**Supplementary File 1** to the manuscript “**Life as a positive fallout of the Moon-forming impact: The coolest start-up from anoxic geothermal fields enriched in zinc and potassium”** by A.Y. Mulkidjanian, D.V.Dibrova and A.Yu. Bychkov.

**S1. Basic features of life.**

S1.1. Biomolecules: RNA, DNA, proteins, sugars, and lipids.

Although living organisms contain diverse biomolecules, the key players of life - as we know it - are polymers of three types: ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs) and proteins (Fig.S1.1). Long DNA molecules store genetic information as strings of nucleotides, RNA molecules help translate information into protein amino acid sequences, and proteins do most of the work in the cell [1].

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Fig. S1.1. Relation between DNA, RNA, and proteins in a cell. Image credit: Wikipedia.

RNA molecules are formed from ribonucleotides - quite complex units that consist of a nucleobase, a sugar ribose and phosphate group(s), see Fig. S1.2. Nucleobases are one- or two-ring moieties made of alternating carbon and nitrogen atoms; because of the high nitrogen content, nucleobases are sometimes called nitrogenous bases or nitrogen bases. There are four main nucleobases in RNA, these are purines adenine (A), guanine (G), and pyrimidines cytosine (C) and uracil (U), all shown in Fig. S1.2. Nucleobases are attached to ribose moieties which have a ring-like structure. When nucleotides join into RNA molecules, phosphate groups link the ribose moieties, so that phosphate groups alternate with ribose moieties to which nucleobases are attached (Fig. S1.2).

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Fig. S1.2. Structure of an RNA segment (left) and RNA molecules with catalytic activity (ribozymes). Image credit: Wikipedia

Formation of polynucleotides in a so-called condensation reaction is accompanied by the release of water molecules (Fig. S1.3).

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Fig. S1.3. Polymerization (condensation) of ribonucleotides

In addition to being the building blocks of RNA, ribonucleotides in their triphosphate forms store energy in the cell, with adenosine triphosphate (ATP) being the most abundant energy-storing moiety, see Fig. S1.4. The cleavage of their phosphate groups is accompanied by the release of free energy that can be utilized by specific enzymes, in particular, for doing mechanical work [2] .

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Fig. S1.4. Nucleotide-containing cofactors

DNA differs from RNA in that it contains deoxyribose as the sugar moiety; deoxyribose has one oxygen atom less than ribose, which makes the DNA polymers less flexible than RNA molecules but 200 times more stable towards occasional backbone breaks by water (hydrolysis, see [3,4]). In addition, DNA contains thymine (T) instead of chemically similar uracil (U), see Fig. S1. 5.



Fig. S1.5. Structure of a double-strand segment of DNA. Left: chemical structure; right, ball-and-stick, presentation. Image credits: Madprime and Zephyris, respectively, Wikipedia

Nucleobases can form so-called Watson-Crick pairs (Fig. S1.2, S1.5.) that are stabilized by hydrogen bonds (H-bonds) where protons link oxygen or nitrogen atoms of two apposed nucleotides [5]. Adenine is pairing with uracil (A-U, in RNA) or thymine (A-T, in DNA), whereas guanine “recognizes” cytosine (G-C, both in RNA and in DNA); these pairs are called complementary bases. Multiple Watson-Crick interactions between chains of complementary nucleotides usually yield double helices. They are stabilized by H-bonds between the bases of opposing chains and stacking interactions between the bases of the same chain (Fig. S1.2, S1.5.).

Rather inflexible DNA molecules usually exist as long double helices (Fig. S1.5.). In contrast, RNA molecules are more versatile. They also form long double helices while storing genome information, as in the RNA viruses. RNA molecules, however, can also fold into compact structures built of many short, intertwined double helices. Some of these structures possess catalytic activity, they are called ribozymes, see Fig. S1.2.

Upon reproduction, the double helix of a DNA molecule (or RNA in some viruses that have no DNA) opens and a chain of complementary nucleotides is lined up for each original chain, which leads to the formation of two similar double helices of DNA, this process is called replication (Fig. S1.6.).

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Fig. S1.6. Replication scheme. Image credit: Wikipedia

Currently, replication is accomplished with the help of proteins. In general, proteins do almost all the hard work in the cell - from forming cellular structures to catalyzing (accelerating) chemical reactions. As shown in Fig. S1.7., proteins are made up of amino acids, of which there are 20 ubiquitous ones. Amino acids are small molecules with an amino group at one end and an acid group at the other. Each time one amino acid binds to the other, the amino group of one amino acid interacts with the acid group of another amino acid with the formation of a new carbon — nitrogen (CN) bond (peptide bond); this reaction is accompanied by the release of a water molecule (Fig. S1.7). A chain of peptide bonds forms the backbone from which the side chains of amino acids protrude. The side chains differ in size, polarity, electrical charge, the presence of aromatic groups, etc. This chemical and structural diversity of amino acid side chains, as well as variations in protein sequences, account for the diversity of proteins and their functions.

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Fig. S1.7. Three levels of protein structure. Image credit. Wikipedia

In many cases, enzymes are assisted by so-called cofactors. These are either ions of such metals as zinc, iron, cobalt, manganese, magnesium, potassium, or complex organic molecules, or combinations of both. Cofactors help to accelerate chemical reactions that cannot be accelerated by amino acid side chains alone. Remarkably, many ubiquitous organic cofactors, in addition to their chemically involved parts, also contain nucleotide moieties, the function of which remain obscure, see some examples in Fig. S1.4.

Although nucleic acids and proteins have quite different structures, they share a common construction principle. In both cases, the homopolymer backbone is built of identical units (sugar-phosphate units and peptide groups, respectively) whereas the variability is owing to dissimilarity of groups that are attached to the backbone (nucleobases and amino acid residues, respectively), cf Fig. S1.2, S1.5, and S1.7.

Sugar molecules, in addition to being constituents of nucleotides (Fig. S1.2 – S.1.5.), can join into polysaccharides that form a protective coat on the surface of cells (Fig. S1.8). Also, polysaccharides are used as food storage products.

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Fig. S1.8. Biological membrane. Shown are two layers of amphiphilic lipid molecules stabilized by hydrophobic interactions between the “fatty” tails, integral membrane proteins, and sugar chains attached to some of membrane proteins. Image credit: Encyclopedia Britannica

Amphiphilic lipid molecules are another major component of living systems. Because of their polar heads and hydrophobic tails, they can form bilayer membranes that can serve as barriers for polar molecules and ions. Such lipid bilayers form the core of the cell membranes (Fig. S1.8. and S1.9.).

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Fig. S1.9. Schematic presentation of a lipid bilayer (Image credit: MDougM, Wikipedia).

**S1.2. Protein synthesis - translation**

DNA, RNA and proteins are coupled via the genetic code that attributes triplets of nucleotides to amino acids; the information stored by a four-letter nucleotide code is “translated” into the 20-letter amino acid alphabet (Fig. S1.10).



Fig. S1.10. The genetic code. Image credit: Wikipedia

As shown in Fig. S1.1., to enable protein synthesis, the double helix of DNA opens and a special enzyme complex - called RNA polymerase – gets access to the gene-coding strand of DNA, aligns complementary ribonucleotides along it, and links them into an RNA polymer called messenger RNA (mRNA). This process is called transcription.

After detaching from the DNA strand, the mRNA molecule can attract the large and small ribosomal subunits (LRS and SRS, respectively). Both ribosomal subunits are formed by long but tightly folded ribosomal RNA (rRNA) molecules intertwined with protein molecules [6,7]. The LRS and SRS eventually "clamp" over mRNA with formation of a full-fledged ribosome, the protein synthesis machine (Fig. S1.11.). Ribosomes slide along mRNA and synthesize proteins from amino acids.

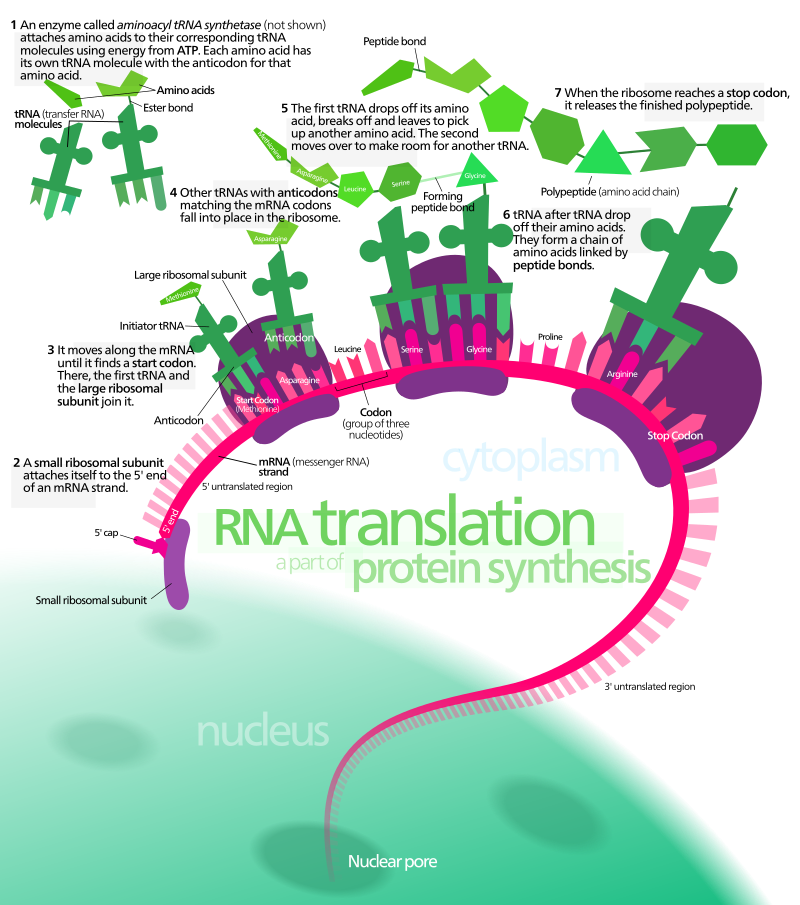


Fig. S1.11., Overview of translation. Image credit: Wikipedia

Each amino acid is delivered by its specific small transfer RNA (tRNA), which carries a nucleotide triplet complementary to the one encoding this amino acid on the mRNA. Only if this triplet properly docks to the respective triplet of mRNA, the amino acid gets attached to the nascent protein chain (Fig. S1.10), In sum, the SRS is responsible for the interaction with mRNA and decoding, whereas the LRS catalyzes the formation of peptide bonds; tRNAs interact with both subunits and functionally link them. Since the four-letter language of DNA and RNA is converted into the twenty-letter language of proteins, this process is called translation.

**S1.3. Ability of biomolecules to self-assemble and self-recover**

Biomolecules described in the previous section share the abilities to self-assemble and self-recover. For instance, heating of a folded RNA molecule (see Fig. S1.2) would lead to its denaturation and loss of a specific (native) structure. However, upon gradual slow cooling, the RNA molecule will recover its initial structure [8]. This native structure is characterized by the maximal number of thermodynamically favorable interactions between the nucleotides; thus, biopolymers tend to achieve the state with lowest energy.

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Fig. S1.12. Schematic presentation of a stepwise self-assembly (folding) of an RNA molecule (from [9])

Short polymers of sugars (oligosaccharides) are also capable of self-assembly [10]; however, this area is not well explored yet.

Much better understood is the self-assembly of lipids. They tend to assemble into structures where hydrophobic tails interact with each other while polar headpieces face the water phase. In particular, when lipids involved have an approximately cylindrical shape, they attain a bilayer structure (see Fig. S1.9) and [11].

Most proteins - but by no means all of them – also tend to fold into their thermodynamically most stable 'native' structures. However, the stabilization of a protein globule (see Fig. S1.7) involves more diverse interactions than in the case of self-assembling DNA, RNA or lipids. Therefore, special protein complexes called chaperones often help other proteins to fold.

The ability to self-assemble is inherent not just to individual biopolymers, but also to their complexes. For example, ribosomes, shown in Fig. S1.11, self-assemble from a few large RNA molecules and numerous proteins in response to the appearance of nascent mRNA.

The peculiarity of all the self-assembling systems considered is that they become disordered as the temperature rises from physiological levels. The double-helical regions of DNA and RNA unwind, proteins unfold, and membrane bilayers become less ordered and eventually disintegrate. This is because the contribution of the entropy factor -TΔS to the Gibbs equation[[1]](#footnote-1) increases with temperature, which favors disorder. The flip side of this peculiarity is that decreasing temperature from physiological levels additionally stabilizes such self-assembling systems.

**S1.4. Cellular and acellular life forms and the nature of the Last Universal Cellular Ancestor (LUCA).**

Living organisms can be either cellular or acellular (Fig. S1.13). Cellular organisms include eukaryotes (e.g. plants and animals) and prokaryotes (e.g. bacteria). Eukaryotes have larger cells containing a separate nucleus that preserves DNA. The smaller prokaryotes have no nuclei, their DNA is usually spread within the cell.

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Fig. S1.13. Cellular life forms Image credit: Wikipedia

Acellular life forms are represented by viruses and viroids. While cellular organisms synthesize nucleic acids and proteins themselves, viruses and viroids hijack cells and force them to perform these syntheses for them.

Most viruses are containers of tightly packed nucleic acids and proteins. Such a “package” is encased either by a protein capsid or by a lipid membrane, which is usually “borrowed” from the previous viral host. Upon a viral attack, the “package” is injected into the cell and reprograms its biosynthetic machinery to produce new viruses.

Viroids seem to be the simplest forms of life. They are circular, single-strand RNA molecules without envelop of any kind. After getting into the cell, they (somehow) force the cell to provide for their reproduction.

The common origin of cellular life forms was clarified by Carl Woese and his colleagues, who compared the nucleotide sequences of the small ribosomal subunits (SRS) sequences of very different organisms from all "kingdoms of life" and found that they were similar, albeit to different extents [12,13]. According to the Tree of Life based on comparison of SRSs (Fig. S1. 14), all cellular organisms belong either to Bacteria, or to Archaea or to Eukarya. Archaea are prokaryotes as Bacteria, however, their machinery for reproduction and protein synthesis resemble those of Eukarya. It is thought that the first Bacteria split from the ancestors of Archaea/Eukarya, which was followed by the separation of Eukarya from the archaeal lineage. Woese defined the universal ancestor at the root of the Tree of Life as the “progenote” [14].

More recently, it has been established that the ancestors of eukaryotes are in fact closely related to the recently characterized so-called Asgard archaea. Apparently, the archaeal ancestors of eukaryotes underwent multiple symbioses/fusions with distinct bacteria before becoming full-fledged complex eukaryotic cells, see Fig. S1.14 and [15,16].

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S1.14. The three-domain tree of life according to Woese [17,18] and the currently accepted two-domain tree [15]. Image credit: Wikipedia (with modifications).

Archaea and Bacteria fundamentally differ in many respects [19-22]. In particular, they have different membrane lipids [23]. Bacterial lipids are built of fatty acids connected to a glycerol-3-phosphate via ester bonds (Fig. S1.12 and S1.15., left) whereas archaeal membrane lipids are comprised of branched isoprenoid tails ether-linked to a glycerol-1-phosphate moiety, an optical isomer of glycerol-3-phosphate (Fig. S1.15., right).

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Fig. S1.15. Organization of two-tail phospholipids in Bacteria/Eukarya (left, phosphatidylethanolamine) and Archaea (right, diphytanylglycerol ether analog of phosphatidylethanolamine), image taken from [24]. Differences in chemical organization are indicated by arrows.

The nature of the “progenote”, which was later dubbed Last Universal Cellular (or Common) Ancestor (LUCA) [25,26], was specified after the deciphering of the first complete genomes and identification of those genes that are present in all freely living cellular organisms and, hence, were present in the LUCA. This set is small; it contains approx. 100 protein and RNA coding genes [19-21]. Three quarters of these genes code for the components of protein synthesis machinery. It turned out that the LUCA could be defined through its ability to synthesize proteins [22].

Remarkably, DNA is not directly involved in the protein synthesis; all the work is done by RNAs and proteins. Furthermore, it should be noted that the seemingly simple reaction of transforming a ribonucleotide into a deoxyribonucleotide by chipping an oxygen atom from a sugar residue (see Fig. S1.2 and S1.5.) is, in fact, a complex transformation that is performed by enzymes, which are different in Bacteria and Archaea. The DNA-processing enzymes involved in transcription and replication (see Fig. S1.1 and S1.6) also differ in Bacteria and Archaea [19-22]. Therefore, it is widely believed that DNA, as a more stable information storage medium than RNA, emerged after the appearance of RNAs and proteins. Consequently, it has been suggested that LUCA may have been an RNA/protein organism, and that Bacteria and Archaea/Eukaryotes mastered the use of DNA separately from each other [22]. Alternatively, LUCA could have already had a primitive DNA genome and could have processed DNA with adapted versions of RNA-processing enzymes [27]; these "provisional" enzymes could have been independently replaced by more specialized DNA-tailored enzymes in bacteria and archaea, respectively, after their separation.

The fundamental difference between the membrane lipids of Bacteria and Archaea makes the nature of the membrane lipids of LUCA murky. It has been suggested that LUCA may have had primitive single-tail lipids [28-30], but the chemical nature of either tails or headpieces of LUCA’s lipids remains unclear.

It is possible to envision the LUCA as a community of mutually dependent organisms. Each organism was able to synthesize some proteins and metabolites but could not synthesize everything it needed. Therefore, it shared some of the synthesized proteins and metabolites by expelling them out of the cell, and instead took the necessary ones synthesized by other organisms. Such mutual interdependence of organisms is typical even for modern life.

**S2. Paradoxes of Life**

Life has many paradoxical features. It is thought that resolving these paradoxes may help to unravel the circumstances of life origin [31-34]. Most of these paradoxes are known to the experts in the field. Still, for those readers who are not deeply involved in the origin of life research, we review here some of the paradoxes of life. In our consideration, we move on from paradoxes involving the simplest organic molecules to paradoxes involving more complex structures and cells.

In most cases, we are not the first to identify these paradoxes of nature, so we also review the solutions that have been proposed for them to date (if they have been proposed at all). In this way, we simultaneously present the main scientifically plausible ideas about the origin of life.

**S2.1. Paradox of the super-reduced state of organic molecules.**

Organic molecules got their name because they are found in organisms and are rare in inorganic nature. In organic molecules, carbon atoms are bonded with hydrogen atoms and to a lesser extent, if at all, with oxygen atoms. Therefore, these molecules are considered to be *reduced* in oxygen. The reducing power of organic molecules usually decreases when their hydrogen atoms are replaced by *oxygen* atoms; this process is called *oxidation*.

In chemistry, the reducing power is characterized by the redox potential, which is defined as [35]:

(Eq. 1)

where *R* is the universal gas constant, *T* the absolute temperature, *F* the Faraday constant, *n* is the number of electrons transferred, and *E*0 is the “standard” redox potential determined at equal concentrations of the reduced and oxidized forms when the last term becomes zero. According to equation (1), the redox potential depends on the concentration of the reactants and decreases with increasing concentration of the reduced form.

Redox potentials are defined relative to the potential of the so-called hydrogen electrode, a platinum plate at which protons of water can be reduced to molecular hydrogen (H2) in a reaction:

***2H+ (in water) + 2e– ↔ H2 (gas)*** (Eq. 2)

where e– denotes an electron. The *E*0 value of this reaction at pH 0.0, 25ºC, and 1 atm pressure of H2 is taken as 0 mV, provided that water is used as a solvent.

This equation can be written also as

***2H2O (liquid) + 2e– ↔ H2 (gas) + 2OH– (in water)* (**Eq. 3)

Redox potentials of reactions involving protons are pH dependent. For redox reactions where the number of electrons transferred is equal to the number of protons transferred, one can use a simplified relation

(Eq. 4)

Hence, at pH 7.0, the *E*07 value for the hydrogen electrode is –59 mV × 7 = – 414 mV. The redox potential of the hydrogen electrode is considered as the low-potential limit of water stability; reducing agents with even lower redox potentials can decompose water into H2 and OH– anions according to Eq. (3).

Another important redox reaction is the decomposition of water at very high potentials:

**O2 (gas) + 4H+ (in water) + 4e– = 2H2O (in water) (5)**

The E07 value of this reaction is +820 mV.

Fig. S2.1. shows typical organic and inorganic redox half-reactions (redox pairs) with the values of their standard redox potentials at pH value of 7.0 (*E*07) which is considered as standard in biochemical literature.

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Figure S2.1. Redox potentials and oxygen fugacity *f*(O2). **A**, Redox potentials of biologically relevant redox reactions. Left, biologically relevant half-reactions; right, operative redox ranges of main redox cofactors. The difference between the redox potentials of two half-reactions corresponds to the free energy of the redox reaction between them. Spontaneous electron transfer occurs when the redox potential of the electron-donating half-reaction is more negative than that of the electron-receiving half-reaction. The plot is based on data compiled from [36-41]. **B**, Stepwise reduction of CO2 to methane, modified from [39]. **C,** *f*(O2)-temperature diagram. Log oxygen fugacity vs temperature at 1 bar pressure for common buffer assemblages, plotted using algorithms compiled by B. R. Frost [42]. The FMQ buffer is characterized by the reaction of partial reduction of Fe3+ to Fe2+ (3Fe2SiO4 + O2 = 2Fe3O4 + 3SiO2), the IW redox buffer is characterized by the reaction of partial reduction of Fe2+ of wustite to metallic iron Fe0, [2(1-x)Fe0 + O2 = 2 Fe(1-x)O], the IQF buffer is characterized by the reaction of reduction of fayalite to metallic iron Fe0, SiO2 + 2Fe0 + O2 = Fe2SiO4, whereas the Zn-ZnO buffer is characterized by the reaction 2Zn0 + O2 = 2ZnO.

The simplest known reaction of converting an inorganic compound into organic is the reduction of CO2 to a formic acid.

As shown in Fig. S2.1B, this reaction requires very strong reducing agents with a redox potential of ≤ –600 mV. In general, the very low redox potentials of most organic molecules are outside the water stability range, as can be seen from Fig. S2.1A. Also paradoxically, water hinders the reduction of CO2, since the oxidation of strong reducing agents by ubiquitous water protons (E07 –410 mV) is thermodynamically and kinetically more favorable than their oxidation by CO2 (see Figs. S2.1). Therefore, researchers perform reductive syntheses of organic molecules - both in the liquid phase and on electrodes - either in anhydrous solutions or in mixtures with a small fraction of water [43,44]. We believe that the very facts that efficient reduction of CO2 to organics is only possible outside the stability range of water is one of the key paradoxes of life.

The dominant source of reducing power in the biosphere is the chlorophyll-based photosynthesis, which uses energy of light to produce stable electron-donating compounds with redox potentials as low as –700 mV [45,46]. Photosynthesis is the most productive of several biogenic pathways of the so-called "autotrophic" reduction of CO2 to organic compounds. Because of the need to avoid the leakage of electrons to water, all autotrophic processes are very complex and involve many interacting enzymes [47].

It is chemically much easier to produce new organic molecules by transforming pre-existing organic molecules; a network of such "heterotrophic" transformations is an essential part of cellular metabolism. Recently, it has been shown that incubation of one of the sugar phosphates involved in glycolysis or pentose phosphate pathway in warm water and in the presence of transition metal catalysts leads to non-enzymatic formation of other intermediates of these pathways, which implies that the chemistry of the respective reactions is not particularly demanding [48-52].

Small amounts of organic molecules are also formed abiotically, during the so-called hydrothermal alteration of hot rocks at sites of geothermal activity. Earth’s rocks contain about 5% iron, mostly as iron oxide, FeO. In the presence of geothermal fluids, at high pressure of the rock, and at temperatures <500ºC, some of the Fe2+ ions within the rock can be oxidized to Fe3+ by protons present in the geothermal fluids. This reaction produces magnetite (Fe3O4) and H2. Various organic molecules (mostly hydrocarbons) were shown to be produced under such conditions, albeit at low yields, presumably from the interaction of CO2 either with H2 or directly with iron oxides of hot rocks. These abiotically produced organic molecules are transported to the surface by geothermal fluids [53-66].

This phenomenon of H2 formation in hot rocks allows the correlation of the redox potential scale for liquid systems at 25°C and pH 7.0 (Fig. S2.1A) with the reducing power of hot solid rocks, which is characterized not by redox potential but by the oxygen fugacity, *f*(O2). Fugacity (*f*) is defined as the effective partial pressure of a gas (in this case, oxygen gas) in thermodynamic equilibrium with a given mineral assemblage, see Fig. S2.1C and [67-69]. Typically, *f*(O2) is reported in log10 units relative to well-characterized mineral redox buffers, specifically fayalite-magnetite-quartz (FMQ) or iron-wüstite (IW), see Fig. S2.1C. The *f*(O2) of today’s Earth’s crust and mantle typically varies around the FMQ value. Since the ability of Earth rocks to reduce water protons to H2 is manifested when their temperature drops below 500°C, the reducing power of the FMQ mineral redox buffer at ~ 500°C (log*f*(O2) ~ –20, see Fig. S2.1C) roughly corresponds to the reducing potential (power) of a hydrogen electrode at 25°C, i.e. –410 mV at pH 7.0, see Fig. S2.1A and [67-69]. The *f*(O2) value decreases with temperature (Fig. S2.1C), so that at t° < 500°C the reducing power of the rock even increases. However, at t° < 250°C, redox reactions in the rock attenuate because of their high activation barriers [70].

When liquid water is interacting with a rock, one can establish a relation between the redox-potential and oxygen fugacity because both this parameters attain meaning under such conditions. For example, for the reaction described by Eq. (5) this relation will look like [69]:

E = 1.228 – 0.5991pH + 0.0148logf(O2) (Eq. 6)

Although *f*(O2) is defined in terms of oxygen partial pressure, the value of *f*(O2) is used as an integral parameter to characterize the reducing power of the entire rock [67-69]. The corresponding integral parameter for complex fluid mixtures, such as the Earth’s water reservoirs or cell cytoplasm, is the redox potential of the medium, E*h* [67,69,71,72]. It can be measured using a chemically inert platinum or gold electrode capable of exchanging electrons with all redox agents present in the solution [67,73,74].

Most modern natural reservoirs in contact with the atmosphere, including the oceans at all depths, have high and positive E*h* values in the range of +600 ÷ +750 mV [71]. This is because oxygen, with its E07 of +820 mV (Eq(5) and Fig. S2.1A), usually acts as the dominant redox buffer.

In contrast, the redox potential inside cells is rather low, around –300 mV [75]. It is thought that the first cells initially lived in highly reduced habitats and then failed to adapt to the oxidation of their environment in response to the appearance of atmospheric oxygen [76]. Therefore, in most cases, cells must keep their cytoplasm much more reduced than the environment, which requires energy.

In addition to hydrothermal alteration, CO2 can be abiotically photoreduced to diverse organic molecules at the surface of several UV-absorbing naturally occurring minerals with broadband semiconductor properties, in particular TiO2 (anatase/rutile), MnS (alabandite), and ZnS (wurtzite, sphalerite), which may have been deposited at sites of geothermal or volcanic activity [39,77-82].

Apart from classical electrochemistry, CO2 can also be reduced by ionizing radiation. This was first shown in the context of origin of life research when Calvin and his colleagues imitated terrestrial radioactivity by irradiating a mixture of hydrogen, water, and CO2 with a helium ion beam. They obtained formic acid and formaldehyde [83]. Gethoff and his colleagues showed in 1960 that hydrogen is not needed as an electron donor to obtain organic molecules in such a system. The ionizing radiation itself generated "hydrated electrons" with high reducing power by interacting with water molecules, so that irradiation of a CO2/water mixture produced organic molecules and H2 [65,84]. Although it is still unclear how the "hydrated electrons" with an apparent E07 of ~ –2.9 V are formed [85,86], their ability to promote the formation of carbon- and nitrogen-containing organic molecules has been demonstrated in various systems [87-89].

Not surprisingly, several origin-of-life scenarios invoke natural ionizing radiation as a source of reducing energy for primordial syntheses. Some of these scenarios consider radioactive isotopes of actinides, such as isotopes of uranium (U) and Thorium (Th) [90,91], while others rely on the radioactivity of the natural potassium isotope 40K [92,93]. Actinide radioactivity is stronger, while 40K is more abundant in the crust [92,93], so there seems to be a kind of tie.

In general, it has been repeatedly shown that high energy inputs, such as UV illumination, γ-irradiation, proton beams, or electrical discharges, can generate organic molecules from simpler inorganic building blocks see [84,88,94-105] and references therein.

**S2.2. Abiotic Syntheses of Organic Molecules and the Tar Paradox.**

The immediate products of stepwise CO2 reduction are simple organic molecules with one carbon atom (C1 compounds) such as formate, formaldehyde, methanol, and methane, see Fig. S2.1B and [94,100]. Oparin speculated that the interaction of such simple organic compounds with hydrogen and nitrogen in the atmosphere may have resulted in increasingly complex organic molecules [106,107]. In 1952, Miller and Urey tested this hypothesis experimentally by sending electrical discharges through a flask containing water vapor, hydrogen, methane, and ammonia. They obtained amino acids and some other organic molecules [95,103].

In all such experiments, however, the reaction products mostly deposit on the walls of the experimental flasks as tar incapable of further chemical transformation. The reason is that randomly formed organic molecules are usually non-polar and tend to stick together, which is what happens in the experiments.

Benner and his colleagues approached this problem experimentally. In addition to their formulation of the "tar paradox" ("Organic systems, given energy and left to themselves, devolve into uselessly complex mixtures" [32]), they showed how tar deposition can be prevented in the case of the so-called formose reaction, discovered by Butlerov as early as 1859 [108]. This reaction is thought to be of key importance for prebiotic syntheses because it produces a viscous mixture of sugars and organic acids with 4 to 7 carbon atoms (C4-C7 compounds) from C1-C3 aldehydes and/or alcohols [109,110]. The Butlerov reaction appears to be unique in producing complex molecules from simpler building blocks without energy input and in water. The reaction proceeds spontaneously under mildly alkaline conditions and is enhanced by metal cations (Ca2+, Mg2+, Na+, K+) as catalysts. The reaction is stimulated by UV light, in the presence of which it proceeds even with a C1-molecule of formaldehyde (COH2, see Fig. S2.2.) as the sole substrate [111]. Biologically most relevant ribose, a component of RNA, makes up about 1% of the product mixture.

Benner and his colleagues showed that the "caramelization" of the product sugars can be prevented by adding high concentrations of borate to the solution. Borate anions (BO2–), by binding to the sugar molecules in a unique way (see Fig. S2.2.), drove the reaction towards the specific formation of C5 sugars, which increased the yield of ribose [112,113].

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Figure S2.2. Formation of linear pentoses under borate and molybdate control (Modified from [114]).

However, most of the C5-sugars obtained in the presence of borate were branched, in contrast to the linear sugars present in RNA and DNA. Therefore, Benner and his colleagues next showed that branched C5-sugars can be linearized in the presence of molybdenum oxide (MoO42-) as a catalyst, see Fig. S2.2. and [114].

In fact, the abiotic formation of any sugar is welcome because sugars and their phosphorylated derivates are interconvertible; it has been shown that incubation of one of the sugar phosphates in warm water and in the presence of transition metal ions as catalysts leads to a slow, non-enzymatic formation of other biologically relevant sugars, which implies that the interconversion of such sugars is not particularly chemically challenging [48-52].

The pioneering research of Benner and his colleagues provides a tentative solution to the "tar" paradox. Abiotically formed organic molecules can avoid getting into the tar by specifically binding to solvent components. This gives them a chance to participate in further catalytic transformations, the direction of which can be controlled by particular molecules present in solution. So, the solvent matters.

**S2.3. Key biomolecules contain many CN bonds which are absent in inorganic nature (paradox of the CN bonds)**

Proteins and nucleic acids are more enriched in nitrogen than other organic molecules. As shown in Fig. S2.3. they contain many CN bonds. Paradoxically, CN bonds are not usual in the inanimate nature. Therefore, the origin of the CN bond in biomolecules has been widely debated.

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Figure S2.3. Biomolecules. The CN bonds are green shaded. A, amino acids and formation of a peptide bond between them; B, Nucleotides, their structure and complementary interactions; C, condensation reactions; D, cyclic nucleotides and mechanisms of their polymerization (modified from [115]); E, Ribonucleotide-containing enzyme cofactors.

As early as 1976, Mukhin and his colleagues has discovered large amounts of HCN in the volcanic lava and attributed them to high-temperature chemical reactions in the throat of volcanoes [55]. As pointed by Calvin, the reaction of cyanide formation from methane and ammonia becomes thermodynamically favorable at T > 1050 K [116].

A promising simple stock compound for making CN bonds is ammonium formate [117]. It does not contain a covalent CN bond but can be converted to the CN bond-containing formamide (HCONH2) either via a single dehydration step or in the course of interaction with urea as studied by Hud and his colleagues, see Fig. S2.4 and [117]. Notably, both ammonium and formate can be produced in geochemical processes. The ammonium is the form in which nitrogen is present in the mantle and is delivered by geothermal vapor [118], see also Section S2.8. below. Formate is generated in the very first step of any abiotic CO2 reduction (see Fig. S2.1B above).

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Figure S2.4. Chemistry of formamide (from [119]). Abbreviations. PI = Photon Irradiation, Pyr = Pyrolysis, DT and DP = high temperature and/or high pressure. Top, relationship between formamide and other prebiotic feedstock molecules, such as HCN, and ammonium formate (NH4+HCOO−); bottom, prebiotically relevant products of formamide chemistry.

Not surprisingly, Saladino, di Mauro and many other researchers have favored abiotic syntheses of biomolecules from formamide (HCONH2), which is sometimes called a “free-living peptide bond” because of the structural similarity (cf with Fig. S2.3 above). It was repeatedly shown that heating of formamide, its illumination by UV light, or its treatment by proton beam yield nitrogen bases and amino acids; the reactions can be boosted by certain minerals [88,89,104,120-135]. These exciting findings prompted the concept of the origin of life in a “Formamide World” based on the ability of formamide (if present) to accumulate upon the evaporation of water because of its high boiling temperature of 210°C [104,129].

Sutherland and his colleagues put more emphasis on nitriles, compounds with a terminal highly reactive CN group, with hydrogen cyanide (HCN) as their simplest representative (Fig. S2.4). These authors have elucidated a network of chemical reactions leading to nucleotides, amino acids and even glycerol, which is the building block both for sugars (via the formose reaction, see Section S2.2 above) and for lipids [136-141].

The molecules containing CN bonds, such as cyanide or formamide, may also have been formed in the primordial atmosphere and Hadean ice by UV light [142,143], solar wind [144], and electric discharges that were simulated already by Miller [95,103].

**S2.4. Biomolecules and their polymers are prone to hydrolysis (the water paradox).**

A common feature of biopolymers, such as RNA, DNA, proteins, and oligosaccharides, is that they are formed in the cell by a polycondensation mechanism, where the addition of each monomer is accompanied by release of a water molecule, see Fig. S2.3C and [32,145]. Furthermore, even the formation of single nucleotides implies condensation of a nucleobase with a sugar into a nucleoside and further condensation(s) with phosphate group(s), see Fig. S2.3C and [146]. Such condensation reactions cannot occur spontaneously in water; on the contrary, water usually shifts the reaction equilibrium towards the breakdown of such polymers. In modern organisms, these polycondensation reactions proceed only when driven by excess free energy provided by bioenergetic reactions. Hence, since water ultimately leads to destruction of DNA, RNA, proteins, and polysaccharides, it could hardly have served as a suitable medium upon their spontaneous emergence at the beginning of times.

To enable condensation reactions at the origin of life, researchers have usually considered fluctuating systems with wet/dry cycling, such as tidal zones, periodically drying pools, geothermal systems with fluctuating activity, deserts periodically wetted by rains, and so on [33,133,147-153].

Another option is represented by eutectic (water/ice) systems where water is temporarily removed from the reaction volume by freezing [154-158]; in such systems spontaneous polycondensation of nucleotides proceeds indeed [159-161].

Yet another option is to envision the formation of the first biopolymers in anhydrous solvents, specifically in formamide that can additionally serve as a building block for biomolecules, see Section S2.3. above and [104,129,150].

While envisioning the formation of complex primordial biomolecules, several researchers have considered so-called impact crater scenarios [132,137,139,162]. In these scenarios, the early evolution of life has been proposed to follow the evolution of a meteoritic crater: as long as the crater was hot, water boiled out and biomolecules could form from formamide/cyanide. As the crater cooled down, the condensation reactions and the formation of the first biopolymers became possible, and finally water from rain and snow could flow into the crater, forming cool ponds that could serve as hatcheries for the first cell-like organisms.

**S2.5. The chicken-and-egg paradox of the first biopolymer: The concept of RNA World**

Proteins are made with the participation of DNA and RNA while DNA and RNA are synthesized by protein enzymes, which is a kind of chicken-and-egg paradox. Currently, most biologists resolve this paradox by assuming that the RNA-like polymers came first. The very first organisms are envisioned as aggregates/consortia of small RNA-like molecules that reproduced themselves and catalyzed some biosynthetic reactions [158,160,161,163-197].

This idea is remarkably old. As early as 1957, after discovering the non-coding, structural rRNA (see Supplementary File 1 and [198]), Belozersky reported at the 1st Congress on the Origin of Life: “it seems rather that ribonucleotides and then RNA originated ﬁrst” [163]. He argued that RNA molecules could both store genetic information (as in RNA viruses) and perform some protein functions (as in ribosomes). Similar concepts were put forward by several other researchers [164-167]. After RNA molecules were found to be able of catalyzing chemical reactions (such RNA molecules are called ribozymes, see [169,170]), Gilbert came up with the vision of the “RNA World … containing only RNA molecules that serve to catalyze the synthesis of themselves” [171].

Furthermore, Chetverin and his colleagues showed that “RNAs themselves can rearrange their sequences under physiological conditions, without the need for group activation or assistance from proteins or ribozymes” [199], which ability might have had a great evolutionarily importance.

The primacy of RNA is also supported by the observation that ribonucleotides are part of many organic cofactors (Fig. S2.3E), which is seen as evidence for the emergence of these cofactors in the primordial RNA World [200,201].

As described in Supplementary File 1, the ribosomal protein synthesis apparatus forms the ancient core of the cell. When the first structure of the ribosome was solved, it was shown that the linking of the two amino acids is accomplished by RNA loops; no proteins are involved [6,7]. It turned out that proteins are made by those rRNA molecules that Belozersky discovered in 1957.

More recently, the evolution of ribosome has been reconstructed by using several different methods, which allowed a deeper look into the primordial RNA world. In each case, an ancient catalytic peptidyl transferase center was identified in the core of the large ribosomal subunit (LRS); this center consists of two pseudo-symmetric regions of only about 60 nucleotides each, see [202-210]. Furthermore, the fragments of this catalytic center have been shown to synthesize dipeptides [211,212]. These reconstructions provide conclusive evidence that the RNA World existed indeed and was inhabited by consortia of RNA molecules of some 50-60 nucleotides.

Also, the evolutionary primacy of RNA is independently supported by the fact that RNA is the *only* biopolymer present in all known life forms, namely cells, viruses, and viroids (see Section S1.3.).

Manfred Eigen was the first to realize the danger of an “error catastrophe” for the RNA World [213,214]. Eigen has calculated that primitive RNA-like replicating entities could not properly transmit information through generations unless they possessed a sophisticated, nearly error-free replication machinery. The maximum length of a correctly replicated chain in a primordial, enzyme-free system was estimated to be about 100 bases, which is obviously insufficient to encode elaborated replication machinery. As a solution to the problem, Eigen and Schuster proposed “hypercycles” in which short RNA and protein oligomers helped each other to maintain information without loss [215-217]. In the following decades, the versatility of RNA molecules as catalysts was fully appreciated, so that some current versions of hypercycles do not invoke proteins anymore, but consider aggregates of short interacting RNA oligomers, see e.g. [177,218]. Quite recently Joyce and his colleagues seemed to succeed in overcoming the error threshold in an experimental, purely RNA-based setup [194].

Current views consider that the first RNA-organisms may have initially proliferated not by self-replication, which implies sequential addition of individual nucleotides along the available template, but in a less streamlined process, with new RNA oligomers assembling from oligomers of shorter length in interaction with templates. We will refer to this mode of assembly as self-copying, to distinguish it from “self-reproduction” which applies to the “duplication” of the whole content of a protocell [219]. Several systems capable of self-copying by cooperation between short RNA oligomers have been obtained and studied experimentally [173,174,192,194].

Further support for the concept of the RNA world was provided by studies of RNA molecules replicating inside lipid vesicles mimicking protocells [185,220-228]. The authors of these studies usually used either enzymes or artificially activated nucleotides to speed up the reactions studied. Nevertheless, these studies are very important as proof of principle. They provide a possibility of studying the transport of metabolites across primitive membranes and interactions of RNA molecules with these metabolites Concurrently, using a protein replicase, Chetverin and his colleagues obtained RNA colonies growing on a solid substrate and studied their properties [229,230].

The key problem with the RNA World concept is the complexity of the ribonucleotides. Each consists of a nitrogenous base, a ribose moiety, and one or more phosphate groups (Fig. S2.3). The closed electronic system of the nitrogen base makes it (photo)stable (see also below) but hinders the formation of the so-called glycosidic bond with the ribose moiety, see [34,231] and references therein. Since it is difficult to assemble a nucleotide from its constituents even under laboratory conditions, it has remained unclear how it could have happened under the primordial conditions in the absence of enzymes.

Among many new pathways of abiotic nucleotide synthesis [34,136,137,232-234], only one, as reported by Carell and colleagues, has so far provided a "unified prebiotically plausible synthesis of pyrimidine and purine RNA ribonucleotides" see Fig. S2.5. and [234]. This synthesis mimics events in a shallow primordial pond that could have undergone wet/dry cycles. Upon successive steps of this synthetic pathway, 3-aminoisoxasole with *T*B as high as 225°C is used first as a low-volatile solvent and later as a key common intermediate which is converted to N-isoxazolyl-urea using Zn2+ ions as catalysts. In another catalytic step metallic Zn is used to reduce nitroso-pyrimidines to formamidopyrimidines.

Earlier, Sanchez and Orgel showed the possibility of stepwise assembly of a nitrogen base on a sugar template [235]. Using a similar approach, Powner and his colleagues developed a novel one-pot synthesis protocol in which the base precursor closed its ring *after* being attached to the sugar [136]. In general, this approach has proven to be a very convenient technique that is now widely used in nucleotide synthesis, see [232] for a review [[2]](#footnote-2).

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Fig. S2.5. Proposed geochemical scenario for the simultaneous synthesis of purine and pyrimidine nucleosides, driven by wet-dry cycles (from [234]). In yellow, the solvent is 3-aminoisoxazole (**4**), which can be enriched from an aqueous solution due to its high boiling point (228 °C).

Remarkably, the pioneering synthesis by Powner and his colleagues [136], yielded a ribocytidine β-2',3'-cyclic phosphate, i.e. a cytidine nucleotide with a phosphate group making not one but two bonds with the sugar moiety, see Fig. S2.3D. Hereafter we will denote such 2',3'-cyclic nucleotides as 2',3'-n>P, where ***n*** is the nucleoside moiety.

Another intensively studied type of cyclic nucleotides are the 3’,5’-cyclic nucleotides (3’,5’-n>Ps, see Fig. S2.3D and [134,135].

The cyclic nucleotides are “pre-loaded” by free energy needed for their polymerization. The enthalpy of scission of the additional phosphate-oxygen bond in cyclic nucleotides is about –40 kJ/mol [236]; this energy can be used for binding to another nucleotide, so the cyclic nucleotides are considered to be “activated”. Notably, their binding to another nucleotide or an RNA oligomer can even proceed without the release of a water molecule (see Fig. S2.3D), thus overcoming the water paradox (see Section S2.4.).

The 2',3'-n>Ps are the immediate products of the RNA hydrolysis by several small “housekeeping” ribonucleases (RNAses) [237,238]. In the 60’s, biotechnologists have attempted to force such RNAses to work in the reverse mode and to synthesize oligonucleotides. RNAses synthesized short oligonucleotides from 2',3'-n>Ps in various solvents including formamide; the oligonucleotide yield was particularly high at 0°C, see [239] for a review. An RNAse itself cannot affect the thermodynamics of the reaction, so polymerization of 2',3'-n>Ps must be thermodynamically favorable at ≤ 0°C also in the absence of RNAses.

Recently, Braun and his colleagues described the non-enzymatic oligomerization of 2’,3’-n>Ps in thermal gradients [240], as well as at heated air-water interfaces at pH range of 7–12; in the latter case, the reaction was marginally enhanced by K+ ions [241]. The 3’,5’-n>Ps have also been shown to spontaneously polymerize under certain conditions, especially on the surface of silicate minerals [134,135]. These observations prove the ability of cyclic nucleotides to polymerize without enzymes, which may have proceeded also on Hadean Earth.

**S2.6. Natural nucleotides are extremely radiation-resistant (photostability paradox).**

Nucleotides (see Fig. S2.3B and S1.2-S1.5) strongly absorb UV light in the range of 260-280 nm. This property underlies the popular belief that solar UV quanta absorbed by DNA can cause hazardous DNA mutations, which sunscreens are designed to prevent. This ability to trap potentially damaging UV quanta by nucleotides is paradoxical because it would seem to compromise the reliability of genetic information storage. Actually, it is not that bad; 99,9% of UV quanta are trapped by nucleoside moieties (nucleobase+sugar, see Section S1.1) that are exclusively photostable against UV radiation compared to structurally similar compounds, such as aromatic amino acids or non-canonic nucleobase analogs [136,242-244]. Canonical nucleosides usually convert the energy of a UV-quantum into heat in about 10-13 s (Fig. S2.6), i.e., much faster than any destructive chemical reactions can occur. It has been argued by several authors that the unique ability to dissipate excitation energy in femtoseconds is due to very fast deformations of the nucleobase rings and in particular to torsional motions around the C–N bonds [243-245]. Other than five canonical bases, the ability to rapidly discard UV quanta is also shared by hypoxanthine, a non-canonical, but natural base of the minor nucleoside inosine, see Fig. S2.6 and [244]. Inosine often plays a key role in ribosomal protein synthesis and is thought to precede guanosine in evolution [246]. It is also paradoxical that this exceptional photostability of natural bases is apparently unrelated to the mechanisms of information transfer.

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Figure S2.6. Excited state lifetimes of natural bases (blue squares) and modified bases (red circles) in aqueous solution measured in femtosecond transient absorption experiments. While the modified bases have lifetimes that span several orders of magnitude (note the logarithmic scale) all of the natural bases have lifetimes of < 1 ps. The image is modified from [244].

One may ask: if nitrogen bases are so efficient at deactivating UV quanta, why is the UV light considered harmful? In fact, the main harm comes from truly dangerous backbone breaks caused by absorption of UV light not by nucleotides proper, but by phosphate moieties that connect the nucleotides, see Fig. S2.3 and [247]. Due to their resonant structures, the phosphate groups also absorb UV light in the same range of 260-280 nm, albeit very weakly [248,249]. However, their photoexcitation yields a reactive phosphate radical that manages to break the sugar-phosphate backbone with a very high quantum yield of about 0.5, i.e. in every second case [247,250].

The nitrogen bases, thanks to their ability to intercept and dissipate UV quanta, efficiently protect the RNA and DNA from these breaks. The extent of this protection can be estimated from the data of Goossen and Kloosterboer who concurrently irradiated glycerol-2-phosphate and AMP with UV light of 254 nm and monitored their photodestruction by measuring the release of phosphate [251]. Phosphate was photo-released ten times faster from glycerol-2-phosphate than from AMP [251], even though the adenine moiety of AMP absorbed 104 times more UV quanta per unit time than the phosphate moiety of glycerol-2-phosphate [247]. Thus, in RNA, nitrogen bases provide at least 105-fold protection of the sugar-phosphate backbone from UV cleavage. This feature explains why deleterious UV damage to the backbones of RNA and DNA molecules is 103–104 times less frequent than photodestruction of the nitrogen bases proper (Cadet and Vigny 1990). Eventually, the bases are sacrificed to prevent the breaks in backbones of RNA and DNA; however, provided that the backbone is not damaged, the cellular repair systems rapidly replace the damaged bases [252].

Hud, di Mauro and their colleagues reported the formation of notable amounts of adenine, guanine, and hypoxanthine upon irradiation of a 10% formamide solution in water with UV light whereby these nitrogen bases were the main products of irradiation [130]. Furthermore, irradiation of liquid formamide with a high-energy proton beam in the presence of powdered meteorites yielded a wide spectrum of organic compounds, the most complex of which were the canonical nitrogen bases [88]. In another set of experiments, the proton beam catalyzed the transglycosylation of pyrimidine nucleobases to yield canonical N1-pyrimidine nucleosides [89]. These data indicate that canonical nitrogen bases and nucleosides, once formed in an energy flux, are more resistant to high-energy radiation than other compounds of comparable complexity.

The photostability of nitrogen bases may have played a key role in the origin of life. Already in 1973 Carl Sagan suggested that they may have initially served as UV-protectors [253]. He argued that the 240-300 nm window was transparent for potentially damaging solar UV radiation before oxygen accumulated in the atmosphere and could be converted to ozone by solar UV quanta, so protection from this radiation may have been a prerequisite for the emergence of the first replicating entities.

In sum, nitrogen bases are not only letters of the genetic alphabet, but also UV-protectors, which may have been their initial function, as has been suggested by Sagan [253] and is elaborated in Section S2.7. below.

**S2.7. Emergence of the first complex molecules (the complexity paradox)**

Although the second law of thermodynamics teaches that the disorder of the universe increases with time, living beings are rather well-ordered. This paradox is usually explained by complexification of organisms on the expense of external energy – brought in by light, contained in the food and so on. Organisms are quite adept at using this energy to drive thermodynamically unfavorable reactions, namely synthesize highly reduced compounds, drive polycondensation reactions in the water phase, or maintain chemical disequilibria across cell membranes. However, the very first organisms had neither energy-harvesting nor energy-transforming systems, yet they had to reach a certain level of complexity to survive. How could external energy have been used to produce increasingly complex molecules without sophisticated energy conversion machinery?

As far as we know, this paradox was first formulated by Carl Sagan who also proposed its solution. As early as 1957, he wrote that “differential survival of polymerized molecules over unpolymerized molecules” under condition of a high-energy flux, such as UV light, could promote the selective accumulation of more complex molecules [254].

Elsewhere, building upon experimental observations from [247,251] considered in Section S2.6, we used Monte-Carlo modeling to investigate how the ability of attached nitrogen bases to protect sugar-phosphate units in RNA-like polymers from UV damage might have affected the complexity of such units, see Fig. S2.7 and [255]. Upon modelling, the binding of nitrogen bases to sugar-phosphate moieties was set as thermodynamically unfavorable (see the caption to Fig. S2.7 for the reaction constants used). When the nitrogen bases were assumed to provide no UV protection, the polymers were short and the extent of nucleobase incorporation into the polymers was close to zero (Fig. S2.7, circles on panels *a* and *b*). In another simulation run, the UV protection was "turned on" so that the binding of a nucleobase to a sugar-phosphate moiety reduced the probability of its UV breakage by a factor of 30, which is a rather modest value compared to the experimentally determined factor of 105, see Section S2.6. and [247,251]. In this case, the sugar-phosphate units began to acquire UV-protecting nitrogen bases, and the length of the polymers increased dramatically (Fig. S2.7, triangles on panels *a* and *b*).

When we simulated the funneling of UV energy into the condensation reactions with efficiency as small as about 10-7, the length of the formed polymer chains increased dramatically and these chains were built predominantly from nitrogen base-possessing nucleotides (Fig. S2.7, squares on panels *a* and *b*). This result was expected and trivial, though.

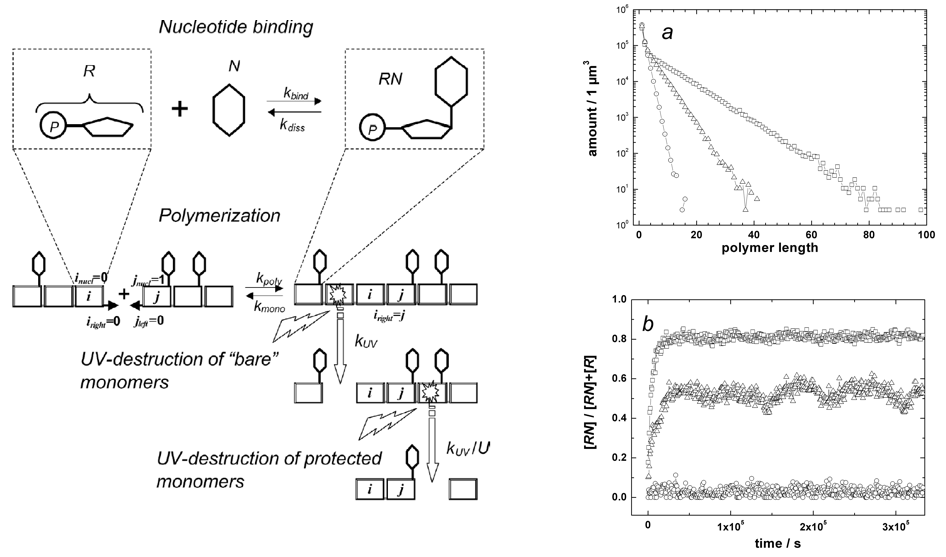


Figure S2.7. **Monte Carlo simulation of a sugar-phosphate polymerization reaction in the presence of nitrogenous bases and under UV-illumination. The image is taken from** [255].Sugar-phosphate polymerization reaction in the presence of nitrogenous bases and under UV-illumination was simulated using the following parameter set: The concentration of monomers in the reaction volume was kept on a constant level of 10-3 M (comparable with their concentration in the cell). The second-order rate constant of polymerization *kpoly* was 3 M-1 s-1 and the first-order rate constant of re-dissociation *kmono* was 10-4 s-1, which corresponds to the equilibrium constant of 30. The rate constants of nucleotide binding (*kbind*) and dissociation (*kdiss*) were 3 × 10-8 s-1 and 10-6 s-1, which corresponded to the equilibrium constant of 3 × 10-2. Under the UV illumination, monomers decomposed with the rate constant of 3·10-3 s-1 irrespectively of their position in the chain. For simplicity, the UV protection factor *U* of 30 was used both for monomers and oligomers. The partial funneling of UV energy was assumed to increase the *kbind* value from 3 × 10-8 s-1 up to 1.2 × 10-7 s-1. *a*, Polymer length distribution at equilibrium. *b*, Fraction of monomers protected by nitrogenous bases as a function of time.

The increase in the relative proportion of longer, nucleobase-carrying polymers solely in response to the "turning on" of the UV-quenching ability of nitrogen bases (Fig. S2.7, triangles on panels *a* and *b*) was not trivial at all, since no radiation energy was allowed to be funneled into any reaction of bond formation in these simulation runs. The increase in the proportion of more complex molecules was solely due to the UV-destruction of less photostable molecules down to building blocks that could then re-enter the polymerization reactions. Since the number of molecules in the Monte Carlo simulation was limited, the relative proportion of more photostable and thus more complex polymers increased under UV light, in accordance with Carl Sagan's prediction [254]. Therefore, and this is the key point, the enrichment in more complex RNA-like polymers proceeded without direct chemical coupling between energy flow and bond formation.

Furthermore, the folding of RNA molecules into double helices was shown to increase their UV stability hundredfold [256], which implies that complementary nucleobase pairs may be specifically photoselected under UV illumination. Based on all these observations, we hypothesized that, in the absence of the ozone layer, the UV-rich sunlight may have promoted the selective accumulation of RNA-like polymers capable of forming double helices [255].

Remarkably, the above described one-pot synthesis of pyridine nucleotides by Powner and his colleagues (see Section S2.5. and [136]) yielded initially a mixture of 2’,3’-cytidine>Ps with several by-products. To get rid of the latter, the authors exposed the mixture to 254 nm UV light for three days. The prolonged UV irradiation resulted in the destruction of the by-products and the partial conversion of the β-ribo*cytidine*-2',3'-cyclic phosphate into β-ribo*uridine*-2',3'-cyclic phosphate (2’,3’-uridine>P), another activated natural ribonucleotide. The authors concluded that “there must be some (photo)protective mechanism functioning with natural nucleotides but not with other pyrimidine nucleosides and nucleotides” [136]. This photoprotective mechanism not just prevented the destruction of natural 2',3'-n>Ps, but maintained the additional high-energy bond (see the red arrow in Fig. S2.3D) despite its susceptibility to spontaneous hydrolysis. The fact that this bond persisted after three days of UV irradiation may indicate that the energy of the UV light specifically restored this additional bond in 2',3'-pyrimidine n>Ps after its eventual breaks. This seminal experiment documents the UV selection of natural nucleotides - in their high-energy, polymerization-prone state – from a mixture of different structurally related compounds.

The emergence of complex, potentially information-carrying molecules by UV selection seems to be related to the so-called Landauer principle [257]. Landauer analyzed the thermal behavior of a physically realistic computer with a limited number of memory units and showed that information cannot be stored without expending energy. He found, however, that energy can be expended in two ways: either to store information in the empty memory units, or to "clean up" the previously filled but no longer needed memory units. Thus, energy can support the emergence of new information - aka increase in complexity - by dismantling the unneeded, less complex entities. In this respect, Landauer's formalism is consistent with our Monte-Carlo simulation, which used a limited number of building blocks [255], as well as with the experiments from [136], where the amount of chemicals in the flasks was obviously limited.

Therefore, the experimentally demonstrated UV-selection of activated natural 2',3'-n>Ps [136], as well as the results of our earlier modeling of UV effects on RNA-like polymers [255], indicate that the emergence of activated nucleotides and their polymers on primordial Earth could have been driven by energy-consuming, selective disassembly of less "perfect" variants, in agreement with the insightful suggestion of Carl Sagan [254]. The Landauer formalism implies that the preferential accumulation of radiation-resistant – and therefore complex - activated 2',3'-n>Ps [136] was powered by the energy of UV light in accordance with the laws of thermodynamics.

Thus, complex molecules, even in their high-energy reactive states, may have emerged and accumulated at the expense of solar UV radiation even before the development of complex biological mechanisms for harnessing energy and directing it to synthetic reactions.

**S2.8. Phenomenon of chemistry conservation and predominance of K+ over Na+ inside the cells (paradox of high intracellular potassium levels)**

Table S2.1 shows the difference between the concentration of inorganic constituents of seawater, the water of the ancient anoxic ocean, cellular cytoplasm, and extracellular media represented by the blood plasma, as compiled from [258-266]. Living cells contain much more phosphate (PO43-), potassium, magnesium, and zinc than the media they reside in. In contrast, the intracellular concentrations of Na+ and Ca+ ions are usually much lower than in the environment. These characteristics are common to archaea, bacteria, and eukaryotes that split some 4 Ga ago (see Section S1.3).

Table S2.1. Molar concentration of life-relevant inorganic substances in different media. Data are compiled from refs. [258-265,267].

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Substance | Cell cytoplasm | Blood plasma | Today’s sea water | Anoxic, sulfidic ocean |
| Na+ | 0.014 | 0.142 | 0.4 | > 0.4 |
| K+ | 0.1 | 0.005 | 0.01 | ~0.01 |
| Mg2+ | 0.1-0.01 (mostly bound) | 0.0015 | 0.05 | ~0.01 |
| Ca2+ | 10-7÷10-6 (free)  10-3 (bound) | 0.002 | 0.01 | ~0.001 |
| Zn2+ | 10-3 to 10-4 | 1.0-1.5 × 10-5 | 10-8 | 10-15 ÷ 10-12 |
| Fe | 10-3 to 10-4 | 0.0015 (transferrin-bound) | 10-8 (mostly Fe3+) | 10-5 (Fe2+) |
| Mn2+ | 10-3 to 10-4 | 10-8 | 10-10 | 10-8 |
| Cu (I) – Cu (II) | 10-5 to 10-4 | 10-5 | 10-9 (Cu2+) | < 10-20 (Cu+) |
| Mo (IV) – Mo (VI) | 1.6×10–7 | 10-8 | 10-7 mostly Mo (VI) | 10-11 to 10 -9 mostly Mo (IV) |
| Cl– | 0.15 | 0.1 | 0.5 | ~ 0.5 |
| PO43–/HPO4 2– | ~0.01 (mostly bound) | 0.001 | 10-6 to 10-9 | <10-5 |
| CO2/HCO3–/H2CO3 | 0.025 | 0.027 | 0.002 | 0.1-0.02 |
| S (free) | ~10-1 (mostly as methionine and cysteine) | 0.0005 (SO42–) | 0.026 (SO42-) | ~10-2 (mostly S2-) |

Unlike biopolymers such as RNA, DNA, and proteins, which are enclosed by the cell, small molecules and ions constantly leak across cell membranes, driven by their concentration gradients. Therefore, cells use membrane-embedded ion pumps to counteract the leakage and maintain gradients of different ions across cell membranes, which require energy.

The membranes of the first cells are thought to prevent the loss of polymers, but to be not particularly impermeable to small molecules and ions. It has been repeatedly noted that semipermeable primordial membranes must have been vital for the very first cells, which, in the absence of various membrane transporters and pumps, had to rely on the diffusion of small molecules and ions through primordial, leaky membranes [29,30,222-225,268-273].

Therefore, it is thought that intracellular inorganic chemistry reflects the (geo)chemistry of the environments in which the first cellular organisms formed [152,274-277], just as the low Eh of the cytoplasm reflects the reduced state of the primordial environments, see section S2.1. and [76]. In response to environmental changes, cells were not able to modify all of those enzymes that originally depended on certain substances as cofactors (see also the following sections S2.9-S2.11). Consequently, although modern cells have colonized a wide variety of environments, they are filled with a medium similar to that in which their common ancestors lived.

This phenomenon of chemistry conservation is very important because it helps to reconstruct the habitats of the first organisms even in the absence of any geological record [152,274-276]. For example, it is safe to say that the formation of the first cells took place under pH-neutral conditions, since the cytoplasmic pH in almost all organisms is neutral or slightly alkaline.

In particular, already more than a hundred years ago, Archibald Macallum recognized that potassium is more abundant in cellular tissues than sodium, in contrast to both seawater and body fluids, such as blood and lymph, see Table S2.1 and [274,278]. It was already accepted at that time that the high salt content of blood and lymph was related to the origin of multicellular organisms in seawater, see [274,278] and references therein. To explain the chemical difference between cytoplasm and seawater, Macallum proposed that "the cell...has endowments transmitted from a past almost as remote as the origin of life on earth" [274][[3]](#footnote-3). Accordingly, Macallum has suggested that the habitats of the first unicellular organisms had more potassium than sodium.

Elsewhere, we have turned to the ubiquitous proteins common to all free-living cellular organisms [152]. These proteins are thought to be present in the LUCA or even its progenitors, see Section S1.3 and [19-21,279,280]. We checked their functional dependence of ubiquitous proteins on inorganic ions (mostly using the data from the BRENDA database [281]) and the presence of inorganic ions in the available structures, the updated version of such an analysis is presented as Table S2.2.

**Table S2.2. Inorganic constituents of ubiquitous proteins.**

the full Excel version of the Table with additional information is provided as Supplementary File 2.

The table provided information on the inorganic constituents and cofactors of 87 orthologous groups of proteins found in all free-living organisms which, by definition, must have been present in the Last Universal Cellular Ancestor (LUCA) [19]. We took protein sequences longer than 50 aa from the PDB database (checked 13.07.2024) and attributed them to the COG database [282] using the last set of profile HMMs for COGcollator ([283], available at http://boabio.belozersky.msu.ru/tools) and hmmscan program (http://hmmer.org/). If two profile HMMs found overlapping hits and the overlap was longer than 5% of the longest of these two hits, we filtered out the weakest hit. To avoid any confusion, we further used only proteins which were attributed to a single COG with an e-value less than 1e-10 according to this procedure. We selected only protein chains which were attributed to the set of 87 aforementioned universal COGs. Total 71014 protein chains belonging to 7767 PDB structures were sampled.

Functional categories are given according to the COG database C — Energy production and conversion, E — Amino acid transport and metabolism, F — Nucleotide transport and metabolism, G — Carbohydrate transport and metabolism, H — Coenzyme transport and metabolism, I — Lipid transport and metabolism, J — Translation, ribosomal structure and biogenesis, K — Transcription, L — Replication, recombination and repair, D — Cell cycle control, cell division, chromosome partitioning, M — Cell wall/membrane/envelope biogenesis, N — Cell motility, O — Posttranslational modification, protein turnover, chaperones, U — Intracellular trafficking, secretion, and vesicular transport.

Other abbreviations: PPi — pyrophosphate, Pi — phosphate ; n/e — absence of an EC number due to the protein being **n**ot an **e**nzyme.

COGs which were missing from the syn3 minimal bacterial genome are marked with the asterisk \* sign. Notably, The ribosome, as a whole requires high levels of Mg2+ and K+ ions, as well as sufficient levels of Zn2+ ions [284-287], see the main text for further references

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **COG** | **Funct.. Cat.** | **COG name** | **EC number (if available)** | **Functionally relevant inorganic anions** | **Functional dependence on monovalent cations** | **Functional dependence on divalent cations** | **Divalent cations in at least some structures** | **Number of structres in the PDB** |
| COG0636 | C | FoF1-type ATP synthase, membrane subunit c/Archaeal/vacuolar-type H+-ATPase, subunit K | 7.1.2.2 | - | - | - | Mn2+(1) | 3469 |
| COG0112 | E | Glycine/serine hydroxymethyltransferase | 2.1.2.1 | - | - | Mg2+ / Ca2+ | Mg2+(2), Ca2+(1) | 385 |
| COG0125 | F | Thymidylate kinase | 2.7.4.9 | - | - | Mg2+ | Mg2+(59), Ca2+(8) | 230 |
| COG0528 | F | Uridylate kinase | 2.7.4.22 | - | - | Mg2+ | Mg2+(33), Mn2+(1) | 221 |
| COG1109 | G | Phosphomannomutase | 5.4.2.8 | - | - | Mg2+ | Zn2+(20), Mg2+(24), Ca2+(7) | 80 |
| COG0149 | G | Triosephosphate isomerase | 5.3.1.1 | - | - | - | Mg2+(5), Ca2+(7) | 622 |
| COG0561 | H | Hydroxymethylpyrimidine pyrophosphatase and other HAD family phosphatases | 2.5.1.3 | PPi | - | Mg2+ | Mg2+(56), Ca2+(8) | 108 |
| COG0575  /COG4589 | I | CDP-diglyceride synthetase | 2.7.7.41 | PPi | K+ | Mg2+ | Mg2+(3), | 5 |
| COG2890 | J | Methylase of polypeptide chain release factors | 2.1.1.297 | - | - | Mg2+ | Mg2+(4), Ca2+(14), Fe2+/3+(6) | 47 |
| COG0024 | J | Methionine aminopeptidase | 3.4.11.18 | - | - | Co2+/Ni2+/ Mn2+/Fe2+/Zn2+ | Zn2+(3), Mg2+(4), Mn2+(64), Ca2+(1), Fe2+/3+(5) | 240 |
| COG0242 | J | Peptide deformylase | 3.5.1.88 | formate | - | Zn2+/Mn2+/  Ni2+/Fe2+ | Zn2+(101), Mg2+(3), Fe2+/3+(13) | 319 |
| COG0533 | J | tRNA A37 threonylcarbamoyltransferase TsaD | 2.3.1.234 | - | - | Zn2+/Mg2+/  Fe2+ | Zn2+(7), Mg2+(7), Ca2+(1), Fe2+/3+(7) | 37 |
| COG0101 | J | tRNA U38,U39,U40 pseudouridine synthase TruA | 5.4.99.12 | - | K+, NH4+ | - | Mg2+(1), | 38 |
| COG0073 | J | tRNA-binding EMAP/Myf domain | n/e | n/e | n/e | n/e |  | 58 |
| COG0013 | J | Alanyl-tRNA synthetase | 6.1.1.7 | PPi | K+ | Mg2+, Zn2+ | Zn2+(14), Mg2+(6), | 68 |
| COG0018 | J | Arginyl-tRNA synthetase | 6.1.1.19 | PPi | K+ | Mg2+ | Mg2+(1), | 28 |
| COG0124 | J | Histidyl-tRNA synthetase | 6.1.1.21 | PPi | K+ | Mg2+ | Mg2+(1), | 85 |
| COG0060 | J | Isoleucyl-tRNA synthetase | 6.1.1.5 | PPi | K+, NH4+ | Mg2+, Zn2+ | Zn2+(19), | 31 |
| COG0495 | J | Leucyl-tRNA synthetase | 6.1.1.4 | PPi | K+, NH4+ | Mg2+, Zn2+ | Zn2+(49), Mg2+(36), Ca2+(1) | 126 |
| COG0143 | J | Methionyl-tRNA synthetase | 6.1.1.10 | PPi | K+, NH4+ | Mg2+, Zn2+ | Zn2+(31), Mg2+(4), | 145 |
| COG0016 | J | Phenylalanyl-tRNA synthetase alpha subunit | 6.1.1.20 | PPi | K+, NH4+ | Mg2+, Zn2+ | Zn2+(2), Mg2+(12), Mn2+(2) | 68 |
| COG0072 | J | Phenylalanyl-tRNA synthetase beta subunit | 6.1.1.20 | PPi | K+, NH4+ | Mg2+, Zn2+ | Mg2+(66), Mn2+(1) | 161 |
| COG0442 | J | Prolyl-tRNA synthetase | 6.1.1.15 | PPi | - | Mg2+, Zn2+ | Zn2+(40), Mg2+(32), Mn2+(2), Ca2+(15) | 193 |
| COG0172 | J | Seryl-tRNA synthetase | 6.1.1.11 | PPi | K+ | Mg2+, Zn2+ | Zn2+(18), Mg2+(10), Ca2+(6) | 119 |
| COG0441 | J | Threonyl-tRNA synthetase | 6.1.1.3 | PPi | K+, NH4+,Rb+ | Mg2+, Zn2+ | Zn2+(53), Mg2+(2), Ca2+(1) | 206 |
| COG0180 | J | Tryptophanyl-tRNA synthetase | 6.1.1.2 | PPi | K+ | Mg2+, Zn2+ | Mg2+(18), Mn2+(1), Ca2+(5) | 284 |
| COG0162 | J | Tyrosyl-tRNA synthetase | 6.1.1.1 | PPi | K+ | Mg2+ | Mg2+(2), | 67 |
| COG0525 | J | Valyl-tRNA synthetase | 6.1.1.9 | PPi | - | Mg2+, Zn2+ | Zn2+(1), | 9 |
| COG0081 | J | Ribosomal protein L1 | n/e | The ribosome, as a whole requires high levels of Mg2+ and K+ ions, as well as sufficient levels of Zn2+ ions, see the text for further references | | | Mg2+(5), | 522 |
| COG0244 | J | Ribosomal protein L10 | n/e | - | 649 |
| COG0080 | J | Ribosomal protein L11 | n/e | Mg2+(2), | 871 |
| COG0102 | J | Ribosomal protein L13 | n/e | Zn2+(2), Mg2+(6), | 1979 |
| COG0093 | J | Ribosomal protein L14 | n/e | Zn2+(3), Mg2+(65), | 1944 |
| COG0200 | J | Ribosomal protein L15 | n/e | Mg2+(16), Mn2+(1) | 1938 |
| COG0197 | J | Ribosomal protein L16/L10AE | n/e | Zn2+(1), Mg2+(1), Mn2+(1) | 1807 |
| COG0256 | J | Ribosomal protein L18 | n/e | - | 1866 |
| COG0090 | J | Ribosomal protein L2 | n/e | Zn2+(1), Mg2+(106), Mn2+(3) | 1940 |
| COG0091 | J | Ribosomal protein L22 | n/e | Mg2+(1), | 1980 |
| COG0198 | J | Ribosomal protein L24 | n/e | Mg2+(64), Mn2+(2) | 1955 |
| COG0255 | J | Ribosomal protein L29 | n/e | - | 1840 |
| COG0087 | J | Ribosomal protein L3 | n/e | Mg2+(78), Mn2+(1) | 1983 |
| COG0088 | J | Ribosomal protein L4 | n/e | Zn2+(3), Mg2+(13), Mn2+(1) | 1991 |
| COG0094 | J | Ribosomal protein L5 | n/e | Zn2+(3), Mg2+(3), | 1789 |
| COG0097 | J | Ribosomal protein L6P/L9E | n/e | Zn2+(3), | 1827 |
| COG0051 | J | Ribosomal protein S10 | n/e | Mg2+(15), | 1783 |
| COG0100 | J | Ribosomal protein S11 | n/e | Zn2+(5), Mg2+(18), | 1905 |
| COG0048 | J | Ribosomal protein S12 | n/e | Zn2+(2), Mg2+(21), | 1930 |
| COG0099 | J | Ribosomal protein S13 | n/e | Zn2+(1), Mg2+(14), | 1817 |
| COG0199 | J | Ribosomal protein S14 | n/e | Zn2+(126), Mg2+(45), | 1362 |
| COG0184 | J | Ribosomal protein S15P/S13E | n/e | Zn2+(1), Mg2+(9) | 1879 |
| COG0186 | J | Ribosomal protein S17 | n/e | Zn2+(7), Mg2+(16) | 1917 |
| COG0185 | J | Ribosomal protein S19 | n/e | Mg2+(8) | 1795 |
| COG0052 | J | Ribosomal protein S2 | n/e | Zn2+(17), Mg2+(75) | 1829 |
| COG0092 | J | Ribosomal protein S3 | 4.2.99.18 | Mg2+(23) | 1764 |
| COG0098 | J | Ribosomal protein S5 | n/e | Zn2+(7), Mg2+(98) | 1885 |
| COG0049 | J | Ribosomal protein S7 | n/e | Zn2+(9), Mg2+(12) | 1899 |
| COG0096 | J | Ribosomal protein S8 | n/e | Zn2+(3), Mg2+(29) | 1842 |
| COG0103 | J | Ribosomal protein S9 | n/e | Mg2+(8) | 1895 |
| COG0012 | J | Ribosome-binding ATPase YchF, GTP1/OBG family | 3.6.5.3 | Pi | K+ | Mg2+\* | Mg2+(3) | 15 |
| COG0480 | J | Translation elongation factor EF-G, a GTPase | 3.6.5.3 | Pi | K+ | Mg2+ | Mg2+(41) | 302 |
| COG0050 | J | Translation elongation factor EF-Tu, a GTPase | 3.6.5.3 | Pi | K+ | Mg2+ | Zn2+(1), Mg2+(58) | 175 |
| COG0231 | J | Translation elongation factor P (EF-P)/translation initiation factor 5A (eIF-5A) | n/e |  | K+ |  | - | 78 |
| COG0361 | J | Translation initiation factor IF-1 | 3.6.5.3 | Pi | K+ | Mg2+ | Zn2+(1), Mg2+(7) | 78 |
| COG0532 | J | Translation initiation factor IF-2, a GTPase | 3.6.5.3 | Pi | K+ | Mg2+ | Mg2+(20) | 86 |
| COG0202 | K | DNA-directed RNA polymerase, alpha subunit/40 kD subunit | 2.7.7.6 | PPi | K+ | Mg2+, Zn2+ | Zn2+(268), Mg2+(54) | 1768 |
| COG0085 | K | DNA-directed RNA polymerase, beta subunit/140 kD subunit | 2.7.7.6 | PPi | K+ | Mg2+, Zn2+ | Zn2+(363), Mg2+(23) | 1132 |
| COG0086 | K | DNA-directed RNA polymerase, beta' subunit/160 kD subunit | 2.7.7.6 | PPi | K+ | Mg2+, Zn2+ | Zn2+(835), Mg2+(710), Mn2+(8), Fe2+/3+(1) | 1171 |
| COG0195 | K | Transcription antitermination factor NusA, contains S1 and KH domains | n/e |  |  |  | Mg2+(1) | 49 |
| COG0250 | K | Transcription termination/antitermination protein NusG | n/e |  |  |  | Fe2+/3+(1) | 136 |
| COG0258 | L | 5'-3' exonuclease Xni/ExoIX (flap endonuclease) | 3.1.11.- |  | K+ | Mg2+,Mn2+ | Zn2+(2), Mg2+(18), Mn2+(20), Ca2+(8) | 104 |
| COG0592 | L | DNA polymerase III sliding clamp (beta) subunit, PCNA homolog | 2.7.7.7 | - | K+ | Mg2+ | Mg2+(3), Ca2+(47) | 368 |
| COG2812 | L | DNA polymerase III, gamma/tau subunits | 2.7.7.7 | - | K+ | Mg2+,Mn2+ | Zn2+(54), Mg2+(36) | 92 |
| COG0358 | L | DNA primase (bacterial type) | 2.7.7.101 | PPi |  | Mg2+,Mn2+ | - | 29 |
| COG0550 | L | DNA topoisomerase IA | 5.6.2.1 | - | K+ | Mg2+, Zn2+ | Zn2+(4), Mg2+(5), Ca2+(1) | 52 |
| COG0468\* | L | RecA/RadA recombinase | 3.5.4.B7 | Pi | K+ | Mg2+,Mn2+ | Mg2+(59), Mn2+(1), Ca2+(27) | 514 |
| COG0513 | L | Superfamily II DNA and RNA helicase | 3.6.4.13 | Pi | - | Mg2+,Mn2+ | Zn2+(4), Mg2+(65), Ca2+(2) | 421 |
| COG0206 | D | Cell division GTPase FtsZ | 3.6.5.6 | Pi | - | Mg2+ | Mg2+(13), Mn2+(2), Ca2+(17) | 202 |
| COG1136 | M | ABC-type lipoprotein export system, ATPase component | 3.6.3.- | Pi | - | Mg2+ | Mg2+(9), Mn2+(1) | 73 |
| COG0084\* | N | 3'->5' ssDNA/RNA exonuclease TatD | 3.1.16.- | - | - | Mg2+ | Zn2+(7), Mg2+(2), Mn2+(1) | 20 |
| COG1215 | N | Glycosyltransferase, catalytic subunit of cellulose synthase and poly-beta-1,6-N-acetylglucosamine synthase | 2.4.-.- | - | - | Mg2+ | Mg2+(22), Mn2+(23) | 138 |
| COG3118 | O | Chaperedoxin CnoX, contains thioredoxin-like and TPR-like domains, YbbN/TrxSC family | 1.8.1.6 | - | - |  | Zn2+(22), Mg2+(5), Ca2+(4), Fe2+/3+(2) | 668 |
| COG0459\* | O | Chaperonin GroEL (HSP60 family) | 5.6.1.7. | Pi | K+ | Mg2+, Mn2+ | Mg2+(141), Ca2+(9) | 3011 |
| COG0492 | O | Thioredoxin reductase | 1.8.1.9 | - | - | - | Mg2+(11), Ca2+(4), Fe2+/3+(1) | 198 |
| COG0201 | U | Preprotein translocase subunit SecY | n/e | n/e |  |  | Zn2+(1) | 92 |
| COG0541 | U | Signal recognition particle GTPase | 3.6.5.4 | Pi | - | Mg2+ | Mg2+(23), Mn2+(1), Ca2+(4) | 123 |
| COG0552 | U | Signal recognition particle GTPase FtsY | 3.6.5.4 | Pi | - | Mg2+ | Mg2+(12) | 87 |

As it follows from Table S2.2. and Table 2 of the main text, the majority of the ubiquitous proteins that can be confidently traced back to LUCA are involved in ribosomal protein synthesis, see also Section S1.3. This synthesis is K+-dependent in all organisms [286,288,289] because the proper functioning of the ribosomal apparatus requires more than 100 mM K+ ions and their predominance over sodium ions ([K+] > [Na+]) [286,287]. Potassium ions serve as cofactors (i) in the peptidyl transferase center, where amino acids are linked by a peptide bond [290], (ii) in the decoding center, where tRNA recognizes the codon of mRNA [291], and (iii) in numerous proteins that assist translation [2,292]. In addition, [K+] deficiency leads to an unspecific overall disintegration of ribosomes [291,293]. This is why all active cells contain more potassium than sodium. Usually, the concentrations of K+ and Na+ ions in active cells are of the order of 100 mM and 10 mM, respectively, giving a K+/Na+ ratio of about 10 [294]. Maintaining such a high K+/Na+ ratio is a costly enterprise, especially in marine environments, so cells use up to half of the available energy to maintain a tenfold excess of K+ ions over Na+ ions in the cytoplasm [295].

Thus, in the hope of identifying the habitats of the first cells, we have searched for environments with a high content of K+ ions and their predominance over sodium ions ([K+]/[Na+] ~ 10.0). We chose the high [K+]/[Na+] ratio as a key search criterion because, unlike absolute ion concentrations, it cannot be distorted by possible evaporation of water.

In seawater, [K+] << [Na+] (Table S1), so that our search criterion bluntly excludes all salty marine environments as potential hatcheries of the first cells. The rivers and lakes also usually contain more sodium than potassium [296]. Also the Earth’s mantle has ten time more sodium than potassium [297] Furthermore, even in meteorites and asteroids, sodium predominates over potassium [298,299]. Thus, the criterion [K+]/[Na+] ~ 10.0 is a rather strong constraint.

The selected criteria were met only by the condensate of geothermal vapor (Table S2.3.). It stems from meteoric water (water from rain and snow) descending through the rock until it reaches a very hot magma chamber that heats the water up to 300-400°C and saturates it with CO2. The heated fluid becomes lighter and rises to the surface, leaching various compounds from the rocks along the way. As the rock pressure near the surface decreases, the fluid begins to boil, causing the vapor and liquid phases to separate (Figure S2.8). The vapor transiently accumulates beneath the surface in so-called vapor-dominated zones, where it fills open fractures. From there, the vapor vents strictly upwards and escapes through thermal springs, which together form a geothermal field above the vapor-dominated zone. In contrast, the liquid phase reaches the surface by penetrating between rock layers (Figure S2.8) and can erupt as geysers even outside the geothermal field.

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Fig. S2.8. Structure of a geothermal field. Image is based on the structure of the Lassen geothermal system, US. Image credit: USGS. A terrestrial geothermal system is fed mostly by water from rain and snow (meteoric water) which, when it is deep underground, mixes with magmatic fluids and becomes heated to 300 to 500 °C; such hot fluids can leach diverse ions from the hot rock. Upon heating, the water becomes lighter and, being enriched in metal cations and such anions as Cl−, HS−, and CO32− ascends toward the surface. At shallower depths, the vapor phase usually separates from the liquid phase, which leads to the typical zoning. This separation is not only physical but also chemical; the gaseous compounds, such as CO2, NH3, and H2S, go into vapor. Also, the large K+ anions prefer to go into vapor. The vapor rises upward and spreads within the rock; the subsurface area that is filled by steam and gas is called the vapor-dominated zone. Part of the vapor condenses near the surface and is ejected by thermal springs (hot vents).

The chemical properties of the liquid and vapor phases are drastically different [300,301]. The vapor usually contains more K+ than Na+ ions, see Table S2.3. and [152], in contrast to the liquid emissions of geysers, which contain mainly Na+ and Cl– ions [302]. The predominance of K+ ions increases with the size of the geothermal field. The [K+]/[Na+] ratio reached 32 in the steam condensate of the Larderello geothermal field in Italy [303], and 75 in the steam condensates of the world’s largest geothermal field in California, USA [304].

The vapor also accumulates those substances that have affinity for the gas phase. These are compounds that can exist as gases, such as hydrogen sulfide (H2S), CO2 and ammonia (NH3). Being less polar than liquid water, vapor also attracts organic molecules formed in hot rocks (see Section S2.1.).

Table S2.3. Concentration of some essential elements in the water of thermal springs and the vapor condensate of the same springs, Mutnovsky volcano, Kamchatka peninsula (data from [152], expanded).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Vent number | S6-14 | S6-15 | S6-16 | S6-17 | S6-18 | S6-19 |
| water | | | | | | |
| T(K) | 94 | 93 | 89 | 93 | 96 | 96 |
| pH | 0.5 | -0.28 | 0.25 | -0.58 | -0.09 | -0.3 |
| Cl (ppm) | 6731 | 9447 | 8716 | 6403 | 5956 | 7288 |
| Na (ppb) | 128609 | 100599 | 79224 | 479027 | 143699 | 121597 |
| K | 89606 | 138879 | 22881 | 882720 | 86835 | 155190 |
| B | 95109 | 54142 | 35927 | 72639 | 83813 | 133910 |
| P | 7399 | 8615 | 6434 | 33689 | 7568 | 9163 |
| Ca | 279893 | 121911 | 455703 | 213657 | 334430 | 168640 |
| Mg | 168491 | 68883 | 118968 | 78648 | 202059 | 98071 |
| Fe | 384075 | 174308 | 245163 | 258688 | 446416 | 250982 |
| Zn | 657 | 324 | 734 | 471 | 830 | 439 |
| Mn | 7355 | 2909 | 3358 | 3942 | 9424 | 4325 |
| Cu | 4.723 | 4.921 | <LOD | <LOD | 7.189 | 4.165 |
| Ni | 140 | 89 | 82 | 96 | 593 | 67 |
| Mo | <LOD | <LOD | <LOD | <LOD | 10.3 | <LOD |
| W | 0.357 | 0.176 | 0.197 | 0.155 | 0.172 | 0.181 |
| condensate | | | | | | |
| pH | 2.29 | 2.19 | 2.54 | 2.03 | 1.05 | 2.03 |
| Cl (ppm) | 9.81 | 10.77 | 5.23 | 4.38 | 10.42 | 1.15 |
| Na (ppb) | 5427 | 128 | 798 | 14.9 | 50.7 | 3082 |
| K | 15787 | 45.5 | 2317 | 22.6 | 37.6 | 8399 |
| B | 2635 | 84.4 | 1092 | 185 | 215 | 4296 |
| P | 18.0 | 5.2 | 11.8 | 2.0 | 6.6 | 4.3 |
| Ca | 567 | 219 | 424 | 30.0 | 90.0 | 289 |
| Mg | 141.0 | 48.7 | 139 | 2.483 | 15.5 | 24.5 |
| Fe | 760 | 216 | 799 | 10.7 | 155 | 99.4 |
| Zn | 19.0 | 3.4 | 12.8 | 6.0 | 6.9 | 10.8 |
| Mn | 9.0 | 2.3 | 7.0 | 0.1 | 1.9 | 2.3 |
| Cu | 3.08 | 0.59 | 1.97 | 0.15 | 0.82 | 0.39 |
| Ni | 16.2 | 0.4 | 9.2 | 0.2 | 1.3 | 0.7 |
| Mo | 0.046 | 0.014 | 0.044 | 0.002 | 0.013 | 0.028 |
| W | 0.006 | 0.009 | 0.003 | 0.006 | 0.067 | 0.002 |

The main reason why the vapor-dominated geothermal fields have not usually been considered as suitable hatcheries for the early life is that the pools and puddles in such fields are highly acidic (with pH values reaching −0.5, see Table S2.3.) and thus inhospitable to life. The reason for the high acidity is the discharge of large amounts of H2S, which is promptly oxidized by atmospheric oxygen to strong sulfuric acid. However, in the absence of oxygen on the primordial Earth, the geochemistry of the geothermal fields must have been quite different. The pH of the vapor condensate must have been neutral or slightly alkaline; H2S and CO2 ascending with the vapor are weak acids, and their acidity must have been balanced by the interaction with basic rocks and concurrent ascending NH3.

Furthermore, at neutral pH, silica must have precipitated around the thermal springs not as mud as today [151,152], but as porous silicate minerals such as sinter and clays, in analogy to today's near-neutral hot springs [305].

Concurrent studies at several sites around the world have revealed the similarity of finger-like (digitate) sinter deposits around thermal springs [306]. Fig. S2.9 shows a sample of such a deposit from the Lower Te Kopia thermal stream in New Zealand at three magnifications, documenting the porosity of the sinter precipitates. These pores are usually inhabited by microbes, see [306-310]. It is tempting to think that similar porous sinter deposits might have formed in Hadean.

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Fig. S2.9. Digitate sinter deposits from the Lower Te Kopia thermal stream at three magnifications. Three images from [306] are combined. A, a digitate sinter sample, the red box indicates the piece that was studied at higher magnification; B, C, secondary electron (SE) images of freshly broken digitate sinter; B, alternating layers of solid silica (ss) and more porous granular silica with scattered kaolinite (k) crystals; C, granular silica (g) at higher magnification.

Notably, sinter deposits evolve with time, as scrutinized by Lynne and her colleagues in [307]. Particularly remarkable is the conversion of nanospheres, via an intermediate stage of aligned nanospheres (Fig. S2.10A), into sharp, 1D blades (Fig. S2.10B).

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Fig. S2.10. Evolution of sinter deposits at Roosevelt Hot Springs, New Zealand. For details see [307] where the images are taken from. A, Oriented rows of sub-aligned nanospheres (<125 nm in diameter), B, variable oriented blades as developed from aligned nanospheres.

From these data, we have earlier proposed that the first cells may have emerged in pools of cold geothermal condensate. In the absence of atmospheric oxygen, the inorganic chemistry of these pools must have been similar to that of cellular cytoplasm, see [81,152] for details.

In the same papers, we have explicitly noted that the compounds with specific affinity for geothermal vapor are otherwise considered to be either the building blocks for abiogenic syntheses of the first biomolecules (H2S, NH3, simple organics) or the catalysts of these syntheses such as borate (see Table S2.3. and [81,152]. Based on this correlation, we speculated that anoxic geothermal fields may have served as the cradles of life itself, with geothermal pools sheltering and nourishing the first, pre-cellular life forms until they evolved into the first cells [81,152].

The evolution of life from the very first self-copying molecules to the stage of protocells in the same habitats is the most parsimonious scenario: otherwise one would have to imagine separate mechanisms for the transfer of the first, still vulnerable, pre-cellular organisms from elsewhere to the geothermal fields and for their accommodation in new habitats.

Our work [81,152] had prompted geologists to look for vestiges of anoxic geothermal fields. Van Kranendonk and his colleagues have discovered them in the 3.48 Ga old Dresser Formation of the Pilbara Craton, Western Australia [311-314], i.e. in the same location where the oldest evidence of life on Earth had been found earlier [315-319]. Analysis of the Dresser Formation deposits revealed the remnants of hot springs surrounded by sinter terracettes. The mineral assemblage includes geyserite, kaolinite/illite, and borate-bearing tourmaline, see Fig. S2.11. [311,313,314]. The stromatolites, made by microbial communities dwelling in basins of these geothermal fields 3.48 Ga ago, are characterized by alternating layers of zinc and nickel, as revealed by Qualitative Synchrotron Radiation X-ray Fluorescence Microscopy [312,314]. These groundbreaking findings indicate that the anoxic geothermal fields existed as early as 3.48 Ga ago and, most likely, were inhabited.

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Fig. S2.11. Schematic representation of the Dresser hot spring system (from [314]).

The on-land geothermal systems have been repeatedly proposed as potential habitats for early life [54-57,91,133,151,222,320-326]. We contributed to this line of thought by recognizing (i) that the vapor zones of Earth's geothermal fields accumulate exactly the chemicals that must have been needed for the first organisms, and (ii) that the K+-rich condensate of geothermal vapor, resembling the cell cytoplasm chemistry and fundamentally different from both salty geyser discharges and seawater, may have been the medium hosting the first organisms [81,152].

**2.2.9. Paradox of the high Mg2+ to Ca2+ ratio in the cell**

Paradoxically, the intracellular concentration of free Ca2+ ions is typically 105 times lower than the Mg2+ concentration (~ 10-7 M vs. ~ 10-2 M), although their concentrations outside the cells are usually comparable (Table S2.1.). Magnesium makes about 20% of the Earth’s core; not surprisingly, many enzymes attributed to the LUCA use Mg2+ ions as cofactors (see Table S2.2.). Intracellular Mg levels do not differ dramatically from those in the cell environment (Table S2.1.), so maintaining physiological levels of Mg2+ is not very costly. In contrast, pumping the in-leaking Ca2+ ions out of the cell costs energy.

The chemistry conservation principle implies that low intracellular Ca2+ levels may reflect the environmental conditions under which the very first cells may have formed. Why, then, were Ca2+ levels in the habitats of the first cells ~104 times lower than in today’s environments?

A possible solution to this [Mg2+]/[Ca2+] paradox can be seen in the recent data of Mustaev and his colleagues [327]. They investigated whether the involvement of the Mg2+ ion as a cofactor in the thirteen evolutionarily ancient RNA and DNA processing enzymes is related to some specific property of Mg2+ ions – or simply to their availability in the environment in which these enzymes originated. To answer this question, the authors measured the activities of these 13 enzymes in the presence of Mg2+, Mn2+, Co2+, Zn2+, Cu2+, Ni2+, Cd2+, Ca2+, and Fe2+, respectively. Each of the enzymes studied could be activated by one or more cations other than Mg2+. The authors then evaluated the solubility of all these divalent cations (at 10 mM) in the presence of common anions such as phosphate (PO43-) and carbonate (CO32-), taken at concentrations of 20 mM and 10 mM, respectively, as the authors anticipated for primordial environments. In these experiments, only the Mg2+ salts remained soluble. The authors concluded that all of these enzymes use Mg2+ as a cofactor because it was the only divalent cation that remained soluble at high primordial carbonate and/or phosphate levels.

It is noteworthy that Ca2+ ions precipitated both phosphate and carbonate ions in the experiments of Mustaev and his colleagues. Based on their data, it is tempting to suggest that the current intracellular concentration of Ca2+ corresponds to the concentration of free Ca2+ ions in the habitats of the first cells. These concentrations must have been much lower than today because of higher concentrations of natural Ca2+-precipitating anions, especially phosphate and water-dissolved (bi)carbonate in equilibrium with the CO2-rich atmosphere. Remarkably, the difference in solubility of Mg and Ca carbonates is exploited in industry when mixed mine wastewater is treated with pressurized CO2 to separate soluble Mg-containing brine from insoluble CaCO3 [328].

From the data of Mustaev and his colleagues, the concentration of bicarbonate in the habitats of the first cells can be estimated in the order of tens of millimoles, i.e. at least 10-100 times higher than in today’s water basins.

**S2.10. Abundance of Zn2+ ions and absence of Fe2+ ions in the evolutionary oldest enzymes (the zinc/iron paradox).**

Many proteins use zinc ions as cofactors. Zn2+ ions can serve both as catalytic cofactors and as structural elements that stabilize the protein folds by linking several amino acid residues [329]. Zn seems to be the only metal whose ions are routinely used by proteins in just such a structural role [330,331]. Furthermore, the ubiquitous proteins common to all free-living cellular organisms and thought to be present in the LUCA mostly use Zn as a transition metal cofactor, see Table S2.2. and [152]. In addition, Zn correlates with the oldest protein folds and is the most abundant transition metal found in the RNA structures, see [152,277,331,332] and references therein.

The total concentration of Zn2+ ions in modern cells is about 2-4x10-4 M, many orders of magnitude higher than in the environment (Table S2.1.). The accumulation of Zn2+ ions inside the cells demands sophisticated ion pumps, ion-tight membranes, and zinc-storing proteins (metallothioneins) [333-335]. Since the first cells were unlikely to have all these gears, they could only recruit Zn2+ ions if they lived in a Zn-rich environment. The recruitment of Zn2+ ions as mere structural elements(!) indicates the high abundance of Zn2+ ions around the first organisms.

However, this inferred abundance of Zn2+ ions in primordial environments conflicts with the poor solubility of common zinc salts (see also the previous Section S2.9. and [327]). Accordingly, the concentration of free Zn2 in primordial anoxic waters has been estimated to be as low as ≤ 10-12 M [260,261]. How, then, could the first cells have gained access to the Zn2+ ions?

A natural process involving the continuous release of Zn2+ ions is one possibility. In this case, biopolymers may have had a chance to capture Zn2+ ions before they precipitated as inorganic salts, such as ZnS (sphalerite) or ZnCO3 (smithsonite). Consequently, we have attributed the steady release of Zn2+ ions to the photochemical properties of primordial geothermal zinc sulfide (ZnS) precipitates [80,277,332,336]. Crystals of ZnS are semiconductors, in which UV light causes a separation of electric charges. The resulting charge separated states can store the energy of the absorbed light for hours. This unique property manifests itself in phosphorescence (afterglow), so that ZnS – widely known as "phosphor" – is used in numerous devices, from various types of displays to 'glow-in-the-dark' toys [337-339]. By accumulating two or more charge separated states, natural ZnS crystals can serve as potent multielectron reducing agents with redox potentials below –1.0 V [77,78]. In particular, illuminated nanocrystals of ZnS, which exhibit the properties of quantum dots (QD) [339,340], can reduce CO2 to formate with quantum efficiency of up to 80% [341-346], see Fig. S2.12. This efficiency is higher than that of the chlorophyll-based photosynthesis of green plants.

In general, ZnS is considered to be the most potent photocatalyst of natural origin. Similar but weaker photochemical activity is inherent in MnS and CdS crystals, as well as in zinc oxide, ZnO [77,78,347]. Of these compounds, CdS crystals are the least biologically relevant because Cd occurs in nature only as a minor admixture to Zn.

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Fig. S2.12. **Primeval ZnS-mediated photosynthesis in sub-aerial, illuminated settings (**from [80], modified). **Right**: Diverse nanoparticles, including particles of ZnS, in a Hadean geothermal pool. **Left**: A schematic presentation of reactions within a photosynthesizing ZnS nanoparticle, as combined with an energy diagram. Initially the absorption of a UV quantum leads to the separation of electric charges. The electrons migrate within the crystal until they are trapped at the surface; the trapped electrons can reduce a CO2 molecule either via two one-electron transfers [342] or, possibly, in a concerted two-electron reaction. The remaining electron vacancy (hole) is initially reduced by the S2- ion of the crystal; however, the ultimate electron equilibration requires external electron donors, e.g. H2S.

Notably, ZnS-, MnS- and CdS-mediated photochemical reduction is accompanied by the disruption of photosynthesizing crystals and the release of Zn2+, Mn2+, and Cd2+ ions, respectively, see Figure S2.12 and [342,348]. With this property, we have explained the exclusive recruitment of Zn2+ and Mn2+ ions by the first proteins – assuming that the habitats of their hosts contained Mn and Zn sulfides and were accessible to the UV-rich radiation of the young Sun (see Section S2.6.). We have called this early Zn-dependent step in the evolution of life the 'Zinc World', whose past existence is evidenced by the almost exclusive dependence of the evolutionarily oldest proteins on zinc [80,152,277,332,336].

However, the geochemistry of these primordial ZnS-rich habitats has remained obscure. In nature, large amounts of ZnS, along with other metal sulfides, are found only at the sites of current or ancient geothermal activity, where the leached metals are/were brought to the surface by very hot geothermal fluids. It has remained unclear whether such ZnS-rich systems existed en masse during the Hadean.

We were also unable to explain convincingly why the evolutionarily oldest proteins avoid using Fe2+ ions as cofactors (Table S2.2). The absence of Fe2+ ions in such proteins is all the more paradoxical because the Earth’s crust and mantle contain a thousand times more iron than zinc. Geothermal settings, although they accumulate more ZnS and MnS compared to the unaltered rock, are always dominated by Fe2+ ions which are also prevalent in geothermal vapor, see Table S2.3.

Furthermore, the sulfides, phosphates and carbonates of divalent iron are moderately soluble. Consequently, the equilibrium concentration of Fe2+ in the primordial anoxic waters has been estimated to be as high as 10-5 M, compared to estimates of ≤ 10-12 M for Zn2+ [260,261].

And yet, the found absence of Fe2+ ions in the evolutionarily oldest proteins [152,277,332] got support from the data of David and Alm on the delayed recruitment of iron as an enzyme cofactor. These authors “mapped the evolutionary history of 3,983 gene families across the three domains of life onto a geological timeline” [349]. One of their findings was that the mass appearance of Fe2+-containing enzymes occurred about 100-200 million years after the appearance of the very first enzymes. To these first enzymes, David and Alm assigned the ubiquitous nucleotide- and phosphate-processing enzymes, many of which are zinc-dependent, see Table S2.2.

A tentative solution to the Zn/Fe paradox is provided by the scenario proposed in Section 3. The scenario clarifies (i) how the primordial Earth became covered by Zn-enriched protocrust, (ii) why and how the evolutionarily oldest proteins predominantly recruited Zn2+ as a transition metal cofactor, and (iii) why the recruitment of Fe2+ ions by enzymes may have occurred with a delay of about 100 million years as evidenced in [349].

**2.2.11. The Phosphate Paradox**

The total concentration of phosphate in cells is on the order of 10 mM (Table 1). Since phosphate groups serve as linkers in RNA and DNA, the formation of the first RNA-like polymers, which are thought to have preceded the origin of cells (see Sections S1.3 and S2.6.), must have occurred in phosphate-rich habitats. However, the concentration of phosphate ions in natural waters such as lakes or oceans rarely exceeds 1 µM because phosphate is precipitated by most divalent metals (see Table S2.1., section S2.9. and [327]). Accordingly, modern organisms invest energy and use specific membrane transporters to accumulate phosphate in their cells.

Thus, any origin of life scenario must provide a plausible and abundant source of soluble phosphorus compounds for the first, not yet sophisticated organisms.

As early as 1955, Gulick argued that reduced phosphorus species such as hypophosphite (PO23-) and/or phosphite (PO33-), which are ~1000 times more soluble than phosphate (PO43-), could have been abundant under primordial reduced conditions [350]; this line of thought was further developed in the following years [351-356]. The shortcoming of the original hypothesis was the lack of a clear source for these reduced phosphorus compounds. Both hypophosphite and phosphite have very low redox potentials, well below the low-potential stability limit of water (see Fig. S2.1, S2.13). Therefore, if dissolved in water, these compounds must have been oxidized by water protons, even in the absence of atmospheric oxygen. Consequently, some origin of life scenarios assumed that reduced phosphorus could have resided in the solid state as a constituent of the relevant minerals and mobilized - as a substrate for primordial reactions - when these minerals were dissolved, e.g. by rainwater, see e.g. [190].

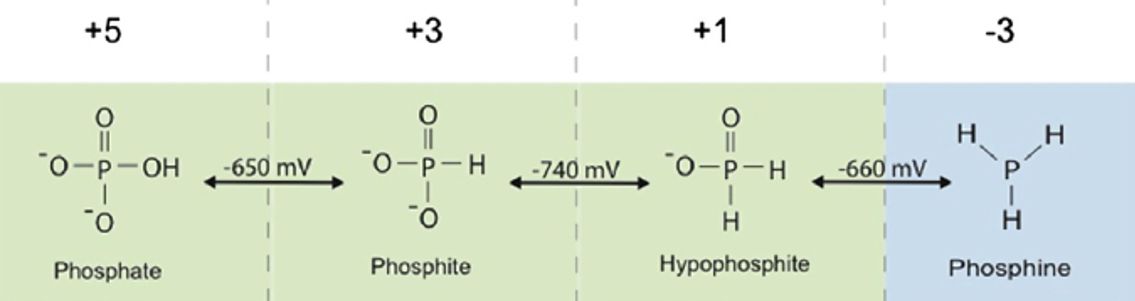


Fig. S2.13. Chemical structures of phosphorus compounds and their redox transformations (modified from [357]). Numbers above each compound indicate the oxidation state of phosphorus. The numbers over the arrows indicate the E07 values or respective redox reactions.

A thinkable possibility is a continuous supply of reduced species of phosphorus by geothermal systems. The phosphorus content of geothermal vapor is, generally, relatively high, see Table S2.3 and [118,302]. The redox state of such geothermally delivered phosphorus was first determined by Foster and her colleagues for the pristine geothermal pool at Hot Creek Gorge near Mammoth Lakes, CA, which the authors considered “representative of early Earth” [358]. The measurement revealed almost equal amounts of phosphite and phosphate, albeit at low levels of ~ 0,05 µM. The pool at Hot Creek Gorge was described as bicarbonate-rich [358], implying a contribution from geothermal vapor. The parity of phosphite and phosphate amounts [358,359] may be due to the state of phosphorus in a hot geothermal vapor being (PO2)n [360-362]. Hydrolysis of such phosphorus species by water yields a mixture of hypophosphite, phosphite and phosphate. Apparently, the redox conditions in the vapor zone beneath Hot Creek Gorge are still sufficiently reducing to maintain phosphorus in a partially reduced state. Later, reduced species of phosphorus of unclarified origin were also discovered in other environmental samples [355,363].

The discovery of highly soluble phosphite in the contemporary environment justifies the existence of enzymes capable of hypophosphite and phosphite oxidation in many prokaryotes [364].

Since well-soluble reduced phosphorus species are still supplied at sites of today’s geothermal activity, their supply by geothermal/volcanic systems of the anoxic primordial Earth seems quite plausible. Further support for this view is provided in Section 3 of the main text.

**2.2.12. Faint young Sun paradox**

We conclude by considering the faint young Sun paradox. While some of the paradoxes considered above have not been formulated as such before, the paradox of the faint young Sun is well recognized, see e.g. [365,366] for recent reviews. As early as 1958, by applying the physical principles governing the structure and evolution of other stars to our Sun, Hoyle showed that the luminosity of Sun must have changed over time, with the young Sun being considerably less luminous than today [365,367]. The consequences for life on Earth were recognized by Sagan and Mullen in 1972, who noted that at Sun's luminosity of 60-70% of today's the average temperature at the Earth's surface must have been about -25°C, well below the freezing point of water (at today's atmospheric pressure, see [368]). Assuming that "liquid water is almost certainly necessary for the origin of life" [368], these authors considered possible means to keep surface temperatures high enough to maintain water in a liquid state. As a solution, they proposed a greenhouse atmosphere with ammonia as the dominant greenhouse gas.

While the analysis by Sagan and Mullen was limited to modeling the Earth's surface temperature as a function of atmospheric composition and solar luminosity [368], a more sophisticated but less well known model by Moroz and Mukhin, while addressing the same problem, considered the Earth’s atmosphere in its interaction with geological processes [323,324]. In the following, we will take a closer look at this modeling, as we will use its results for the evolutionary scenario as presented in Section 3 of the main text.

Moroz and Mukhin assumed an initially dry Earth with an atmospheric pressure of 10-3 atm. With such a thin atmosphere, the Earth's surface temperature was determined by the radiation from the faint young Sun. For these conditions, average surface temperatures around -50°C were calculated, in agreement with earlier estimates by Sagan and Mullen [368]. The temperature must have gradually risen due to the warming of the Sun and the greenhouse effect of the atmosphere built up through volcanic outgassing. The authors argued that ammonia, originally proposed by Sagan and Mullen as a primordial greenhouse gas, could not perform this function because of its photolability in the UV range. Instead, they proposed water vapor and CO2, the main photostable products of volcanic outgassing, as greenhouse factors.

Moroz and Mukhin proposed that CO2 must have accumulated in the atmosphere, increasing the greenhouse effect (Fig. S2.14), while the water vapor would have initially frozen as ice and snow and, by increasing the Earth's albedo, must have retarded the warming of the Earth. The frozen water could not have contributed to the buildup of atmospheric pressure.

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Fig. S2.14. Mean temperature TS of the Earth’s surface as a function of the CO2 abundance in the atmosphere at three realistic albedo values A, as calculated in [324]. The dashed line indicates 273K.

Moroz and Mukhin assumed that the greenhouse effect from CO2 accumulation in the atmosphere must have eventually enabled the melting of the frozen water. The liquid water might have trapped the dissolved CO2 through the process of so-called chemical weathering. Dissolved in water, CO2 gets converted to carbonic acid (HCO3– or CO32–, depending on the pH), which leaches out of the rock lining the water basins. The released metal cations, mainly Ca2+ ions, interact with (bi)carbonate ions, which leads to the precipitation of insoluble carbonates and thus to the sequestration of CO2. However, chemical weathering must have continued only until the CO2 concentration dropped to the point where water froze. As a result, after its initial rise, atmospheric CO2 must have been maintained at a constant level that provided average global temperatures around the melting point of water. Moroz and Mukhin suggested that such a negative feedback loop must have slowed down both the rise in atmospheric pressure and the development of the greenhouse, which implied a rather mild, if not frosty, climate throughout the Hadean [323,324].

Moroz and Mukhin calculated that the average temperature must not have exceeded the melting point of water until solar luminosity reached about 0.8 of its present value, about 4 Gy ago. The authors predicted that even after this point, the emergent life, by consuming CO2, could interfere with the constantly operating CO2 feedback loop and cause further glaciation episodes. Evidence for such glaciation periods was later found, see [369] for a recent review.

Notably, Moroz and Mukhin were the first to identify the CO2 feedback loop as an important climate factor; similar ideas were later popularized by Walker and his colleagues [370]. This feedback loop still maintains the Earth's mild climate and is considered the key element in current climate models.

It is regrettable that the papers of Moroz and Mukhin did not receive the attention they deserved when published. Moroz and Mukhin did not specifically promote their model, as they were busy organizing the exploration of Venus by the Soviet "Venera" landers in the 70s and 80s [371-373]. Still, the unsurpassed success of that program, in which eight "Veneras" landed safely and sent back data and images despite temperatures of ~500°C and pressures of ~100 bar, proves the expertise of Moroz and Mukhin in dealing with planetary atmospheres.

Moroz and Mukhin did not consider the presence of a liquid ocean as a prerequisite for the origin of life because they favored the origin of life around dryland volcanic systems [54,55,323,324]. Accordingly, it was acceptable to them that the average temperature on the young Earth could initially have been well below the freezing point of water.

As argued above in Sections S2.1 and S2.4., liquid water in large amounts was the last thing needed for the emergence of life. Therefore, from the viewpoint of the origin of life at terrestrial volcanic/geothermal systems, which we share, the faintness of the young Sun must have even promoted the emergence of the first organisms by eliminating liquid water through its freezing. Thus, we see no reason to regard the well-publicized "faint young sun paradox" as a paradox.

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1. ΔG = ΔH - TΔS [↑](#footnote-ref-1)
2. As Fiore and Strazewski pointed out, “we should refrain from intuitively assuming that there is only “one way to RomeNA”, and we are certain that with further progress in prebiotic systems chemistry…organic chemists are looking into a brighter future than we have ever been able to imagine” 232. Fiore, M.; Strazewski, P. Bringing Prebiotic Nucleosides and Nucleotides Down to Earth. *Angew Chem Int Ed Engl* **2016**, *55*, 13930-13933, doi:10.1002/anie.201606232.. [↑](#footnote-ref-2)
3. Macallum wrote insightfully in this regard: "...the very earliest organisms must have been of the micellar or ultramicroscopic kind... These had as yet no nuclei, and an enclosing membrane could only have been of the most elementary character". "...as the cell is older than its media as now constituted, the relative proportions of the inorganic elements found in it are of more ancient origin than the relative proportions of the same elements found in the media, blood plasmas, and lymph..." 274. Macallum, A.B. The paleochemistry of the body fluids and tissues. *Physiol. Rev.* **1926**, *6*, 316-357.. [↑](#footnote-ref-3)