Origins of Life: The *Garakuta World*

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Abstract

Since the 1920s, a large number of studies have been conducted, and most monomers found in proteins and nucleic acids have been synthesized abiotically and/or detected in extraterrestrial bodies. However, the question of how the first life arose from non-living matter remains controversial. One popular theory is the RNA world hypothesis, but it still faces challenges in explaining how the first functional oligonucleotide formed abiotically.

Alternative hypotheses propose that life may have arisen from simpler, less sophisticated molecules rather than complex ones like proteins or RNA. For instance, when a mixture of simple molecules (e.g., CO, N_2, and H_2O) is irradiated with high-energy particles, macromolecular organic compounds can form. These compounds, though containing some amino acid moieties, are largely uncharacterizable and have been referred to as "*Garakuta molecules*." Interestingly, *Garakuta molecules* exhibit some functional properties, such as esterase activity. This has led to the proposal of the *Garakuta world* hypothesis, suggesting that life could have emerged from these *Garakuta molecules*.

Key words

Origins of Life, the Garakuta World, Radiation, Amino Acid Precursors, Catalytic Activity

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1. Introduction

In 1924, Oparin published his first book on the origin of life in Moscow [1]. Haldane presented a similar idea in 1928 [2]. Both proposed that life on Earth arose through the evolution of organic compounds, known as the chemical evolution hypothesis. Experimental verification of this hypothesis began in the 1950s. Miller [3] showed that amino acids could be formed abiotically by spark discharges (simulating lightning) when strongly reducing gas mixtures containing methane and ammonia were used. However, it was later estimated that the early Earth's atmosphere was not strongly reducing, but only weakly so. Nevertheless, amino acids could still have formed, considering the energy from cosmic rays and solar energetic particles [4,5].

In addition to endogenously formed amino acids, the extraterrestrial delivery of amino acids is also a possibility. Various amino acids have been identified in carbonaceous chondrites [6]. Research has shown that amino acids can form in simulated extraterrestrial environments, such as interstellar dust in molecular clouds [7] and the interiors of meteorite parent bodies [8].

Nucleic acids (which are polymers of nucleotides), alongside proteins, are among the most important bioorganic compounds. Nucleotides consist of nucleobases, sugars, and phosphate. Both nucleobases and sugars have been synthesized abiotically [9,10] and found in carbonaceous chondrites [11,12].

Thus, we can conclude that most monomers of biological significance were available in early Earth environments. However, a long process was required to transition from these monomers to "life."

2. The RNA World

In the current terrestrial life system, proteins serve as catalysts (enzymes) to maintain metabolism, while nucleic acids (DNA and RNA) are self-replicating molecules that hold biological information. It is assumed that RNA formed prior to DNA, raising the significant question of whether proteins or RNA were the first biomolecules.

Since the 1980s, ribozymes—RNAs with catalytic activity—have been discovered [13]. Following these discoveries, Gilbert [14] proposed an origin-of-life hypothesis known as the RNA world hypothesis: In the very beginning of the terrestrial life system, RNA served as both a catalytic and self-replicating molecule, independent of proteins. Over time, this RNA world could evolve into a world where proteins took on catalytic roles (the RNP world), followed by the emergence of DNA, which stores biological information more securely (the DNP world).

The RNA world hypothesis has garnered considerable support from molecular biologists, but it has notable limitations. One of the largest issues is the abiotic formation of the first RNA, which appears to be quite difficult. The initial step is the abiotic synthesis of ribonucleotides. As previously mentioned, the prebiotic formation of the organic components of ribonucleotides (the four nucleobases and ribose) might be feasible. However, achieving the "right connection" of a nucleobase, ribose, and phosphate is challenging due to the many isomers of nucleosides. For example, canonical uridine has non-canonical isomers such as α-uridine and α- and β-pseudouridine, while canonical 5'-uridine (UMP) has isomers like 2'-, 3'-UMP and 2', 3'-cyclic UMP. Powner et al. [15] demonstrated an alternative method for synthesizing canonical cytidylic acid using only prebiotically available small organic molecules like cyanamide. However, this method is considered less "prebiotic" because (i) it requires a very high concentration of each starting material, and (ii) each reaction step must occur under precisely controlled and varying conditions after purifying the starting molecules.

Even if canonical nucleotides could be formed, the next challenge is that only activated nucleotides (e.g., nucleoside phosphoimidazolide [16]) are suitable for polymerization. It is uncertain whether such activated nucleotides could form abiotically. If they do arise, it may be possible to condense them with the help of metal ions, clays, and/or complementary templates [17].

The formation of oligonucleotides is not the ultimate goal of the RNA world. The final and most significant hurdle is the creation of nucleotides with self-replicating activity.

Totani [18] calculated the likelihood of functional RNAs forming, concluding that one planet might host such RNAs for every 10^{40} stars. This scenario appears quite rare in our observable universe, which contains only about 10^{23} stars.

3. Alternative Scenarios for the Origins of Life

The scenario that proteins were the first biological molecules has been proposed by a number of investigators. For example, Ikehara [19] proposed that the first life was generated from proteins composed of four amino acids: glycine, alanine, aspartic acid, and valine. This concept is known as the GADV-protein world hypothesis. The merit of this hypothesis lies in the ease of abiotic synthesis of these four amino acids and the relative simplicity of amino acid condensation compared to that of nucleotides. However, such protein world hypotheses have been criticized for their lack of self-replication ability.

Proteins and RNAs are sophisticated biomolecules, and their prebiotic formation was quite challenging. As a result, several scenarios for the origins of life have been proposed, where simpler materials played roles in catalysis and/or self-replication. Cairns-Smith [20] proposed that the self-replication of clay minerals was the first step toward "life," which would eventually lead to organic life. Wächtershäuser [21] presented his idea that the first metabolism—a cycle of chemical reactions—started on iron sulfide minerals.

Calvin [22] explained his view on the evolution of biocatalysts: ferric ion (Fe^{3+}) has peroxidase activity (decomposition of H_2O_2), but this activity is extremely low (10^{-5} mL^{-1} s^{-1}). If porphyrin coordinates with it to form heme, its catalytic activity is enhanced by 1,000 times. When a protein molecule is attached to the porphyrin, forming a modern catalase molecule, the activity increases further by 10^7 times. Thus, we do not have to wait for the first primitive catalytic molecules until the formation of canonical proteins or RNAs.

Dyson published the second edition of "Origins of Life" [23] in 1999, where he introduced the Garbage Bag World hypothesis. He noted that the formation of sophisticated biomolecules is quite difficult, but it is plausible that a random collection of prebiotically formed organic molecules could be contained in a sort of "bag." Such a "garbage bag" could gradually evolve into a more efficient system. The first life did not emerge from sophisticated organic molecules.

4. Formation and merits of *Garakuta Molecules*

It has been reported that amino acids were formed in both terrestrial and extraterrestrial environments. In Miller's spark discharge experiments, he found that HCN and HCHO were formed prior to the synthesis of amino acids. He concluded that amino acids were generated through the Strecker synthesis [24]. The Strecker synthesis is a well-known reaction for synthesizing α-amino acids, involving hydrogen cyanide, aldehydes, and ammonia to yield aminonitriles (Fig. 1(a)). Amino nitriles are hydrolyzed to produce amino acids, making them precursors to amino acids.

The Strecker synthesis is so widely recognized that many researchers assume that the abiotic α-amino acids found in carbonaceous chondrites [6] and carbonaceous asteroids [25] were formed via the Strecker synthesis. However, it is important to note that

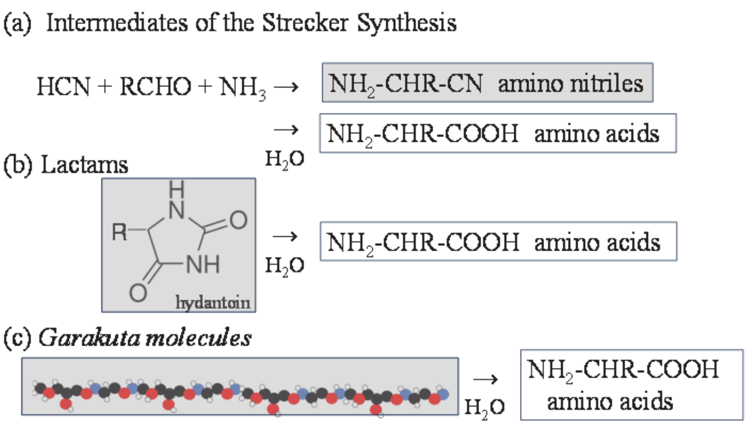

Fig. 1. Amino acid precursors.

aminonitriles were not identified in the reaction products or in extracts from carbonaceous chondrites.

In carbonaceous chondrites, lactams (cyclic amide compounds; e.g., hydantoin, Fig. 1(b)) have been detected. Since lactams can often yield amino acids, they may be candidates for abiotic amino acid precursors [26].

We analyzed amino acids and their precursors formed in experiments simulating early Earth and extraterrestrial environments. When a mixture of CO, N_2, and H_2O was irradiated with high-energy protons, various amino acids were identified in the hydrolysates of the products, while only traces of amino acids were detected in the unhydrolyzed samples [27]. This indicates that not free amino acids, but rather amino acid precursors, were formed during the irradiation experiments.

When a mixture of CO, CH_4, and N_2 (10:10:80 v/v) was irradiated with protons, various amino acids were also detected in the hydrolysate. The unhydrolyzed sample was analyzed using gel filtration HPLC to estimate its molecular weights, revealing peaks at 2300, 4400, and 6000 daltons [28]. This suggests that macromolecular amino acid precursors were directly formed by the irradiation. These macromolecules were not peptides, as only a small percentage of the original molecules were converted to amino acids after acid hydrolysis. This implies that while the macromolecules contain amino acid segments, the majority of their structure consists of non-amino acid moieties. We refer to these as *Garakuta molecules* (Fig. 1(c)). "Garakuta" is a Japanese term that translates to a type of garbage, yet it implies potential usefulness. Indeed, a *garakuta-ichi* (flea market) can be a treasure trove, where valuable items can be discovered among eclectic offerings.

When a gas mixture of CO, NH_3, and H_2O was irradiated with high-energy protons, complex molecules containing amino acid precursors were formed. We refer to this mixture as CAW, which serves as an analog for interstellar complex organics. The stability of CAW as an amino acid precursor under various energy exposures (e.g., UV light and radiation) was compared with that of free amino acids and simple amino acid precursors like hydantoins and aminonitriles [29]. Results showed that *Garakuta molecules* were more robust than free amino acids and simple amino acid precursors during delivery to Earth.

When CAW was irradiated with circularly polarized UV light (CP-UVL), the alanine formed after hydrolysis of the irradiated product showed an enantiomeric excess, though the overall alanine yield remained unchanged [30]. It has often been suggested that enantiomeric excesses induced by CP-UVL could result from the asymmetric decomposition of free amino acids in space. However, our findings suggest that *Garakuta molecules* may harbor seeds of homochirality through UV-induced alteration rather than UV decomposition.

Some proteinogenic amino acids, such as histidine and tryptophan, have complex structures, making it challenging to explain their prebiotic formation. Recently, Kuroda and Kobayashi proposed a possible formation mechanism for these complex amino acids, considering the presence of *Garakuta molecules* in planetary environments [31].

As shown above, *Garakuta molecules* offer multiple advantages in chemical evolution pathways. In the next chapter, I will discuss the functional role of *Garakuta molecules* in more detail.

5. Catalytic Activity of *Garakuta Molecules*

We examined the function of such *Garakuta molecules*. The target molecules were synthesized by irradiation of a mixture of CO (350 Torr), N_2 (350 Torr) and H_2O (20 Torr) with 3 MeV protons. The product exhibited esterase activity (catalytic activity to hydrolyze fluorescein diacetate to fluorescein). When the product was hydrolyzed in 1 mM HCl at 110°C, the activity increased by 30% during the initial 20 minutes and then decreased to 60% [32,33]. It is known that small molecules such as imidazole have esterase activity. Then the product was fractionated by HPLC (column: TSK Gel SP-2SW) (Table 1). Imidazole appeared in fraction G (25-30 min), and this fraction accounted for 26% of the total activity. The fraction B (3-6 min) showed 45% of the

Table 1.　Esterase activity found in the product of proton irradiation of a mixture of CO, N_2 and H_2O after fractuibatuib by HPLC (Ref. 32 with the modification)

Fraction name	retention time (min)	Activity (A)* (relative value)	TOC** (mmol)	A/TOC ($mmol^{-1}$)	Note
A	1 — 3	6.6	1.26	5.2	
B	3 — 6	45	7.20	6.3	Near void volume
C	6 — 9	0	5.42	0	
D	9 — 14	6.6	8.97	0.74	
E	14 — 20	11	2.43	4.5	
F	20 — 25	5	2.95	1.7	
G	25 — 30	26	1.95	13.3	Imidazole elutes here

* Relative value (total = 100); ** total organic carbon

total activity, which seemed to be derived from macromolecular compounds [34].

The same product also exhibited phosphatase activity (catalytic activity to hydrolyze 4-methylumbelliferylphosphate to 4-methylumbeliferone) [35, 36]. Hydrolysis of phosphate esters is more challenging than that of carboxylate esters, so small molecules like imidazole cannot perform the former. It has been proposed that detection of phosphatase activity could be used as a tool for detecting life in extraterrestrial environments as well as in extreme terrestrial environments [37]. It has been shown that catalytic *Garakuta molecules* could be easily formed under prebiotic conditions, although their activity was much lower than that of modern enzymes.

6. The *Garakuta World* Hypothesis

In the past 70 years of studies on chemical evolution, it has been hypothesized that evolution proceeds step by step from small molecules to larger ones. As shown in Fig. 2, the first step of protein synthesis is the formation of amino acids through Strecker synthesis, involving hydrogen cyanide, aldehydes, and ammonia. Following the formation of such fundamental monomers as amino acids, nucleobases and sugars, peptides, nucleosides, nucleotides, and oligonucleotides are formed sequentially [38]. These steps involve condensation reactions, which is why many RNA world supporters prefer land springs over oceanic environments (such as submarine hydrothermal vents),

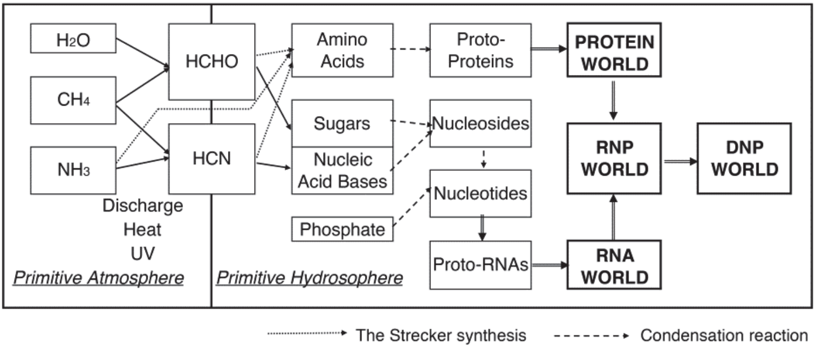

Fig. 2.   Conventional scenario of chemical evolution.

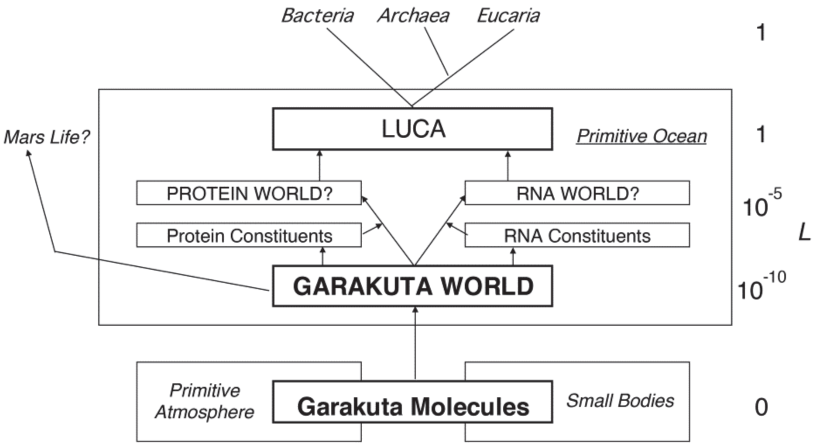

Fig. 3.   The *Garakuta world* hypothesis.

as water is more easily removed in the former [39].

As discussed in Chapters 4 and 5, macromolecules with catalytic activities, including amino acid precursors, could be formed directly from simple molecules. This suggests that there may be another chemical evolution pathway aside from the step-by-step approach.

Fig. 3 illustrates the concept of the *Garakuta world* Hypothesis [40]. *Garakuta molecules*, which are macromolecules including amino acid precursors, are abiotically formed in planetary atmospheres and in space. These molecules accumulate in primitive oceans, where further evolution occurs—particularly in submarine hydrothermal systems. They become encapsulated in globules, and globules containing functional molecules are naturally selected and evolved. Some molecules may exhibit autocatalytic activities, increasing in number and representing a prototype stage of self-replication. Ultimately, self-replicating molecules are formed and selected from a large "library."

All terrestrial organisms share a common life system that began with the appearance of the last universal common ancestor (LUCA). I refer to the present life system as the $L = 1$ system. Prior to LUCA, organisms with more primitive life systems existed, characterized as having $0 < L < 1$. I will refer to the stage of $0 < L << 1$ as the *Garakuta world*. Mere lifeless organic molecules ($L = 0$) are easily decomposed by various energies (such as heat, UV radiation, or other forms), but the *Garakuta world* ($L > 0$) could persist by maintaining itself through autocatalysis and other functions.

Today, Earth is covered with living organisms ($L = 1$), making it quite difficult for organisms with $L < 1$ to survive. However, in a world where only $L = 0.01$ organisms exist, $L \geq 0.01$ organisms could potentially thrive.

Finding evidence for the *Garakuta world* hypothesis (as well as other hypotheses like the RNA world hypothesis) is quite challenging. However, discovering different life systems on extraterrestrial bodies such as Mars [41] and Europa (a Moon of Jupiter) [42] could provide crucial insights. Such findings, along with the discovery of relics of chemical evolution on bodies like Titan (a Moon of Saturn) [43], may help answer the question of how life originated on Earth and elsewhere.

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Competing interests

The author declares no competing interests.

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