The origin of life; the first self-replicating molecules were RNA nucleotides.

K. Ohsaka
Freelancer, CA USA

Abstract

Difficulty to efficiently synthesize RNA nucleotides by joining their subunits in modern labs under simulated prebiotic Earth environments leads us to propose an alternative process by cross complimentary self-replication with help of abiotic catalysts such as minerals which are known to be good catalysts and certainly existed on prebiotic Earth. The process took place in areas with cyclic environmental changes such as tideland where wet & dry cycles repeated due to the rise and fall of the tide. Self-replication of the nucleotides (monomers) and polynucleotides (polymers) may be considered as the origin of evolving life, and also the reason for heredity of RNA. The homochirality of RNA was naturally established during polymerization. Self-replication enabled transfer of molecular information and allowed mutation and natural selection, essential evolutional processes of life.

1. Introduction

Life has been evolving through self-replication, mutation and natural selection processes. Popular thought suggests that life is originated from polymerization of RNA nucleotides, which is corroborated by circumstantial evidence and some experimental results, and is known as the RNA world [1,2]. There are ongoing efforts to synthesize RNA nucleotides with nucleobases adenine (**A** for short), uracil (**U**), guanine (**G**) and cytosine (**C**) in modern labs starting from simple molecular components presumably existed on prebiotic Earth [3-7]. It seems that entire processes leading to synthesis of three molecular subunits of RNA nucleotides, i.e., the nucleobases, ribose sugar (**S)**, and phosphate group (**P**) took place in prebiotic Earth. Alternatively, some intermediate products might be originated from outer space and delivered to Earth. Evidence found in meteorites suggests this possibility [8]. In contrast, the last process, the synthesis of the RNA nucleotides by joining the subunits is turned out to be difficult because they must be joined together with the proper regiospecific and stereospecific configurations and the formation of covalent bonding requires overcoming a high activation energy [9]. Thus, there had to be a process that arranged the subunits and reduced the activation energy for efficient formation of the nucleotides.

Once the concentration of RNA nucleotides had reached a certain level, polymerization took place, and single strand polynucleotides were synthesized on prebiotic Earth. An experiment using an abiotic catalyst under a simulated prebiotic condition shows that single strand polynucleotides can grow up to 50 nucleotide units [10]. The max length is determined by the stability of the polynucleotide that is constantly subjected to dissociation (polymer chain breakup). The max length is well short compared to the length of known short functional RNAs (around 100 units). The dissociation rate linearly increases as the polynucleotide grows in length. In order to grow further, a process that helped stabilization of the polynucleotide had to be operated in prebiotic Earth.

Self-replication is an autocatalytic process and a special property of certain molecules, by which the molecules can catalyze their own formation as a template, thus potentially exponential multiplication is possible. An example of self-replication of molecules is found in literature. It is an amino adenosine triacid ester (AATE) [11] which can copy itself by attracting another ester molecule to its adenosine end, and an amino adenosine molecule to its ester end. These two molecules then react to form another AATE. The self-replicating process works because of a weak bonding, known as hydrogen (H) bonding, which easily breaks up. It is notable that H bonding plays the same role in a complemental self-replication (the self-replication) of DNA. In this paper, the self-replication is distinguished from other syntheses that form the nucleotides by joining the subunits.

Catalysts are widely used to increase yields in modern chemical syntheses. They speed up chemical reactions by reducing activation energies. We think that abiotic catalysts played an essential role for the formation of RNA nucleotides and the polymerization on prebiotic Earth. Clay minerals are known to be good catalysts and certainly existed on prebiotic Earth [12,13]. Their roles in the process were twofold. First, they provided surfaces on which the subunits were selectively adsorbed and immobilized. This reaction consequently increased the concentration of the subunits on the surface and allowed the subunits to join each other more frequently. Secondly, they strained and aligned the subunits and nucleotides in proper orientations for formation of a transient covalent bonding so that particular reactions became easier due to reduction of the activation energy.

There is an interesting hypothesis that extant RNA and DNA are products of prebiotic evolution in self-replicating polynucleotides [14,15]. We think that this hypothesis is also applicable for the formation of prebiotic nucleotides, i.e., we hypothesize that there were various proto nucleotides initially formed by the self-replication, but most of them could not survive and eventually only canonical nucleotides populated. In the following, we describe the formation of RNA nucleotides and their polymerization by self-replication on prebiotic Earth.

2. Formation of RNA Nucleotides

Life is defined as an evolving entity that self-replicates, occasionally mutates and adjusts to its environment; thus, we think that the origin of life is the formation of RNA nucleotides and present a sequence of nucleotide multiplication on prebiotic Earth [16]. Nucleotide possesses an autocatalytic ability because it forms a pair with another nucleotide by H bonding which is easy to form and break up. The process is also assisted by an abiotic catalyst that reduces the activation energy for multiplication. We assume that the subunits of the nucleotides of terrestrial or exterrestrial origin existed in prebiotic oceans. The process took place in areas with cyclic environmental changes such as tideland where wet & dry cycle repeated due to tidal cycle. The subunits dissolved in ocean water were selectively adsorbed on the surface of clay minerals on the floor of tideland. Since fresh subunits were supplied every tidal cycle, the concentration of the subunits on the mineral surface gradually increased. Once it had reached a certain level, the synthesis triggered by a high energy such as lightning occurred to form a nucleotide by joining three subunits. It repeated and the concentration of the nucleotides slowly increased in a linear manner. As a competing process, the self-replication also operated at the same time, but the production rate was insignificant in the beginning when the concentration of template nucleotides was low. However, when it reached a certain level, the rate became significant and it started to

rapidly increase in an exponential manner. Various proto-nucleotides formed in the beginning, but a group of nucleotides gradually dominated due to easier self-replication and better stability comparing with other nucleotides, and eventually became the canonical RNA nucleotides. The sequential steps of the self-replication of the canonical RNA nucleotides are shown in Fig 1 and explained in the following using a nucleotide with **A** as a template nucleotide.

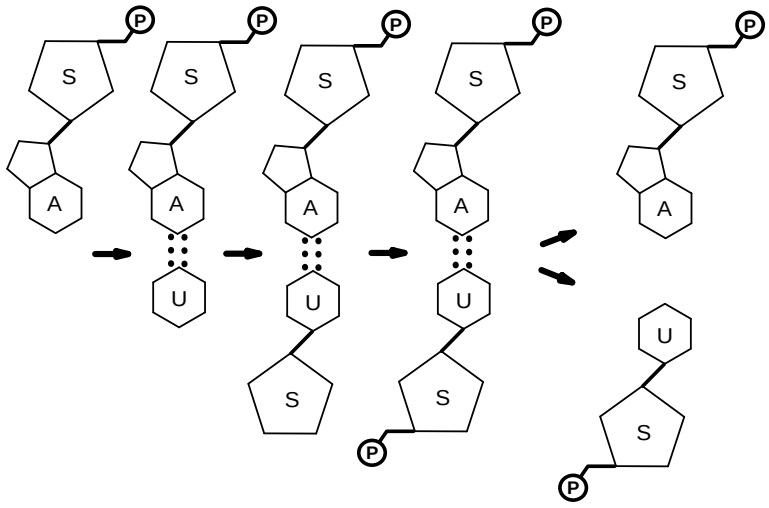

Fig 1. Nucleotide formation by self-replication, where **P** is phosphate group, **S** is sugar, and H bonding is represented by dot lines.

Step 1: A near-by complementary **U** joins with **A** of the template nucleotide by H bonding. This bonding is almost instantaneously formed on contact because the activation energy is low. Now, **U** is immobilized, which makes ribose to join easier. Step 2: Due to a subtle structural constraint, normally only a free D-ribose joins **U** by covalent bonding, thus a nucleoside with **U** is formed. This reaction is possible because the template nucleotide is preferentially oriented on the mineral surface and in a state of reduced activation energy for the bonding. Step 3: A free **P** joins the D-ribose by covalent bonding to form the second nucleotide. Again, the activation energy is reduced due to a preferential orientation. Step 4: Lastly, the H bonding between the template and the second nucleotide are broken by cyclic variation of the environment due to tidal cycle, resulting in two separate nucleotides.

Steps 1- 4 repeat and the template nucleotide with **A** and the second nucleotide with **U** produce the third nucleotide with **U** and the fourth nucleotide with **A**, respectively. These two self-replications proceed independently. The same process also proceeds on the (**G**)-(**C**) pair of nucleotides. The process continues as long as the subunits are supplied.

3. Polymerization of RNA Nucleotides

Once the concentration of RNA nucleotides had reached a certain level, polymerization started. Both single strand polymers and double strand polymers were formed by synthesis and self-replication, respectively. Single strand polymers initially dominated, but as the concentration of template polymers increased, the double strand polymers started to dominate. Dissociation rate of

polymers was linearly proportional to the length of the polymer. As a result, beyond a certain length, the double strand polymers only remained because H bonding between pair nucleotides contributed to better stability. In the following, the sequential steps of the self-replication of RNA polymers are schematically shown in Fig 2 and explained using a single strand dimer with **A** and **U** as a template for simplicity [17].

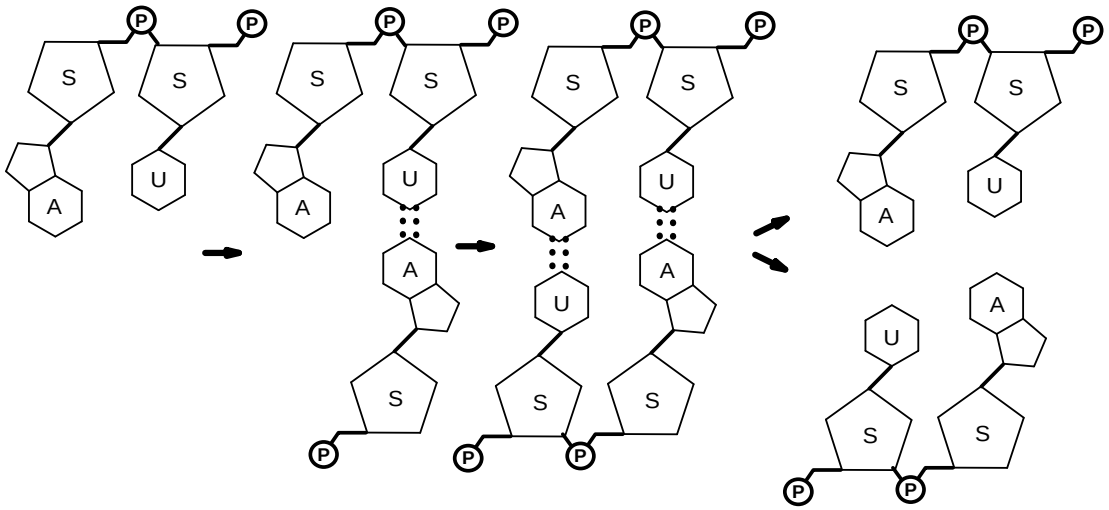

Fig 2. Polymerization of RNA nucleotides and multiplication by self-replication.

Step 1: The single strand dimer is adsorbed and properly aligned on a clay mineral which acts as a catalyst and the activation energy is reduced. Step 2: Third nucleotide with **A** joins with the complementary base **U** of the template dimer by H bonding when the two bases come to close each other. Step 3: Fourth nucleotide with **U** joins with the unbonded complementary **A** of the template dimer by H bonding and then third and fourth nucleotides join together by covalent bonding and release a water molecule. This condensation process is always in the 5'-to-3' direction. As a result, a double strand dimer forms. Step 4: The cyclic environmental change causes breaking up of H bond between the bases and the template dimer and its complimentary dimer separate from each other. The self-replication of the template dimer completes. The process independently repeats on each of the two separated dimers and the number of the replicated dimers increases.

As the concentration of self-replicated dimers increases, there is a chance of synthesis by which a nucleotide joins at an end of one of the single strand dimers resulting in formation of a trimer (there is a small chance of formation of a tetramer by joining two dimers together). If the trimer (or tetramer) is stable, the self-replication of the trimer starts. Joining of free complementary nucleotides always starts from one end of the trimer and finishes at the other end, forming a double strand trimer. The trimer and its complementary trimer are separated by the wet & dry cycle and continue to self-replicate independently. Single strand polymer generally grows by joining a nucleotide at an end of the strand, but the chance of joining a polynucleotide increases as their concentration increases, resulting in increase of the length by multiple units. If the resultant polymer is beyond a certain length, it will be dissociated unless it turns to a double strand by self-replication. If a mutant polymer is formed and turned out to be better fit with the environment, it continues to grow and replaces the original polymer. Polymers that fail to reach sufficient lengths to be functional eventually dissociate and become feeds for other growing polymers.

4. Discussion

All life self-replicates. It copies genetic information, passes onto offspring and evolves by occasional mutation. This is the reason why the RNA world took off when the nucleotides emerged that could make copies of themselves. As they joined together, new self-replicating polynucleotides emerged and became RNAs. Some were better at self-replicating themselves than others. The RNAs competed against each other, and the most successful won out. This was the molecular level Darwinian evolution where the fittest molecules survived. Over many years, these RNAs multiplied and evolved to create an array of functional RNAs. Significant developments were replacement of **U** with thymine (**T)** and ribose with deoxyribose that formed DNA which was much more stable than RNA and further advanced evolution. In the subsequent evolution, the main roles of DNA and RNA became storing genetic information, and transcripting and translating the information, respectively.

We hypothesized that tidal land was the place where the self-replication of nucleotides and RNAs took place in prebiotic Earth, however estuary was also likely place where the self-replication was possible because pH fluctuation due to tide could promote break up of RNA polymers. The proposed process can explain features associated with RNA, such as homochirality and heredity. Homochirality was established during the nucleotide formation and polymerization. Canonical subunits and other subunits were involved in the formation of proto-nucleotides. For example, both D-ribose and L-ribose could initially involve in the formation of proto nucleotides. The proto-nucleotides with L-ribose might be less stable, thus canonical nucleotides with D-ribose eventually dominated due to a thermodynamic advantage. During the polymerization, if remaining non-canonical nucleotides joined, the growth stopped, therefore long polymers were exclusively made from canonical nucleotides, thus homochirality established. In other words, homochirality progressively started in the nucleotide formation and completed in the polymerization by crowding out less advantaged nucleotides and polymers. Since polymerization proceeded by self-replication, heredity naturally established.

Double strands created by the self-replication process were inherently more stable than the corresponding single strand because H bonding contributed to stability, thus extending the length of the polymers. Although the majority of extant RNAs are single stranded and formed by DNA transcription, by which a portion of DNA nucleotide sequence is copied into RNA nucleotide, double stranded RNAs are also found in various biological systems. The RNAs can be in the double strand form transiently or permanently. The “cloverleaf” shape of tRNA is an example. It is formed when a single RNA molecule has a complementary sequence in a different part of the molecule. Another example is double stranded RNA viruses which are not technically living things because they cannot reproduce themselves, but they are considered proto-life.

Certain RNAs, namely ribozymes, can self-replicate [13]. The self-replication involves two ribozymes, each is composed of two subunits, and acts as a catalyst to form the other. The self-replication process is cyclic, in that the first one joins the two subunits that comprise the second one to form a new copy of the second one; while the second one similarly joins the two subunits that comprise the first one to form a new copy of the first one. This is the highly evolved form of

the self-replication of RNA and may have its origin in the prebiotic self-replication of RNA nucleotides.

The effective clay minerals for the formation of nucleotides from the subunits have not been identified yet. The minerals in micron or submicron size (still large comparing molecular size) are particularly of interest because they can be carried around by the tide and more often encountered with the subunits. Selective adsorption of prebiotic molecules in oceans depends on atomic structure and composition of the surface of the minerals. For RNA nucleotides, montmorillonite seems a viable candidate. It has been shown to catalyze the formation of single strand RNA polymers [18]. Experimental investigations on montmorillonite and similar minerals may be the first step to identify suitable clay minerals for the formation of nucleotides.

The formation of nucleotides from the subunits is a condensation process where two water molecules are released on the reaction. The self-replication reaction starts with bonding of a complimentary pair base, and then D-ribose joins the base to form a nucleoside. Next, a phosphate group joins the nucleoside. The activation energy for H bonding is about 1.6 kJ/mole, which is less than the thermal energy at RT, therefore the reaction is instantaneous on contact. The activation energy for covalent bonding is 55 ~ 75 kJ/mole, which is reduced by the catalytic minerals. After the complemental nucleotide is formed, the pair of nucleotides undergoes separation. The breaking up at the site of the H bonding requires energy input that exceeds the energy of H bond that is in the range of 15 ~ 30 kJ/mole. Also, the energy of physisorption is in the range of 12 ~ 40 kJ/mole. Therefore, thermal energy at RT that is approximately 2.5kJ/mole is not sufficient for breaking up. Potential sources of energy to surmount the H bond and physisorption energies are UV light energy (~ 100 kJ/mole) when the surface of the mineral is directly exposed to the sunlight. The strength of UV light rapidly weakens as the depth of sea water increases due to absorption. In comparison, covalent bond is stronger (in the range of 400 ~ 500 kJ/mole) and UV light may not be strong enough. Thus, higher energy sources such as impact of rain drops and shear flow of water around the mineral surface due to tidal cycle are required to break up the bond.

Growth rate of single strand of polynucleotide is linearly proportional to the concentration of the nucleotides, so if the concentration is high and a suitable abiotic catalyst is available, it may grow to a functional RNA. However, it is statistically unlikely to have other polynucleotides with the same sequence nearby. In contrast, if a functional RNA undergoes self-replication, multiple copies of the polynucleotide are available for assisting chemical reactions. For the functional RNA to take hold, many copies of the polynucleotide are necessary.

In this paper, we described self-replication of Watson-Click base pair nucleotides, where **A**-**U** base pair and **C**-**G** base pair were joined by 2 and 3 H bonds, respectively. However, we realize that **C**-**U** and **A**-**G** base pairs can be joined by 2 H bonds if we disregard the 5'-to-3' direction. For the nucleotide self-replication, the direction may not be a strict requirement. We are not sure whether this could happen in prebiotic environment.

The first functional RNAs were likely to be biotic catalysts (ribozymes) that engaged in accelerating the formation of themselves through formation of nucleotides from the subunits and self-replication of the nucleotides and the polynucleotides [19]. These catalysts replaced the abiotic catalysts such as minerals, were much more efficient and allowed the reactions to take place at

various places besides tideland and were different from enzymes that were proteins and formed with help of RNA in a later stage of evolution. There are competitive hypotheses of the origin of life in literature [20,21]. Hydro vent in deep ocean bed where life started as a simple metabolic process and hot spring where fresh water contributed for the formation of original life form of proto cells. These hypotheses are plausible in some respects but not in others. We think that these life forms are well advanced compared with the nucleotides, thus these places are occupied in a later stage of evolution of life by adaptation.

5. References

1. Leslie E. Orgel, Prebiotic chemistry and the origin of the RNA world, Critical Reviews in Biochemistry and Molecular Biology 2004, 39(2), 99.
2. Ben K. D. Pearce, Ralph E. Pudritz, Dmitry A. Semenov and Thomas K. Henning, Origin of the RNA world: The fate of nucleobases in warm little ponds, Proc. Natl. Acad. Sci USA 2017, 114(43), 11327.
3. Annabelle Biscans, Exploring the Emergence of RNA Nucleosides and Nucleotides on the Early Earth, Life 2018, 8(4), 57.
4. H. L. Barks, R. Buckley, G. A. Grieves, E. Di Mauro, N. V. Hud, and T. M. Orlando, Guanine, adenine, and hypoxanthine production in UV-irradiated formamide solutions: Relaxation of the requirements for prebiotic purine nucleobase formation, Chembiochem 2010, 11 (9), 1240.
5. M. Ruiz-Bermejo, C. Menor-Salvan, S. Osuna-Esteban and S. Veintemillas-Verdaguer, Prebiotic Microreactors: A Synthesis of Purines and Dihydroxy Compounds in Aqueous Aerosol, Orig. Life Evol. Biosph. 2007, 37 (2), 123.
6. Mahipal Yadav, Ravi Kumar and Ramanarayanan Krishnamurthy, Chemistry of Abiotic Nucleotide Synthesis, Chem. Rev., 2020, 120, 4766.
7. Stuart A. Harrison & Nick Lane, Life as a guide to prebiotic nucleotide synthesis, NATURE COMMUNICATIONS, 2018, 9(1), 5176.
8. Ben K. D. Pearce, Ralph E. Pudritz, Dmitry A. Semenov and Thomas K. Henning, Origin of the RNA World: The Fate of Nucleobases in Warm Little Ponds, Proc. Natl. Acad. Sci USA 2017, 114.
9. Pierre-Alain Monnard, Taming Prebiotic Chemistry: The Role of Heterogeneous and Interfacial Catalysis in the Emergence of a Prebiotic Catalytic/Information Polymer System, Life 2016, 6, 40.
10. Tracey A. Lincoln and Gerald F. Joyce, Self-Sustained Replication of an RNA Enzyme, SICENCE 2009, 323, 1229.
11. John Emsley, Artificial Molecule Shows ‘Sign of Life’, New Scientist, 1990, April 28, 38.
12. James P. Ferris, Montmorillonite-catalysed formation of RNA oligomers: the possible role of catalysis in the origins of life, Phil. Trans. R. Soc. B Biol Sci. 2006, 361, 1777.
13. Robert M. Hazen and Dimitri A. Sverjensky, Mineral Surfaces, Geochemical Complexities, and the Origins of Life, Cold Spring Harb Perspect Biol. 2010, 2.
14. Nicholas V. Hud, Searching for lost nucleotides of the pre-RNA World with a self-refining model of early Earth, Nature Communications 2018, 9, 5171.
15. Brian J. Cafferty, David M. Fialho, Jaheda Khanam, Ramanarayanan Krishnamurthy and Nicholas V. Hud, Spontaneous formation and base pairing of plausible prebiotic nucleotides in water, Nature Communications 2016, 7, 11328.

16. K. Ohsaka, The origin of life; the first self-replicating molecules were nucleotides, PeerJ Preprints 7: e27919v1.
17. K. Ohsaka, The origin of life: Oligomerization of RNA nucleotides on prebiotic Earth, EcoEvoRxiv Preprints:10.32942/osf.io/mrzp9.
18. James P. Ferris, Mineral Catalysis and Prebiotic Synthesis: Montmorillonite-Catalyzed Formation of RNA, Elements 2005, 1, 145.
19. G. F. Joyce, Evolution in an RNA World, Cold Spring Harb Symp Quant Biol. 2009, 74, 17.
20. Bruce Damer and David Deamer, The Hot Spring Hypothesis for an Origin of Life, ASTROBIOLOGY, 2020, 20 (4), 429.
21. Norio Kitadai and Shigenori Maruyama, Origins of building blocks of life: A review, Geoscience Frontiers 2018, 9, 1117.