]. The first successful prebiotic amino acids synthesis was carried out with an electric discharge and a strongly reducing model atmosphere of CH_4, NH_3, H_2O, and H_2 [52]. The result of this experiment was a large yield of a racemic mixture of amino acids, together with hydroxy acids, short aliphatic acids, and urea. One of the surprising results of this experiment was that the products were not a random mixture of organic compounds; rather, a relatively small number of compounds were produced in substantial yield. Moreover, with a few exceptions, the compounds were of biochemical significance.

The mechanism of synthesis of the amino and hydroxy acids formed in the spark discharge experiment was investigated [52,53]. The presence of large quantities of hydrogen cyanide, aldehydes and ketones in the water flask, which were clearly derived from the methane, ammonia, and hydrogen originally included in the apparatus, showed not only that the amino acids were not formed directly in the electric discharge, but were the outcome of a Strecker-like synthesis that involved aqueous phase reactions of highly reactive intermediates. Detailed studies of the equilibrium and rate constants of these reactions demonstrated that both amino- and hydroxy acids can be synthesized at high dilutions of HCN and aldehydes in a simulated primitive ocean. The reaction rates depend on temperature, pH, HCN, NH_3, and aldehyde concentrations, and are rapid on a geological time scale; the half-lives for the hydrolysis of

the intermediate products in the reactions, amino- and hydroxy nitriles, are less than a thousand years at $0\,^\circ$C, and there are no known slow steps [56].

A few years after the Miller experiment, Juan Oró, who had been studying the synthesis of amino acids from an aqueous solution of HCN and NH_3, reported the abiotic formation of adenine [63]. The synthesis is indeed remarkable. If concentrated solutions of ammonium cyanide are refluxed for a few days, adenine is obtained in up to 0.5% yield along with 4-aminoimidazole-5 carboxamide and the usual cyanide polymer [63,65]. This reaction, proceeds through the self-condensation of HCN to give diaminomaleonitrile, which according to [65], then reacts with formamidine to give adenine. Although in principle adenine may be consider as a mere pentamer of HCN, under dilute aqueous solutions adenine synthesis involves the formation and rearrangement of other precursors such as 2-cyano and 8-cyano adenine [91].

In the scheme suggested by Oró [63], the limiting step is the reaction of diaminomaleonitrile with formamidine, but as demonstrated by Ferris and Orgel [18], this can be bypassed by a two photon photochemical rearrangement of diaminomaleonitrile that proceeds readily with sunlight to give high yields of amino imidazole carbonitrile. An additional possibility is that tetramer formation may have occurred in the primitive Earth in an eutectic solution of HCN–H_2O, which could have existed in the polar regions of an Earth of the present average temperature. High yields of the HCN tetramer have been reported by cooling dilute cyanide solutions to temperatures between -10 and $-30\,^\circ$C for a few months [74]. Production of adenine by HCN polymerization is accelerated by the presence of formaldehyde and other aldehydes, which could have also been available on the prebiotic environment [91].

The prebiotic synthesis of guanine, the other major purine present in extant living systems, was first studied in an experimental setting involving high concentrations of a number of precursors, including ammonia [75]. It has been proposed that together with guanine, other purines including hypoxanthine, xanthine, and diaminopurine could have been produced in the primitive environment by variations of the adenine synthesis using aminoimidazole carbonitrile and aminoimidazole carboxamide [76]. A reexamination of the polymerization of concentrated NH_4CN solutions has shown that in addition to adenine, guanine is also produced at both -80 and $-20\,^\circ$C [47]. It is probable that most of the guanine obtained from the polymerization of NH_4CN is the product of diaminopurine, which reacts readily with water and undergoes a hydrolytic deamination to give guanine and some isoguanine. The yields of guanine in this "one-pot" reaction synthesis of purines yields are 10–40 less than those of adenine, guanine, and a simple set of amino acids dominated by glycine have also been detected in dilute solutions of NH_4CN which were kept frozen for 25 years at -20 and $-78\,^\circ$C, as well as in the aqueous products of spark discharge experiments from a reducing experiment frozen for 5 years at $-20\,^\circ$C [48]. Moreover, formamide, which is an hydrolytic product of HCN and is formed abundantly from the pyrolytic decomposition of HCN polymers, has been shown to react with HCN to produce adenine and formylpurine derivatives [72]. This reaction, which is enhanced in the presence of mineral catalyst, including silica, alumina, zeolite, and kaolin, is also known to yield cytosine and 4-hydroxypyrimidine [71,73].

The abiotic synthesis of cytosine in an aqueous phase from cyanoacetylene (HCC–CN) and cyanate (NCO^-) has been described [19,74]. Cyanoacetylene is abundantly produced by the action of a spark discharge on a mixture of methane and nitrogen, and cyanate can come from cyanogen (NCCN) or from the decomposition of urea (H_2N–CO–NH_2) However, since it is rapidly hydrolyzed to CO_2 and NH_3, the high concentrations of cyanate (>0.1 M) required in this reaction may be unrealistic.

Orotic acid, which is a biosynthetic precursor of uracil, was identified, albeit in low yields, among the hydrolytic products of hydrogen cyanide polymers [21]. On the other hand, the reaction of cyanoacetaldehyde, which is produced in high yields from the hydrolysis of cyanoacetylene, with urea, first studied

by Ferris et al. [20], produces no detectable levels of cytosine. However, when the same non-volatile compounds are concentrated in the laboratory modelling of "evaporating pond" conditions simulating primitive evaporating lagoons or pools on drying beaches on the early Earth, surprisingly high amounts of cytosine (>50%) are observed [68]. A related synthesis under evaporating conditions uses cyanoacetaldehyde with guanidine, which produce diaminopyrimidine [20] with very high yields [69]. Although it is unlikely that high amounts of diaminopyrimidine were present in the primitive Earth, both uracil and very low yields of cytosine result from its hydrolysis. The effectiveness of formamide as a prebiotic precursor of a mixture of both purines and pyrimidines in the presence of TiO_2 [71,73] suggest that in such environments simple minerals could have also promoted the synthesis of nucleobases in the primitive environment from hydrolytic products of HCN and other reactants that may have been easily available.

It is unlikely that high amounts of diaminopyrimidine were present in the prebiotic environment. However, a wide variety of other modified nucleic acid bases may have been available in the early Earth. The list includes isoguanine, which is a hydrolytic product of diaminopurine [47], as well as other modified purines which are the outcome of side reactions of both adenine and guanine with a number of different amines under the concentrated conditions of a drying pond [46], including a number of methylated bases.

6. How did organic compounds accumulate in the prebiotic soup?

The easiness of formation under reducing conditions ($CH_4 + N_2$, $NH_3 + H_2O$, or $CO_2 + H_2 + N_2$) in one-pot reactions of amino acids, purines, and pyrimidines strongly suggest these molecules were present in the prebiotic broth. Experimental evidence suggests that urea, alcohols, sugars formed by the non-enzymatic condensation of formaldehyde, a wide variety of aliphatic and aromatic hydrocarbons, urea, carboxylic acids, and branched and straight fatty acids, including some which are membrane-forming compounds, were also components of the primitive soup. The remarkable coincidence between the molecular constituents of living organisms and those synthesized in simulation 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 4.5×10^9 years-old Murchison carbonaceous meteorite, which also yields evidence of liquid water in its parent body [14].

These results are extremely encouraging, but it should be emphasized that 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 agreement on the composition of the primitive atmosphere; opinions vary from strongly reducing ($CH_4 + N_2$, $NH_3 + H_2O$, or $CO_2 + H_2 + N_2$) to neutral ($CO_2 + N_2 + H_2O$). In general, non-reducing atmospheric models are favoured by planetary scientists, while prebiotic chemists lean towards more reducing conditions, under which the abiotic syntheses of amino acids, purines, pyrimidines, and other compounds are very efficient.

Although Miller and Urey originally rejected the idea of nonreducing conditions for the primitive atmosphere, a number of experiments were later on carried out in his laboratory using CO and CO_2 model atmospheres [77]. It was found that not only were the yields of the amino acids reduced, but that as the atmosphere became less reducing and more neutral, the yields of synthesized organic compounds decreased drastically and glycine was basically the only amino acid synthesized [56]. 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

nonreducing atmospheres is the formation of hydrogen cyanide (HCN), which is an intermediate in the Strecker pathway and an important precursor compound for the synthesis of nucleobases [21,63]. However, localized high concentrations of reduced gases may have existed around volcanic eruptions and in these localized environments reagents such as HCN, aldehydes and ketones could have been produced, which after dissolving into the primitive oceans could have taken part in the prebiotic synthesis of organic molecules.

Because of problems associated with the direct Miller–Urey type syntheses on the early Earth, different hypotheses for the abiotic synthesis of organic compounds has been proposed. One possibility that has been suggested resulted from the discovery of hydrothermal vents, which have been proposed as the site where prebiotic synthesis took place and life originated [11,32]. A further refinement of this hypothesis has led Everett Shock and his coworkers to argue, based on calculations of thermodynamic-based equilibria, that such environments favor the formation of compounds such as amino acids at high temperatures [81], especially in vents associated with off-axis systems [40].

As recognized long ago by Harvey [29], a major advantage of high temperatures is that the chemical reactions would go faster, and the primitive enzymes, once they appeared, could have been less efficient. However, the price paid is manifold: such high-temperature regimes would lead to (a) reduced concentrations of volatile intermediates, such as HCN, H_2CO and NH_3; (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 NH_4HCO_2; (c) instability of reactive chemical intermediates like amino nitriles ($RCHO(NH_2)CN$), 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 [4,54,55].

Survival of nucleic acids is limited by the hydrolysis of phosphodiester bonds [50], and the stability of Watson–Crick helices (or their pre-RNA equivalents) is strongly diminished by high-temperatures. For an RNA-based biosphere the reduced thermal stability on the geologic timescale of ribose and other sugars is the worst problem [42], but the situation is equally bad for pyrimidines, purines and some amino acids. As reviewed elsewhere [55], the half-life of ribose at $100\,°C$ and pH 7 is only 73 min, and other sugars (2-deoxyribose, ribose-5-phosphate, and ribose 2,4-biphosphate) have comparable half-lives [42]. The half-life for hydrolytic deamination of cytosine at $100\,°C$ lies between 19 and 21 days [25,45,80], although at $100\,°C$ the half-life of uracil is approximately 12 years [45]. At $100\,°C$ the thermal stability of purines is also reduced: between 204 to 365 days for adenine [23,45,79], with comparable values for guanine [45]. These results imply that if the origin of life was sufficiently long, all the complex organic compounds in the ocean, whether derived from home-grown synthesis or from exogenous delivery, 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.

The difficulties involved with the endogenous synthesis of amino acids and nucleobases have led to the development of alternatives. It is likely, for instance, that geological sources of hydrogen, such as pyrite, may have been available; in the presence of ferrous iron, a sulfide ion (SH^-) would have been converted to a disulfide ion (S^{2-}), thereby releasing molecular hydrogen [51]. In addition, an analysis of Oro's 1961 suggestion on the role of cometary nuclei as sources of volatiles to the primitive Earth, has lead to the reassessment of the proposal that the exogenous delivery of organic matter by asteroids, comets and interplanetary dust particles (IDPs) could have played a significant role in the prebiotic accumulation of the compounds necessary for the origin of life [8]. If this idea is correct, impacts on the early Earth

could have led to devastating conditions which made it difficult for life to originate, but also delivered the raw material necessary for setting the stage for the origin of life. It is also possible that the impacts of iron-rich asteroids enhanced the reducing conditions, and that cometary collisions led to localized environments favouring organic synthesis. Based on what is known about prebiotic chemistry and meteorite composition, if the primitive Earth was non-reducing, then the organic compounds required must have been brought in by interplanetary dust particles, comets, and meteorites, a hypothesis that requires that a significant percentage of meteoritic amino acids and nucleobases could survive the high temperatures associated frictional heating during atmospheric entry, and become part of the primitive broth.

This eclectic view in which the prebiotic soup is formed by contributions from endogenous syntheses, extraterrestrial organic compounds delivered by comets and meteorites, and pyrite-mediated CO reduction does not contradict the heterotrophic theory. Even if the ultimate source of the organic molecules required for the origin of life turns out to be comets and meteorites, recognition of their extraterrestrial origin is not a rehabilitation of panspermia (e.g., the hypothesis that life existed elsewhere in the Universe and had been transferred from planet to planet, eventually gaining a foothold on the Earth), but an acknowledgement of the role of collisions in shaping the primitive terrestrial environment.

7. Prebiotic polymers and the RNA world

Regardless of its ultimate sources, the organic material that may have accumulated on the early Earth before life existed very likely consisted of a wide array of different types of compounds, including many of the simple compounds that play a major role in biochemistry today. How these abiotic organic constituents were assembled into polymers and then into the first living entities is currently one the most challenging areas of research in the study of the origin of life. There is no evidence of abiotically produced oligopeptides or oligonucleotides in the Murchison meteorite, but condensation reactions clearly took place in the primitive Earth. Synonymous terms like 'primitive soup', '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 to parts of the hydrosphere where the accumulation and interaction of the products of prebiotic synthesis may have taken place. These include not only membrane-bound systems, but also oceanic sediments, intertidal zones, shallow ponds, fresh water lakes, lagoons undergoing wet-and-dry cycles, and eutectic environments (e.g., glacial ponds), where evaporation or other physicochemical mechanisms (such as the adherence of biochemical monomers to active surfaces) could have raised local concentrations and promoted polymerization [3].

Simple organic compounds dissolved in the primitive oceans or other bodies of water would need to be concentrated by some mechanism. Selective adsorption of molecules onto mineral surfaces could have promoted their polymerization, as suggested by laboratory simulations using a variety of simple compounds and activated monomers [17,30,31]. 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 [17], the general length range considered necessary for primitive biochemical functions.

Since 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

[49,82]. However, as the length of polymers formed on mineral surfaces increases, they become more firmly bound to the mineral [22,62]. 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, or by concentrated salt solutions [30], a process that could take place in tidal regions during evaporation or freezing of seawater and that would have led to the release of polymers.

As summarized elsewhere [2], direct concentration of dilute solutions of monomers could also be accomplished by evaporation and by eutectic freezing of dilute aqueous solutions. The evaporation of tidal regions and the subsequent concentration of their organic constituents has been proposed in the synthesis of a variety simple organic molecules [57]. Salty brines may have also been important in the formation of peptides and perhaps other important biopolymers as well. As summarized by Rode [70], salt-induced peptide formation reaction may provide an abiotic route for the formation of peptides directly from amino acids in concentrated NaCl solutions containing Cu(II). Yields of di- and tripeptides in the 0.4–4% range have been reported using starting amino acid concentrations in the 40–50 mM range. Clay minerals such as montmorillonite apparently promote the reaction, which could have taken place in evaporating tidal pools and where the required concentrated salty brines would have been easily available. It has been shown that the freezing of dilute solutions of activated amino acids at $-20\,^{\circ}\text{C}$ yields peptides at higher yields than in experiments with highly concentrated solutions at 0 and $25\,^{\circ}\text{C}$ [49], and recent studies have shown that eutectic freezing is especially effective in the non-enzymatic synthesis of oligonucleotides [37].

It is very unlikely, however, that the RNA world would have arisen from such process. 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 of that the first living organisms were largely based on ribozymes, an hypothetical stage called the RNA world [27,35]. This possibility is now widely accepted, but the chemical lability of RNA components suggests that this molecule was not a direct outcome of prebiotic evolution, but may have been one of the evolutionary outcomes of what are now referred to as pre-RNA worlds. However, the chemical nature of the first genetic polymers and the catalytic agents that may have formed the pre-RNA worlds that bridged the gap between the prebiotic broth and the RNA world are completely unknown and can only be surmised. Modified nucleic acid backbones have been synthesized, which either incorporate a different version of ribose or lack it altogether. Experiments on nucleic acid with hexoses instead of pentoses, and on pyranoses instead of furanose [15], suggests that a wide variety of informational polymers is possible, even when restricted to sugar phosphate backbones. One possibility that has not been explored is that the backbone of the original informational macromolecules may have been atactic (e.g., disordered) kerogen-like polymers such as those formed in some prebiotic simulations. There are other possible substitutes for ribose, including open chain, flexible molecules that lack asymmetric carbons. One of the most interesting chemical models for a possible precursor to RNA involves the so-called peptide nucleic acids (PNAs), which have a polypeptide-like backbone of achiral 2-amino-ethyl-glycine, to which nucleic acid bases are attached by an acetic acid [58]. Such molecules form very stable complementary duplexes, both with themselves and with nucleic acids. Although they lack ribose, their functional groups are basically the same as in RNA, so they may also be endowed with catalytic activity.

Identification of adenine, guanine, uracil and other nucleobases in the Murchison meteorite supports the idea that these bases were present in the primitive environment. However, it is likely that other hetero-

cycles capable of forming hydrogen bonding were also available. The Watson–Crick base-pair geometry permits more than the four usual nucleobases, and simpler genetic polymers may not only have lacked the sugar-phosphate backbones, but may also have depended on alternative non-standard hydrogen bonding patterns. The search for experimental models of pre-RNA polymers will be rewarding but difficult; it requires the identification of potentially prebiotic components and the demonstration of their non-enzymatic template-dependent polymerization, as well as coherent hypothesis of how they may have catalyzed the transition to an RNA world.

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

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 compartmentized into proto-type cells [28].

As hypothesized elsewhere [2], 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 had been greatly diminished. Many of the components of the primordial soup may have been extensively converted into polymers including those associated with living entities, and the raw materials needed to sustain life may have been largely exhausted. This implies that the origin of simple metabolic-like pathways must have been in place in order 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 ribozyme-mediated and protein-based enzymatic processed began to dominate [44].

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. No ancient incipient stages or evolutionary intermediate of these molecular structures are known but, as discussed below, in some cases the existence of graded intermediates can be deduced.

It is possible that the invention of protein synthesis and the encapsulation of reaction machinery needed for replication may have taken place during the RNA world [2]. The fact that RNA molecules are capable of performing by themselves all the reactions involved in peptide-bond formation suggests that protein biosynthesis evolved in an RNA world [95], i.e., that the first ribosome lacked proteins and was formed 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 solely of RNA [59]. As underlined by Kumar and Yarus [41], four of the central reactions involved in protein biosynthesis are catalyzed by ribozymes, and their complementary nature suggests suggestive that they may have first appeared in the RNA world. 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 [68].

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, provide evidence of the existence of a more primitive, less-regulated version of protein synthesis 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 proteinic components [26,83] strongly supports the possibility of an older ancestral protein synthesis apparatus prior to the emergence of elongation factors.

The same is true of other enzymes. 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 lifeform that existed prior to the separation of the Bacteria, the Archaea and the Eucarya, i.e., the last common ancestor (LCA) or cenancestor. Universal gene-based phylogenies ultimately reach such single universal entity, which very likely was part of a population of similar entities 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. As reviewed elsewhere [13], the cenancestor should be seen as 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 the three major cell lineages. Recognition that cellular genomes are historical documents recording at least part of past evolutionary events has allowed important insights into simpler biological systems that appear to have lacked DNA genomes, but that can be considered basically RNA/proteins cells far removed, if not time, at in complexity with respect the first living systems.

9. Conclusions

The understanding of the origin of life requires, wrote John D. Bernal several decades ago, requires a scientist with a deep knowledge in geology, chemistry, biology, astrophysics, theoretical physics, paleontology and philosophy. Since such polymaths are rare, we must either work in multidisciplinary teams or focus our attention in a particular issue within the framework and methodologies of one of these fields. It is true that physical and biological sciences should be seen as conceptual allies. However, Darwinism has successfully resisted reduction to physics, and the development of complex system systems dynamics advocated by many theoreticians has failed to provide manageable descriptions of the origin of life. 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.

As implied here, the most successful applications of physical sciences in the understanding of prebiotic evolution have resulted from those areas directly related to the reconstruction of the primitive environment, i.e., astrophysics, planetary sciences, and the like, as well as those pertaining the formation and stability of monomers and polymers of biochemical significance, including the physicochemistry of membrane-forming compounds. As emphasized in this review, the study of the emergence of life remains a chemical problem in which the transition from the results of purely physical and chemical processes

on the synthesis, accumulation and stability of simple biochemical monomers and polymers gave rise in still poorly understood processes to replicative systems capable of undergoing natural selection.

It is likely that no 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 a reducing atmosphere, metal sulfide-mediated synthesis in deep-sea vents, and exogenous sources such as comets, meteorites and interplanetary dust. 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. Our ideas on the prebiotic synthesis of organic compounds are based largely on experiments in model systems. The robustness of this type of chemistry is supported by the occurrence of most of these biochemical compounds in the Murchison meteorite. This makes it plausible, but does not proves, 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 in the primitive Earth.

Thus, if convincing processes can be demonstrated for the origin of life on Earth, then it is reasonable to conclude that life is the natural outcome of an evolutionary process, and that it may have appeared elsewhere in the Universe. Although we do not know how the transition from the non-living to the living took place, most of the modern scenarios start out with relative simple organic molecules, now known to be widely distributed, which are readily synthesized, and hypothesized to undergo further evolutionary changes leading into self-maintaining, self-replicative systems from which the current DNA/protein-based biology resulted.

Acknowledgements

References

[1] Anders E. Prebiotic organic matter from comets and asteroids. Nature 1989;342:255–7.

[2] Bada JL, Lazcano A. The origin of life—some like it hot, but not the first biomolecules. Science 2002;296:1982–3.

[3] Bada JL, Lazcano A. In: Ruse M, editor. The Harvard companion of evolution. Cambridge: Harvard Univ. Press; 2005. Submitted for publication.

[4] Bernhardt G, Luedmann HD, Jaenicke R, Koeing H, Stetter KO. Biomolecules are unstable under black smoker conditions. Naturwissenschaften 1984;71:583–6.

[5] Botta O, Bada JL. Extraterrestrial organic compounds in meteorites. Surv Geophys 2002;23:411–67.

[6] Brasier M, Green OR, Jephcoat AP, Kleppe AK, van Kranendonk MJ, Lindsay JF, Steele A, Grassineau NV. Questioning the evidence for Earth's earliest fossils. Nature 2002;416:76–9.

[7] Chyba CF. Impact delivery and erosion of planetary oceans in the early inner Solar System. Nature 1990;343:129–33.

[8] Chyba CF, Sagan C. Endogenous production, exogenous delivery, and impact-shock synthesis or organic compounds, an inventory for the origin of life. Nature 1992;355:125–32.

 [9] Cody GD, Boctor NZ, Filley TR, Hazen RM, Scott JH, Sharma A, Yoder Jr HS. Primordial carbonylated iron–sulfur compounds and the synthesis of pyruvate. Science 2000;289:1337–40.
[10] Cooper G, Kimmich N, Belisle W, Sarinana J, Brabham K, Garrel L. Carbonaceous chondrites as a source of sugar related organic compounds for the early Earth. Nature 2001;414:879–83.
[11] Corliss JB, Baross JA, Hoffman SE. An hypothesis concerning the relationship between submarine hot springs and the origin of life on Earth. Oceanol Acta 1981;4(Suppl.):59–69.
[12] Deamer DW, Pashley RM. Amphiphilic components of the Murchison carbonaceous chondrite, surface properties and membrane formation. Origins Life Evol Biosph 1989;19:21–38.
[13] Delaye L, Becerra A, Lazcano A. In: Ribas de Pouplana L, editor. The genetic code and the origin of life. Landes Bioscience: Georgetown; 2004. p. 34–47.
[14] Ehrenfreund P, Irvine W, Becker L, Blank J, Brucato J, Colangeli L, Derenne S, Despois D, Dutrey A, Fraaije H, Lazcano A, Owen T, Robert F. Astrophysical and astrochemical insights into the origin of life. Reports Prog Phys 2002;65:1427–87.
[15] Eschenmoser A. Chemical etiology of nucleic acid structure. Science 1999;284:2118–24.
[16] Fenchel T. Origin and early evolution of life. Oxford: Oxford Univ. Press; 2002.
[17] Ferris JP. Montmorillonite catalysis of 30–50 mer oligonucleotides, laboratory demonstration of potential steps in the origin of the RNA World. Origins Life Evol Biosph 2002;32:311–32.
[18] Ferris JP, Orgel LE. An unusual photochemical rearrangement in the synthesis of adenine from hydrogen cyanide. J Am Chem Soc 1966;88:1074.
[19] Ferris JP, Sanchez RP, Orgel LE. Studies in prebiotic synthesis: III. Synthesis of pyrimidines from cyanoacetylene and cyanate. J Mol Biol 1968;33:693–704.
[20] Ferris JP, Zamek OS, Altbuch AM, Freiman H. Chemical evolution: XVIII. Synthesis of pyrimidines from guanidine and cyanoacetaldehyde. J Mol Evol 1974;3:301–9.
[21] Ferris JP, Joshi PD, Edelson EH, Lawless JG. HCN, a plausible source of purines, pyrimidines, and amino acids on the primitive Earth. J Mol Evol 1978;11:293–311.
[22] Ferris JP, Hill AR, Liu R, Orgel LE. Synthesis of long prebiotic oligomers on rnineral surfaces. Nature 1996;381:59–61.
[23] Frick L, Mac Neela JP, Wolfenden R. Transition state stabilization by deaminases, rates of nonenzymatic hydrolysis of adenosine and cytidine. Bioorg Chem 1987;15:100–8.
[24] Garcia-Ruiz JM, Hyde ST, Carnerup AM, Christy AG, van Kranendonk MJ, Welham NJ. Self-assembled silica-carbonate structures and detection of ancient microfossils. Science 2003;302:1194–7.
[25] Garrett ER, Tsau J. Solvolyses of cytosine and cytidine. J Pharm Sci 1972;61:1052–61.
[26] Gavrilova LP, Kostiashkina OE, Koteliansky VE, Rutkevitch NM, Spirin AS. Factor-free, nonenzymic, and factor-dependent systems of translation of polyuridylic acid by *Escherichia coli* ribosomes. J Mol Biol 1976;101:537–52.
[27] Gilbert W. The RNA world. Nature 1986;319:618.
[28] Hanczyc MM, Fujikawa SM, Szostak JW. Experimental models of primitive cellular compartments, encapsulation, growth, and division. Science 2003;302:618–22.
[29] Harvey RB. Enzymes of thermal algae. Science 1924;60:481–2.
[30] Hill AR, Böhler C, Orgel LE. Polymerization on the rocks, negatively charged D/L amino acids. Origins Life Evol Biosph 2001;28:235–43.
[31] Holm NG, editor. Marine hydrothermal systems and the origin of life. Dordrecht: Kluwer Academic; 1992.
[32] Holm NG, Andersson EM. Abiotic synthesis of organic compounds under the conditions of submarine hydrothermal systems: a perspective. Planet Space Sci 1995;43:153–9.
[33] Huber C, Wächtershäuser G. Activated acetic acid by carbon fixation on, Fe, Ni, S under primordial conditions. Science 1997;276:245–7.
[34] Huber C, Wächtershäuser G. Peptides by activation of amino acids with CO on, Ni, Fe, S surfaces and implications for the origin of life. Science 1998;281:670–2.
[35] Joyce GF. The antiquity of RNA-based evolution. Nature 2002;418:214–21.
[36] Kamminga H. The origin of life on Earth, theory, history, and method. Uroboros 1991;1:95–110.
[37] Kanavarioti A, Monnard PA, Deamer DW. Eutectic phases in ice facilitate nonenzymatic nucleic acid synthesis. Astrobiology 2001;1:481.
[38] Kasting JF. Earth's early atmosphere. Science 1993;259:920–6.
[39] Kauffman SA. The origins of order: self organization and selection in evolution. New York: Oxford Univ. Press; 1993.

[40] Kelley DS, Karson JA, Blackman DK. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. Nature 2001;241:145–9.

[41] Kumar RK, Yarus M. RNA-catalyzed amino acid activation. Biochemistry 2001;40:6998–7004.

[42] Larralde R, Robertson MP, Miller SL. Rates of decomposition of ribose and other sugars, implications for chemical evolution. Proc Natl Acad Sci USA 1995;92:8158–60.

[43] Lazcano A. A.I. Oparin, the man and his theory. In: Plogazov BF, Kurganov BI, Kritsky MS, Gladilin KL, editors. Evolutionary biochemistry and related areas of physicochemical biology. Moscow: Bach Institute of Biochemistry and ANKO Press; 1995. p. 49–56.

[44] Lazcano A, Miller SL. On the origin of metabolic pathways. J Mol Evol 1999;49:424–31.

[45] Levy M, Miller SL. The stability of the RNA bases: implications for the origins of life. Proc Natl Acad Sci USA 1998;95:7933–8.

[46] Levy M, Miller SL. The prebiotic synthesis of modified purines and their potential role in the RNA world. J Mol Evol 1999;48:631–7.

[47] Levy M, Miller SL, Oró J. Production of guanine from NH_4CN polymerizations. J Mol Evol 1999;49:165–8.

[48] Levy M, Miller SL, Brinton K, Bada JL. Prebiotic synthesis of adenine and amino acids under Europa-like conditions. Icarus 2000;145:609–13.

[49] Liu R, Orgel LE. Efficient oligomerization of negatively-charged D/L amino acids at −20 °C. J Am Chem Soc 1997;119:4791–2.

[50] Lindhal T. Instability and decay of the primary structure of DNA. Nature 1993;362:709–15.

[51] Maden BEH. No soup for starters? Autotrophy and origins of metabolism. Trends Biochem Sci 1995;20:337–41.

[52] Miller SL. A production of amino acids under possible primitive Earth conditions. Science 1953;117:528.

[53] Miller SL. Production of some organic compounds under possible primitive Earth conditions. J Am Chem Soc 1955;77:2351–61.

[54] Miller SL, Bada JL. Submarine hot springs and the origin of life. Nature 1988;334:609–11.

[55] Miller SL, Lazcano A. The origin of life—did it occur at high temperatures? J Mol Evol 1995;41:689–92.

[56] Miller SL, Lazcano A. In: Schopf JW, editor. Life's origin, the beginnings of biological evolution. Berkeley: California Univ. Press; 2002. p. 78.

[57] Nelson KE, Robertson MP, Levy M, Miller SL. Concentration by evaporation and the prebiotic synthesis of cytosine. Origins Life Evol Biosph 2001;31:221–9.

[58] Nielsen P. Peptide nucleic acid, PNA, a model structure for the primordial genetic material? Origins Life Evol Biosph 1993;23:323–7.

[59] Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. The structural basis of ribosome activity in peptide bond synthesis. Science 2000;289:920–30.

[60] Oparin AI. Proiskhozhedenie zhizni. Moscow: Moscckovskii Rabochii; 1924 (reprinted and translated in Bernal JD, the origin of life. London: Weidenfeld and Nicolson; 1967).

[61] Oparin AI. The origin of life. New York: MacMillan; 1938.

[62] Orgel LE. Polymerization on the rocks, theoretical introduction. Origins Life Evol Biosph 1998;28:227–34.

[63] Oró J. Synthesis of adenine from ammonium cyanide. Biochem Biophys Res Comm 1960;2:407–12.

[64] Oró J. Comets and the formation of biochemical compounds on the primitive earth. Nature 1961;190:442–3.

[65] Oró J, Kimball AP. Synthesis of purines under primitive Earth conditions. I. Adenine from hydrogen cyanide. Arch Biochem Biophys 1961;94:221–7.

[66] Oró J, Lazcano A. Comets and the origin and evolution of life. In: Thomas PJ, Chyba CF, McKay CP, editors. Comets and the origin and evolution of life. New York: Springer-Verlag; 1997. p. 3–27.

[67] Perutz M. I wish I'd made you angry earlier, essays on science, scientists, and humanity. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2003.

[68] Robertson MP, Miller SL. An efficient prebiotic synthesis of cytosine and uracil. Nature 1995;375:772–4.

[69] Robertson MP, Levy M, Miller SL. Prebiotic synthesis of diaminopyrimidine and thiocytosine. J Mol Evol 1996;43:543–50.

[70] Rode BM. Peptides and the origin of life. Peptides 1999;20:773–86.

[71] Saladino R, Crestini C, Costanzo G, DiMauro E. Advances in the prebiotic chemistry of nucleic acid bases. Implications for the origins of life. Curr Org Chem 2004;8.

[72] Saladino R, Crestini C, Costanzo G, Negri R, DiMauro E. Bioorg Med Chem 2001;9:1249–53.

[73] Saladino R, Ciambecchini U, Crestini C, Costanzo G, Negri R, DiMauro E. ChemBiochem 2003;4:514–21.

[74] Sanchez RA, Ferris JP, Orgel LE. Conditions for purine synthesis, did prebiotic synthesis occur at low temperatures? Science 1966;153:72–3.

[75] Sanchez RA, Ferris JP, Orgel LE. Studies in prebiotic synthesis: II. Synthesis of purine precursors and amino acids from aqueous hydrogen cyanide. J Mol Biol 1967;30:223–53.

[76] Sanchez RA, Ferris JP, Orgel LE. Studies in prebiotic synthesis: IV. The conversion of 4-aminoimidazole-5-carbonitrile derivatives to purines. J Mol Evol 1968;38:121–8.

[77] Schlesinger G, Miller SL. Prebiotic synthesis in atmospheres containing CH_4, CO, and CO_2. I. Amino acids. J Mol Evol 1983;19:376–82.

[78] Schopf JW. Microfossils of the early Archaean Apex chert, new evidence for the antiquity of life. Science 1993;260:640–6.

[79] Shapiro R. The prebiotic role of adenine, a critical analysis. Origins Life Evol Biosph 1995;25:83–98.

[80] Shapiro R, Klein RS. The deamination of cytidine and cytosine by acidic buffer solutions, mutagenic implications. Biochemistry 1966;5:2358–62.

[81] Shock EL. Geochemical constraints on the origin of organic compounds in hydrothermal systems. Orig Life Evol Biosph 1990;20:331–67.

[82] Sowerby SJ, Mörth C-M, Holm NG. Effect of temperature on the adsorption of adenine. Astrobiology 2001;1:481–8.

[83] Spirin AS. Ribosome structure and protein synthesis. Benjamin–Cummings: Menlo Park; 1986, 414 pp.

[84] Stoks PG, Schwartz AW. Uracil in carbonaceous meteorites. Nature 1979;282:709–10.

[85] Stoks PG, Schwartz AW. Nitrogen-heterocyclic compounds in meteorites: significance and mechanisms of formation. Geochim Cosmochim Acta 1981;45:563–9.

[86] Stoks PG, Schwartz AW. Basic nitrogen-heterocyclic compounds in the Murchison meteorite. Geochim Cosmochim Acta 1982;46:309–15.

[87] Tice MM, Lowe DR. Photosynthetic microbial mats in the 3.416-Myr-old ocean. Nature 2004;431:522–3.

[88] Urey HC. On the early chemical history of the Earth and the origin of life. Proc Natl Acad Sci USA 1952;38:351–63.

[89] van Zullen MA, Lepland A, Arrhenius G. Reassessing the evidence for the earliest traces of life. Nature 2002;418:627–30.

[90] Van der Velden W, Schwartz AW. Search for purines and pyrimidines in the Murchison meteorite. Geochim Cosmochim Acta 1977;41:961–8.

[91] Voet AB, Schwartz AW. Prebiotic adenine synthesis from HCN, evidence for a newly discovered major pathway. Bioorganic Chem 1983;12:8–17.

[92] Wächtershäuser G. Before enzymes and templates, theory of surface metabolism. Microbiol Rev 1988;52:452–84.

[93] Wilde SA, Valley JW, Peck WH, Graham CM. Evidence from detrital zircons for the existence of continental crust and oceans on the Earth 4.4 Gyr ago. Nature 2001;409:175–8.

[94] Zhabotinski AM. Self-oscillation concentrations. Moscow: Nauka; 1974.

[95] Zhang B, Cech TR. Peptidyl-transferase ribozymes, trans reactions, structural characterization and ribosomal RNA-like features. Chem Biol 1998;5:539–53.