

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

Kenichi Ohsaka¹

¹Affiliation not available

February 22, 2024

Introduction

A notion of life being originated from polymerization of RNA nucleotides seems to be corroborated by circumstantial evidence and some experimental results, however, how it was actually proceeded is not well understood. There are ongoing efforts to synthesize RNA nucleotides in modern labs starting from simple molecular components presumably existed on prebiotic Earth [1-4]. It appears that entire processes leading to formation of the subunits of RNA nucleotides, i.e., ribose sugar, phosphate and nucleobases (adenine (A for short), uracil (U), guanine (G) and cytosine (C)) could be naturally proceeded in prebiotic Earth environment following thermodynamic principles. Alternatively, some intermediate products might be originated from outer space and delivered to Earth. Evidence provided by meteorites suggests this possibility [5]. On the other hand, according to experiments performed in modern labs, the final process, the formation of the RNA nucleotides by joining the subunits is found to be thermodynamically difficult because the subunits must be joined together with covalent bonding that requires to overcome a high activation energy. Thus, there had to be a process that modified the activation energy and promoted the formation of the nucleotides to produce enough quantity for the subsequent polymerization.

Once the concentration of RNA nucleotides had reached a certain level, the polymerization took place, and a single strand of RNA polymer was formed on prebiotic Earth. An experiment a simulated prebiotic condition shows that the polymer can grow up to 40 nucleotide units. The max length is determined by stability of the polymer that is constantly subjected to dissociation (bond breaking). The length is well short comparing the length of known short functional RNAs (70~100 units). The dissociation rate linearly increases as the polymer grows in length. In order to grow further, a mechanism that produced longer polymers had to be operated in prebiotic Earth.

Autocatalytic reaction is a special property of certain molecules, by which the molecules are multiplied exponentially instead of linearly. Self-replication of RNA enzyme without help of biotic catalysts is known [6]. Also, the self-replication of synthetic molecule has been demonstrated [7]. The molecule is an amino adenosine triacid ester (AATE), which copies 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 copying process works because of a weak bonding, known as hydrogen bonding which can be broken even by thermal vibration. Hydrogen bonding plays the same role in the self-replication of DNA.

It is conceivable that self-replication with a help of non-biotic catalysts might play an essential role for the formation of RNA nucleotides and the polymerization of the nucleotides on prebiotic Earth [8]. Catalysts are widely used to increase yields in modern chemical syntheses. We speculate that non-biotic catalysts played an essential role in the formation of the nucleotides and the polymerization on prebiotic Earth. Metals and clay minerals are known to be good catalysts and certainly existed on prebiotic Earth [9, 10]. Their roles in the process were twofold. First, they provided surfaces on which the subunits were selectively adsorbed. This reaction consequently increased the concentration of the subunits on the surface and allowed the subunits to

react each other more frequently. Secondly, they strained and aligned the subunits and nucleotides in proper orientations so that particular reactions became easier due to reduction of the activation energy.

Formation of Nucleotides

We hypothesize a process for multiplication of nucleotides on prebiotic Earth [11]. It is posited that the subunits of the nucleotides of terrestrial or extraterrestrial origin existed in prebiotic oceans. The multiplication of nucleotides took place at tideland or estuary at which wet & dry cycle or pH fluctuation occurred due to tidal cycle. The subunits dissolved in the oceans were selectively adsorbed on clay minerals at the bottom of tideland or estuary. Since fresh subunits were supplied in every tidal cycle, the concentration of the subunits on the mineral surfaces were gradually increased. Once the concentration reached a certain level, it was extremely rare, but a reaction that joined three subunits, say, base (U), D-ribose and phosphate occurred, and an original nucleotide formed. The rate of the reaction is proportional to the concentration of reactants but independent of the concentration of the nucleotides. The nucleotides might degrade before the next reaction occurred; thus, a significant increase in the concentration of the nucleotides was unlikely if the formation was solely relied on the synthesis reaction unless the concentration of the reactants were unnaturally high. In order to increase the concentration, the nucleotides must be formed faster than degradation. As a plausible process, we propose a cross complementary self-replication of the nucleotides. Although various proto-nucleotides including extant RNA nucleotides were produced in the prebiotic Earth, the extant nucleotides eventually dominated. The sequential steps of the formation of the extant RNA nucleotides are described in the following:

Step 1: For example, a free complementary base (A) resided close to the original nucleotide is joined to the base (U) by hydrogen bond. This reaction is spontaneous because the bonding does not require to overcome an activation energy for establishing the bonding.

Step 2: A free D-ribose is joined to the base (A) by covalent bond. A nucleoside is formed. This reaction is possible because the original nucleotide is preferentially oriented and in a state of lower activation energy for the bonding.

Step 3: Next, a free phosphate is joined to the D-ribose by covalent bond to form the second nucleotide. Again, the activation energy is lowered due to the preferential orientation.

Step 4: The hydrogen bond between the original and the second nucleotides is broken by cyclic variation of the environment due to tidal cycle, resulting in two separate nucleotides.

Steps 1-4 repeat and the original nucleotide with (U) and the second nucleotide with (A) produce the third nucleotide with (A) and the fourth nucleotide with (U), respectively. Two different nucleotides are self-replicated. The process continues as long as the subunits are supplied.

The same process also occurs to the base (G) and (C) pair. If the rate of complementary self-replication exceeds the rate of degradation of existing nucleotides, the concentration of the nucleotides is expected to increase exponentially.

Polymerization of Nucleotides

Once the concentration of RNA nucleotides had reached a certain level, polymerization took place, and a single strand of RNA polymer was formed on prebiotic Earth. The sequential steps of the polymerization process are as follows [12]:

Step 1: Two nucleotides join together and form a dimer. The dimer is adsorbed and properly aligned on a clay mineral which acts as a catalyst and lowers the activation energy for polymerization.

Step 2: Although polymerization normally proceeds by joining third nucleotide at an end of the dimer resulting in a trimer, due to a unique structure of the nucleotides, the process differently proceeds by bonding the base of third nucleotide with a complementary base of the dimer first because this bonding does

not need to overcome an activation energy and occurs instantaneously when the two bases come to close each other.

Step 3: Next, fourth nucleotide with base which is complementary to the remaining unbonded base of the dimer is bonded and then third and fourth nucleotides join. As a result, a double strand dimer is formed.

Step 4: The wet & dry cycle of environment causes breaking up of hydrogen bond between the bases and the dimer and its complimentary dimer are separated. The complementary self-replication of the dimer is completed.

Step 5: The process independently continues further on each of the two separated dimers and the number of the replicated dimer that is more stable increases.

Step 6: Although the self-replication reaction dominates, as the number of replicated dimers increases, there is a chance of polymerization process by which a nucleotide joins at an end of one of the 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 more stable than the dimers, the self-replication of the trimer starts. Bonding of free complementary nucleotides starts from one end of the trimer and finishes at the other end, forming a double strand trimer.

Step 7: The trimer and its complementary trimer are separated by the wet & dry cycle. The self-replication of the trimer is completed.

The process continues and the length of the longest polymer steadily increases as long as the free nucleotides are available. Growth of the length reaches a limit when the dissociation rate of the polymer exceeds the self-replication rate.

Discussion

The hypothesized process can explain features associated with RNA, homochirality [13] and heredity. Homochirality was established during the nucleotide formation and the polymerization. Canonical subunits and other subunits involved in formation of proto-nucleotides. These were formed and dissolved many times and the canonical nucleotides eventually dominated. During the polymerization, if remaining non-canonical nucleotides were attached, the growth stopped, therefore long polymers were exclusively made from the canonical nucleotides, thus homochirality was established. In other words, the homochirality progressively started in the nucleotide formation and completed in the polymerization by crowding out less advantaged nucleotides and polymers. Since the polymerization proceeded by the self-replication, heredity was naturally established. Double strands created by the self-replication was inherently more stable than the corresponding single strand because the hydrogen bonding contributed for stability, thus extending the length of the polymers.

With the advance of RNA molecule replication, evolution became possible due to occasional copying mistakes. Various variants were formed from the original molecules and some of them were turned out to be more stable and gradually dominated. This was the molecular level Darwinian evolution where the most fit molecule survived. A significant change was replacement of uracil (U) with thymine (T) that formed DNA which was much more stable than RNA and further advanced the evolution.

The valid clay minerals for the formation of nucleotides from the subunits have not been found yet. Selective adsorption of prebiotic molecules in oceans depends on atomic structure and composition of the minerals. For RNA nucleotides, montmorillonite seems a good candidate [14]. It has been shown to catalyze the formation of RNA polymers. Experimental investigations on montmorillonite and similar minerals may be the first step to identify right clay minerals for the formation of the 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 complementary pair base, and then D-ribose is joined to the base to form a nucleoside. Next, a phosphate is joined to the nucleoside. Bonding is possible due to activation energy modification by the catalytic minerals. After the complementary

nucleotide is replicated, the two nucleotides undergo separation. The breaking up at the site of the hydrogen bond requires energy input that exceeds the energy of hydrogen bond which is about 10 ~ 30 kJ/mole. Thermal energy at RT is approximately 2.5kJ/mole, which is not sufficient. Potential sources of the energy to surmount the hydrogen bond energy are UV energy when the surface of the mineral is directly exposed to the sun light, and flow of water molecules around the mineral surface due to tide cycle. The strength of UV rapidly weakens as the depth of sea water increases due to absorption. On the other hand, covalent bond is much stronger (400 ~ 500 kJ/mole) compared with hydrogen bond, thus it is unlikely to easily break up due to tidal cycle. Higher energy source like lightning is required.

Growth rate of single strand of RNA polymer 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 the second polymer with the same sequence nearby. On the other hand, if a functional RNA undergoes the self-replication, multiple copies of the polymer are available for assisting chemical reactions. For the functional RNA to take hold, many copies of the polymer are needed.

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 polymers. 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 estuary and were different from enzymes that were proteins and formed with help of RNA in a later stage of evolution. Over many years, some RNAs self-replicated and evolved to a variety of functional RNAs. At some later stages of evolution, DNA and proteins were formed with help of RNA and took over the jobs of storing genetic information and driving chemical reactions, respectively.

There are competitive hypotheses of the origin of life in literature [15]. 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 [16]. We think that these places are occupied later stage of evolution of life by adaptation.

References

1. Leslie E. Orgel, Prebiotic Chemistry and the Origin of the RNA World, Critical Reviews in Biochemistry and Molecular Biology 2004, 39, 99.
2. Annabelle Biscans, Exploring the Emergence of RNA Nucleosides and Nucleotides on the Early Earth, Life 2018, 8, 57.
3. 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, 1240.
4. M. Ruiz-Bermejo, C. Menor-Salvan, S. Osuna-Esteban and S. Veintemillas-Verdaguer, Prebiotic microreactors: A synthesis of purines and hydroxy compounds in aqueous aerosol, Orig. Life Evol. Biosph. 2007, 37, 123.
5. 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.
6. Tracey A. Lincoln and Gerald F. Joyce, Self-Sustained Replication of an RNA Enzyme, SICENCE 2009, 323, 1229.
7. T. Tjivikua, P. Ballester and J. Rebek, Self-replicating system". Journal of the American Chemical Society. American Chemical Society (ACS). 1990, 112 (3): 1249.
8. 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.

9. James P. Ferris, Montmorillonite-catalysed formation of RNA polymers: the possible role of catalysis in the origins of life, *Phil. Trans. R. Soc. B* 2006, 361, 1777.
10. Robert M. Hazen and Dimitri A. Sverjensky, Mineral Surfaces, Geochemical Complexities, and the Origins of Life, *Cold Spring Harb Perspect Biol.* 2010, 2.
11. K. Ohsaka, The origin of life: the first self-replicating molecules were nucleotides, *PeerJ Preprints* 7: e27919v1.
12. K. Ohsaka, The origin of life: Oligomerization of RNA nucleotides on prebiotic Earth, *EcoEvoRxiv Preprints*:10.32942/osf.io/mrzp9.
13. V. I. Goldanskii, V. A. Avetisov and V. V. Kuzmin, Chiral purity of nucleotides as a necessary condition of complementarity, *FEBS Lett* 1986, 207, 181.
14. James P. Ferris, Mineral Catalysis and Prebiotic Synthesis: Montmorillonite-Catalyzed Formation of RNA, *Elements* 2005, 1, 145.
15. Norio Kitadai and Shigenori Maruyama, Origins of building blocks of life: A review, *Geoscience Frontiers* 2018, 9, 1117.
16. Hemachander Subramanian, Joel Brown and Robert Gatenby, Prebiotic competition and evolution in self-replicating polynucleotides can explain the properties of DNA/RNA in modern living systems, *BMC Evolutionary Biology* 2020, 20, 75.