

POSSIBLE PATHWAYS BEFORE AND AFTER THE RNA WORLD ON THE BASIS OF  
EXPERIMENTAL AND THEORETICAL EVIDENCES

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(Received October 31 2005 Accepted December 5 2005)

**Abstract**

Although the RNA world hypothesis is important as a model of the emergence of life-like systems, this hypothesis has some drawbacks. This hypothesis was already evaluated from the hydrothermal origin of life hypothesis in our recent paper. On the other hand, a unified theory for the origin and evolution of life has been attempted to develop on the basis of our recent investigations concerning the subjectivity of life and the molecular machinery of assigning genotype and phenotype. In the present paper, the RNA world hypothesis is further evaluated on the basis of the unified theory as well as the experimental evidences concerning the chemical evolution under hydrothermal environments.

**Keywords**

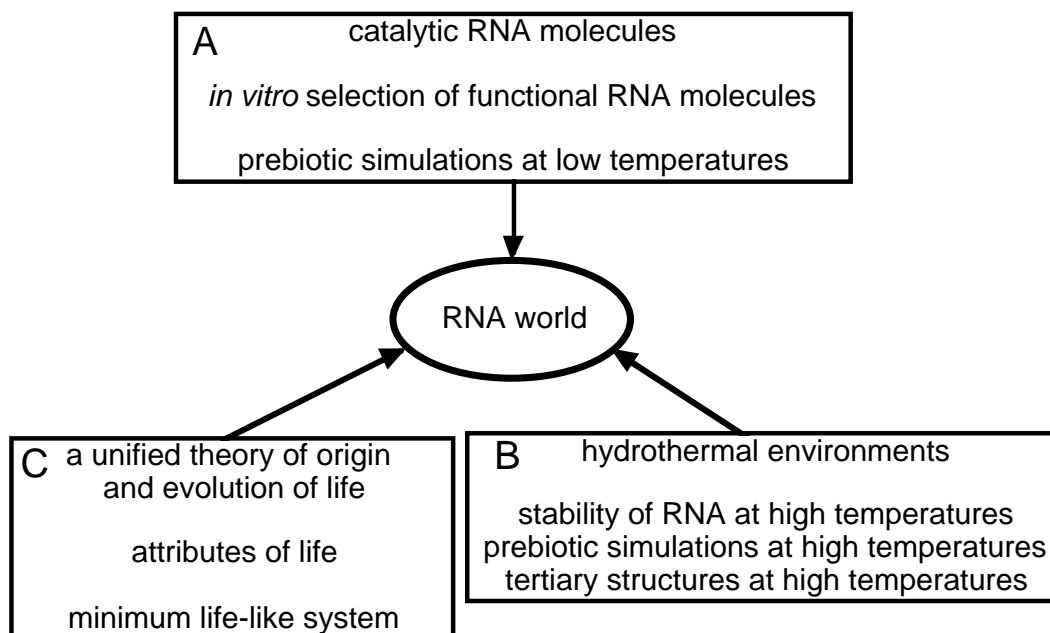
hydrothermal reactions, RNA, RNA world, a unified theory of origin and evolution of life, enzyme

**1. Introduction**

Although the RNA world hypothesis is important [1,2], it has some drawbacks (Fig. 1 (A)). The RNA world hypothesis appears to be inconsistent with the hypothesis that life originated under hydrothermal environments in the primitive ocean (the hydrothermal origin of life hypothesis) [3]. RNA molecules are regularly supposed (1) to be less stable than DNA and proteins and (2) to be difficult to maintain tertiary structures since biologically important interactions, such as hydrogen bonding and hydrophobic interaction, become weak at high temperatures (Fig. 1 (B)) [4,5]. However, most of the

simulation experiments have been carried out at low temperatures. In addition, there have been no practical techniques for the investigations of chemical evolution of RNA under hydrothermal conditions. In our group, (1) monitoring techniques of hydrothermal reactions [6-9], (2) the stability of RNA molecules [8,10-14], (3) prebiotic formation of RNA at high temperatures [15-17], (4) tertiary structures of nucleic acids under hydrothermal conditions [18], have been studied.

If a life-like system entirely consisting of RNA molecules have emerged on primitive earth, naturally the system would be more labile than the present cell



**Figure 1.** Evaluation of the RNA world hypothesis from different aspects. While the RNA world hypothesis is supported by the viewpoint A, some drawbacks remain from the viewpoints B and C.

type systems because of the low stability of RNA. In addition, an essential point of view of the RNA world hypothesis is that RNA molecules could have played important or central roles for the emergence of life. Thus, this hypothesis is not in conflict with the speculation that other prebiotic materials would have facilitated the RNA world. However, there have been few systematic investigations on the roles of such materials for the chemical evolution of RNA.

In our previous paper we have discussed the possibility of an RNA world from the standpoint of the stability of RNA and the prebiotic simulations of RNA formation (Fig. 1 (B)) [19]. In this paper, additional discussions are attempted on the basis of the experimental evidences and a unified theory on the origin and evolution of living organisms, which was deduced from our continuous investigations on the origin and evolution of life (Fig. 1 (C)) [20-22].

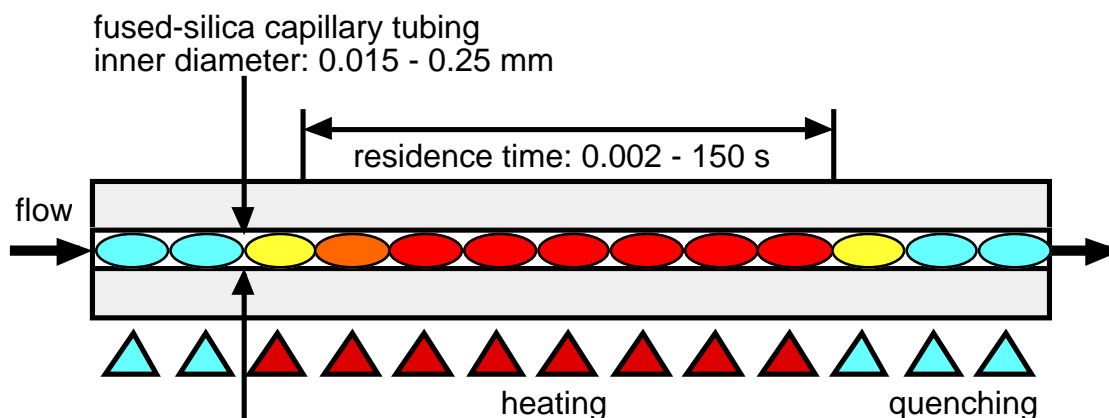
## 2. Chemical evolution of RNA under hydrothermal systems

### 2. 1. Monitoring methods of hydrothermal reactions

While hydrothermal reactions were normally investigated using batch reactors, it was difficult to monitor hydrothermal reactions within the millisecond time scale. To monitor such rapid reactions, the flow reactor monitoring systems are becoming practical techniques [23-25]. We have invented a real-time monitoring method of hydrothermal reactions using a micro-flow reactor system assembled with fused-silica capillary tubing, which enables the monitoring reactions at 0.002 - 150 s at temperatures over 300 °C [6-8]. Furthermore, this technique was applied to conduct *in situ* monitoring UV-visible absorption spectra under hydrothermal conditions [9]. These methods have facilitated the investigation of hydrothermal reactions at the millisecond – second time scale (Fig. 2).

### 2. 2. The degradation and the prebiotic formation of RNA

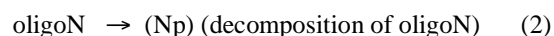
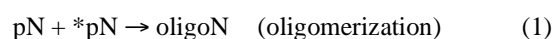
The stability of RNA monomers, dimers,



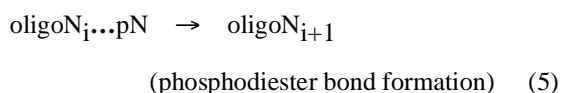
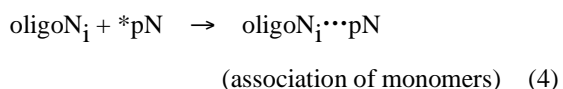
**Figure 2.** Monitoring system of hydrothermal reactions. Samples are immediately heated up in a fused-silica capillary tubing with inner diameter 0.015 – 0.25 mm and immediately quenched by cooling. This principle enables monitoring hydrothermal reactions within 0.002 - 150 s.

oligomers, and polymers has been investigated using the flow reactor system. The half-lives of these molecules are in the time ranges 2 - 70 s at 200 °C and 0.01 - 0.9 s at 300 °C [8,10-14]. These reactions appeared to be so fast under hydrothermal conditions that RNA molecules could not have survived for a geological time scale [19].

On the other hand, simulation reactions for the formation of RNA oligomers on the primitive earth conditions have been extensively investigated using the phosphorimidazolides of nucleotide monomers [26-31]. However, most of the studies were carried out at 25 °C and no investigations were performed at over 37 °C [31]. We have carried out kinetic investigations at temperatures up to 80 °C on (1) the template-directed formation of oligoguanylate on a polycytidylic acid template [15], (2) the cyclization of oligonucleotides [16,17], and (3) the oligocytidylate formation in the presence of  $Pb^{2+}$  [32]. It was confirmed that these reactions proceed at 75 - 80 °C. The reactions are expressed by the following equations [15,33],



where pN, \*pN, and oligoN indicate nucleotide monomer, activated nucleotide monomer, and oligonucleotide. The accumulation of oligoN is determined by the relative magnitude of the processes. In general, the oligomerization in the prebiotic RNA formation models can be described by the following equations (4,5).



where  $K_{ass}$  is the association constant in equation (4) and  $k_{phos}$  is the formation rate constant in equation (5). The over all rate constant of oligomerization ( $k_{form}$ ) is expressed in equation (6).

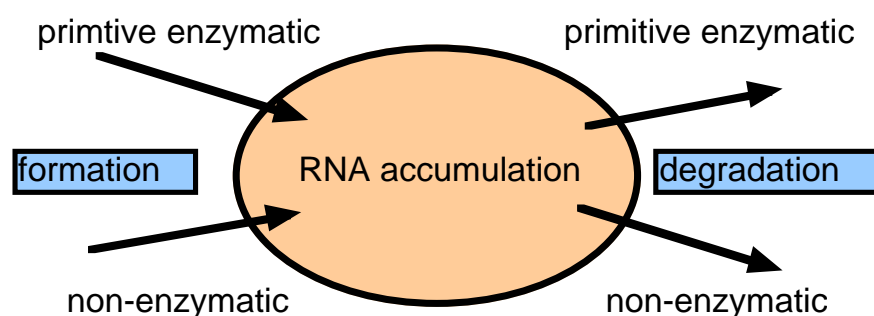


All three different prebiotic formation reactions of oligonucleotides proceeded at 75 - 80 °C although the efficiency of the reactions decreases with increasing temperature [15-17,32]. This fact indicates that the magnitude of  $k_{form}$  could be greater than that of degradation under hydrothermal conditions if the magnitude of  $K_{ass}$  is sufficiently large under

hydrothermal environments. For instance, the kinetic analysis of the template-directed reaction suggested that the low efficiency of oligoN formation at 80 °C is due to the weak association between activated nucleotide monomer (\*pN) and oligoN because hydrogen bonding and hydrophobic interaction decrease with increasing temperature. Thus, the template-directed formation of RNA could be possible under hydrothermal conditions, if the association between activated nucleotide monomer (\*pN) and oligoN is facilitated at high temperatures.

It is normally assumed that the degradation rates of RNA are so fast that the accumulation of RNA under hydrothermal conditions could not have been difficult. The temperature of the primitive ocean is still speculative so that the possibility to consider the cold origin of life is also important. In addition, experiments on simulated hydrothermal vent systems in deep ocean might suggest that prebiotic molecular systems could have survived by the circulation between cold water and hydrothermal vents. Nevertheless, it is indispensable to examine whether RNA systems could have emerged and survived at high temperatures. In this paper, the relationship between the stability of RNA and the prebiotic formation of RNA should be discussed from the

following standpoint. The evaluation that the degradation rates of RNA are so fast is determined on the basis of our perception and it does not seem to be based on a scientific viewpoint. For instance, the metabolism of RNA molecules in modern organisms is normally controlled at the millisecond time range [11,19]. Based on our considerations on the stability of RNA, the following viewpoints were proposed on the accumulation of RNA under hydrothermal environments [11,19]. First, the stability of RNA should be evaluated on the basis of the relative rates of the prebiotic formation and decomposition. Our data suggested that the rate of prebiotic formation reactions of RNA could have been greater than that of degradation of RNA under hydrothermal conditions at around 300 °C. Thus, oligonucleotides could have formed at high temperatures if a sufficient amount of the activated monomer have been supplied. Second, the comparison of the reaction rates concerning the formation and degradation of RNA with and without prebiotic enzymes is necessary since enzymes are essential for controlling biochemical reactions in organisms (Fig. 3). In addition, it has been pointed out that large differences are essential between the reaction rate constants with enzymes ( $k_{cat}$ ) and those without enzymes and the enzymatic reaction rates are



**Figure 3.** The accumulation of RNA under hydrothermal conditions should be evaluated from two different viewpoints. First, the accumulation should be evaluated by both the rates of the prebiotic formation and degradation of RNA. Second, the accumulation should be evaluated by both the rates with and without prebiotic enzymes.

in a narrow range for controlling biochemical reactions [33]. We have attempted the comparison of a number of reaction rate constants with and without enzymes including thermophilic enzymes [19]. First, this trend was possible to extend to temperatures as high as thermophilic enzymes survive. Second, it was found that there is a large difference between the enzymatic reaction rates and the reaction rates without enzymes at high temperatures 200 – 300 °C. This fact implies that a sufficient difference between the reaction rates with and without primitive enzymes could have been developed under hydrothermal environments. In other words, primitive enzymes could have emerged at such high temperatures. Primitive RNA and/or other molecules could be considered as candidates of primitive enzymes [19].

### 2. 3. Tertiary structures of RNA at high temperatures.

The weak interactions, such as hydrogen bonding and hydrophobic interaction, play important roles in biochemical reactions. Tertiary structures of RNA molecules, such as Watson-Crick double helix, several structures of tRNA, rRNA, and ribozymes are particularly important. However, these structures disappear with increasing temperature since the interactions become weak at high temperatures. However, there have been no practical techniques to measure the strength of the interactions at high temperatures over 100 °C. Thus, we have attempted to detect such interactions using our *in situ* UV-visible spectrophotometric system [9]. The UV-visible absorbance changes for the intercalation of ethidium bromide (EB) into double-helical DNA were measured in the presence of different salts at 481 nm at 25 - 200 °C [18]. The absorbance changes

of EB as a function of temperature were substantially consistent with the  $T_m$  values which were determined using a differential scanning calorimeter (DSC). Nevertheless, the change in absorbance at 100 - 130 °C was observed by the flow system, which was not detectable by DSC, and this finding indicates that an unknown weak interaction exists between EB and DNA at 100 - 130 °C. Since the measurements by DSC or conventional spectrophotometer are difficult or impossible at over 100 °C, details on this phenomenon are currently investigated using the new method.

Although the values of  $T_m$  for DNA and RNA are normally lower than 100 °C, the double helix and a variety of tertiary structures of nucleic acids should be maintained in hyperthermophilic organisms at over 100 °C. Thus, we are currently investigating what prebiotic environments and materials could have facilitated the formation of such tertiary structures of nucleic acids at high temperatures. In addition, it would be important to focus the role of electrostatic interaction for hyperthermophilic organisms and primitive life-like system under hydrothermal conditions since hydrogen bonding and hydrophobic interaction are weak.

Conclusively, for instance, the reason that RNA is not easily accumulated under simulated hydrothermal conditions does not seem to be due to the decomposition of RNA formed by the prebiotic pathways. The decrease of biologically important interactions at high temperatures is critical for the prebiotic formation model reactions. In other words, RNA molecules could not have accumulated under hydrothermal environments unless some additives have facilitated the weak interactions. In addition,

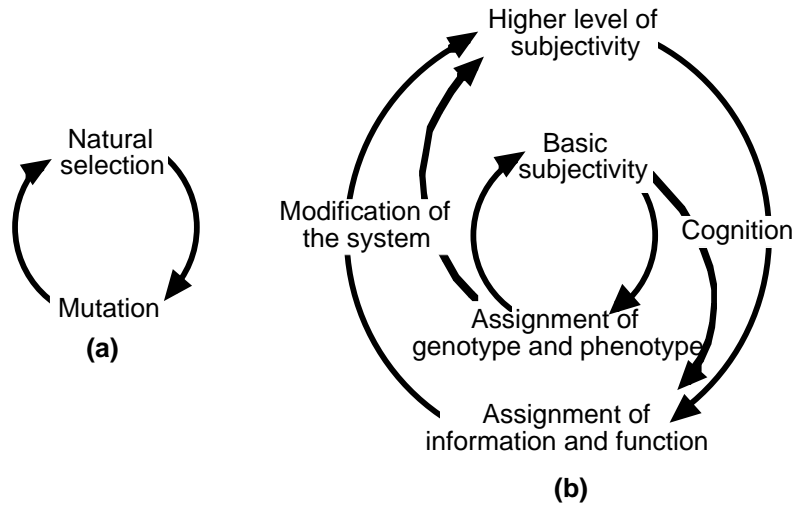
one should consider that the life-like systems have emerged in thermodynamically open systems (Fig. 3). Since our investigations have been carried out for RNA systems entirely consisting of RNA molecules, we have attempted to search whether prebiotic protein-like molecules could have facilitated the prebiotic formation and degradation of RNA molecules. It was found that the thermal condensation products of amino acids as protein-like molecules possess both catalytic and inhibitory activities for the formation and degradation of RNA, while the activities were indeed weak [34-38].

### **3. An attempt to develop a unified theory for the description of chemical evolution and biological evolution.**

#### **3. 1. The definition of life and the hypothesis on the mechanism of evolution**

In this paper and a previous paper [19], the RNA world hypothesis has been evaluated from empirical data concerning the hydrothermal origin of life. These analyses suggest that RNA systems would have been possible under hydrothermal environments if facilitated with effective adjuncts. Naturally, the experimental analyses of the RNA world hypothesis are important, but theoretical analyses would be also indispensable. Here, the evaluation of the RNA hypothesis is attempted on the basis of a unified theory of origin and evolution of biosystems on the earth. Attempts to define "life" would provide insight into the origin of life problem. A few years ago, I have proposed a definition of life that "Life is the system that has subjectivity" [20-22]. The term "subjectivity" was originally adopted as a term of philosophy, where one supposes subjectivity of

humans because of consciousness. Later, the term was extended to use as a term indicating an attribute of organisms beyond the nature of humans by IMANISHI Kinji for the first time. Imanishi has pointed out that organisms cause subjective actions towards environments for their survival although organisms do not have naturally consciousness. In addition, Imanishi argued that subjective actions of organisms towards environments extensively contribute to evolution. Such nature of organisms may not be easily accepted on the basis of the conventional views of biological science. Nevertheless, it was necessary to construct a unified theory to describe behaviors of life-like systems at different hierarchies on the basis of the definition of life [20-22]. To develop such a theory, it was attempted to find the correlation between the subjectivity of organisms and the assignment method between genotype and phenotype. Life-like systems on the earth were classified into three categories, that is, the cell type system (Class I), the virus type system (Class II), and other systems (Class III) on the basis of both the subjectivity and the assignment methods between genotype and phenotype for these systems, where the correlation between the subjectivity and the assignment methods was observed. The principle of the assignment method between genotype and phenotype was extended to the assignment between information and function at higher hierarchies of life-like systems and the correlation between the subjectivity of life-like systems and the assignment methods was investigated. Conclusively, it was hypothesized that the assignment methods between information and function by organisms contribute to the evolution of organisms,



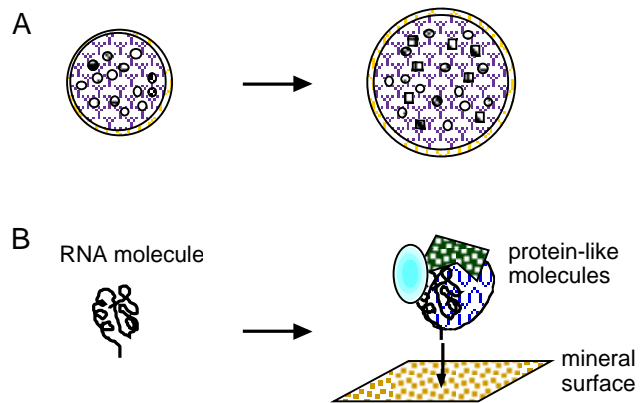
**Figure 4.** A proposed mechanism of evolution of biosystems. (A) indicates Darwinian evolution using a cyclical relationship between natural selection and mutation. (B) indicates the proposed mechanism using a cyclical relationship between “the subjectivity of life” and “the assignment between information and function”. The principle of “the assignment between genotype and phenotype” at the molecular level is extended to higher hierarchies of organisms, where the term is generalized as “the assignment between information and function”.

in which the assignments are caused by the subjective actions of organisms towards environments (Fig. 4).

### 3. 2. A problem of RNA system

According to the proposed theory, a life-like system that is regarded as alive should have possessed a nature that the system can subjectively introduce the environment as a part of the organisms and can adapt itself to the environmental changes for survival. In other words, it can be assumed that the life-like system should have held capability to keep continuity (individuality) and stability as a single system [39]. Question is whether RNA world type systems could have possessed such a nature or not under the primitive earth conditions. However, RNA molecules are normally unstable compared with DNA molecules and proteins. Although the closed vessels in the cell type organisms would be obviously important to expedite the continuity and stability as single biosystems, an RNA

system entirely consisting of RNA molecules could hardly have possessed such functions. Presumably, the adsorption of the RNA molecular system on a mineral surface or with protein-like molecules might have enabled to keep the continuity as a single biosystem (Fig. 5). Naturally, the essence of the RNA world hypothesis is that RNA molecules could have played important roles. Thus, it does not deny the contribution of other molecules. However, there have been few studies on the roles of other molecules for RNA systems. From this viewpoint, the role of some



**Figure 5.** The continuity of life-like systems can be preserved by cells (A), but also it might be maintained by minerals or protein-like molecules (B).

additional molecules should be evaluated as well as the viewpoint of the behavior of RNA under hydrothermal conditions. Thus, systematic investigations are necessary to search the roles of such molecules of maintaining RNA systems under the primitive earth conditions.

On the other hand, a catalytic network consisting of protein-like molecules, in which its reproduction is weakly catalyzed by other protein-like molecules in the network, can be categorized as a system that preserve information concerning the structure of the catalysts. This type of protein-like molecules can be regarded as a candidate of primitive enzymes. Such a catalytic network system could be stable and efficiently preserve the continuity as a single system. As mentioned above, weak inhibitory and/or catalytic activities of thermal condensation products of amino acids as protein-like molecules have been detected for both the formation and degradation of RNA molecules [34-38]. Naturally, these activities are much weaker than those of real enzymes. Presumably, the enzymatic activities would have been evolved in synchrony with the development of the coding systems and the synthesis of primitive enzymes. In addition, this type of protein-like molecules might have enhanced the weak interactions for the formation and replication of RNA molecules as mentioned above. While plausible materials and environments to facilitate the accumulation of RNA molecules at high temperatures have not yet been discovered, we are currently searching such conditions using several prebiotic materials. Naturally, the hypothesis that life emerged under cold environments and/or the hypothesis that prebiotic molecules formed under hydrothermal environments

could have survived within the surrounding cold ocean are important. Nevertheless, the scenario described in this paper intimates that first life-like systems could have emerged, survived, and evolved under hydrothermal environments.

#### **4. Conclusions.**

In this paper, the RNA world has been evaluated on the basis of the experimental analysis concerning the hydrothermal origin of life and a unified theory of the origin and evolution of biosystems on the earth. These analyses imply that an RNA world entirely consisting of RNA molecules is unlikely to occur and some adjuncts could have facilitated the emergence of RNA systems under primitive earth conditions. RNA systems possessing functions to preserve information and enzymatic abilities seem to be fairly advanced systems and it is important to find more primitive systems, such as a system consisting of protein-like molecules, which could have preserved ambiguous information and weak catalytic abilities.

#### **Acknowledgments**

The author greatly appreciates the financial support from the local government of Osaka Prefecture for attendance to the ISOLAB'05 meeting.

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