The Diffuse Organism as the First Biological System

Nikolay P. Kolomiytsev and Nadezhda Ya. Poddubnaya
Department of Biology
Cherepovets State University
Cherepovets, Russian Federation
npkolomitsev@mail.ru
poddoubnaia@mail.ru

Abstract

This article presents a new hypothesis on the origin of life on Earth. According to this hypothesis, life arose within the limits of a particular material system representing a set of specific local environments integrated by a common circulating liquid medium where relatively short RNA molecules, viroid-like particles, are replicated with great accuracy. In each of the local environments, the synthesis of certain substances that are required for accurate replication and survival of the RNAs is carried out. The system, which we called "diffuse organism," is, in essence, a very rough and bulky analog of the structural–functional organization of the cell's biosynthetic machinery. The diffuse organism was an organismal and evolving system at the same time. It seems that only such a system that has emerged in the only specimen as a result of a set of chance events operating under a system of universal physical and chemical laws was able to give rise to life and evolution by means of biological selection. The outlined scenario for the origin of life allows us to narrow down the still insuperable gap between prebiological chemistry and the first living systems without devising conceptions unrelated to the realities of life.

Keywords

accurate replicator, adaptive evolution, diffuse organism, Eigen's paradox, first biological system, major transitions, non-heritable gene, organism–population duality, origin of life, viroid

December 9, 2009; revised and accepted March 22, 2010

What is life? Where and how did it arise? What were the first living beings? During millennia, these questions have tickled inquisitive people's fancy. However, when we try to solve the problem, we often do not realize the enormous complexity of the phenomenon of life and attempt to answer all the questions at once. Meanwhile, similar challenges are seldom solved in one step. Their solutions usually extend for long years and fall into stages logically connected with each other, and the correct answers for the questions of a preceding stage will determine in many respects the correct answers to the questions of the next stage. The solution of the origin-of-life problem, then, naturally falls into the following stages: (1) Identifying one single property of living things whose appearance has determined the transition from the nonliving to the living state of matter; (2) establishing the material basis of this property; (3) figuring out in which material system the required structural elements could be formed; and (4) finding out in which environments and chemical processes such a material system could arise, after previously having solved the extremely hard problem (standing apart from the scheme outlined here) of the synthesis of all precursors of biological macromolecules and their polymerization. Only after that will it be possible to throw a clear light upon where and when life has originated.

In a previous paper (Kolomiytsev and Poddubnaya 2007), we have tried to answer the first two questions of the scheme. We have given reasons for believing that the evolutionary mechanism providing adaptive changes of material objects, i.e., a stable cumulative tendency in their minutest useful changes, is the very factor that makes living matter fundamentally different from nonliving. Switching on the mechanism, "biological selection" has been directly related to the acquisition of the property of inheritance (the specific high fidelity of replication) by self-reproducing nucleic acid polymers. Without inheritance, natural selection by itself, and even together with variability and a high rate of reproduction, would play a destructive rather than a creative role. No sooner had adaptive evolution begun to operate than there were no impossible things for the part of the material world involved in the process to improve its conditions of existence, if only the speed of environmental changes gave it enough time to adapt. Now we will try to find an answer to the third question of the scheme and to form a view about a material system in which the phenomenon of inheritance could appear for the first time.

The Appearance of "Accurate Replicators"

A diverse range of homochiral polymers that are amenable to template synthesis, i.e., such polymers whose monomers cannot only be joined in chains but can also selectively interact with each other by means of weak (e.g., hydrogen) bonds, may in principle be capable of sufficiently accurate self-reproduction via or without the intermediate stage of the reverse-complement sequence. However, speaking about the phenomenon of inheritance—whose emergence was the starting event of life—is worthwhile only in relation to those self-reproducing irregular heteropolymers that possess the capacity to carry the information required to specify their variable properties, such as the ability to copy itself more or less faithfully. To accomplish this, their chains should have sizes that allow them to encode sufficient information. Between two such polymers known to us, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), the relatively simpler one, being obligatory for all living forms without any exceptions, and, to all appearances, preceding DNA in evolution is ribonucleic acid. RNA is usually considered as having provided the first replicators, including those that have given rise to living matter (Eigen and Schuster 1979; Gilbert 1986; Diener 1989; Spirin 2002).

However, it is difficult to admit that the first homochiral RNAs were capable to replicate with sufficient accuracy to switch on the mechanism of adaptive evolution. In the literature, we find very little diversity in ideas about how the specific high fidelity of the RNA replication could have been achieved for the first time. Manfred Eigen appears to have been the first to study the problem. The concept of "catalytic hypercycle" proposed by Eigen (1971) and improved by him and Schuster (Eigen and Schuster 1979) postulates the possibility of the natural formation of particular systems that consist of a set of replicative complexes connected in a cycle by polypeptides (specific replicases or specific cofactors of a common protein with polymerase activity), which are translation products of the replicative complex RNAs. The translation product of each member of the cycle catalytically increases RNA replication rates of the following member, thereby providing opportunities for further structure improvements (and an increase in the replication fidelity) of the polynucleotides. However, the possibility of the evolutionary development of a hypercycle and the maintenance of its stability require that the RNA matrices connected in the cycle reproduce themselves with high enough fidelity, i.e., they evidently must already possess the capacity for adaptive evolution. It is also necessary to note that the principle of the hypercycle was primarily introduced to account for the circumstance that small RNA molecules having comparatively little information can evolve into a cooperative system that would provide a sufficient amount of information for the build-up of good enough translation and replication machinery. Most other ideas concerning the problem of how the high fidelity of polynucleotides replication was achieved are based in one way or another on Eigen's ideas. But the question is whether so much heritable information was necessary for the spontaneous generation of life.

We allow for the possibility that there was (and perhaps still is) some period in the history of our planet when its environment, at least somewhere in the "lost worlds," used to allow various complex chemicals, including relatively long

polypeptides and RNAs, to form spontaneously and coexist for some time. In living organisms, the complex forms of these biopolymers are interrelated in their formation (Eigen 1971). But there are valid reasons to believe that rather simple proteic fragments could be formed in the environment of the primitive Earth more easily than nucleotides (de Duve 1997; Morchio et al. 2001; Leman et al. 2004) and were already present when the latter appeared. This could have contributed to the formation of nucleotides or to the acceleration of such formation (Morchio et al. 2001). Noncatalytic condensation and inorganic catalysts also could have played a not so minor role in the synthesis of various biopolymers and their predecessors (Paecht-Horowitz et al. 1970; Rao et al. 1980; Wächtershäuser 1988; Ferris 1993; Ferris et al. 1996; Smith 1998; Martin and Russell 2003). However, no one has found conditions as yet that could result in the formation of ribonucleotides on the primitive Earth, and these compounds have never been created in prebiotic synthesis experiments (Zubay and Mui 2001). In experiments on the synthesis of RNA oligonucleotides, activated monomers obtained from living forms were always used (Ferris 1993, 2002; Ferris et al. 1996). At the same time, a feasible prebiotic pathway to the purines and effective conditions for phosphorylation and polyphosphorylation have been found in recent years (Lohrmann 1977; Joyce 1989; Zubay and Mui 2001). There are also some other advances in bioorganic synthesis. We should not believe that all the problems could be solved by modern biology.

Among various protein-like molecules synthesized in the "lost worlds," the ones possessing an enzymatic activity could take part in the assembly of a primitive, as yet uninformed peptide-synthesizing machinery (de Duve 1997), and others with multifunctional replicase activity could carry out—independently or in cooperation with the mineral catalysts, e.g., with clays (Rao et al. 1980; James and Ellington 1998; Ferris 2002)—the inexact and unrepeatable replication of some RNA polymers. As a result, the mixed pools of the various molecules of RNA, polypeptide, and primitive protein participating jointly in the template synthesis processes may have been generated and existed in these locations for some time.

The primitive proteins, including those that possess a replicase activity, could be the translation products only of the largest RNA molecules capable of encoding the required genetic information. These molecules, however, were able to provide less continuity to their properties through generations than others, because of the numerous copying errors (Eigen and Schuster 1979). We may hazard a conjecture that "genetic information" (data on the order of amino acids in the sequence of a particular polypeptide, encoded in the order of ribonucleotide bases in the sequence of RNA) may have occurred shortly before the appearance on the scene of the RNA molecule having the capacity to replicate with high accuracy. If it be so, the emergence of genetic information is not to be directly associated with the origin of life. Carriers of genetic information that do not possess inheritance can be called "non-heritable genes." Protein-like molecules with multifunctional replicase activity that arose from such RNAs became a common property of the pool and then could carry out the replication of any RNA molecules. In the mixed pools of polynucleotides and polypeptides, then, there could be an opportunity for reproducing both comparatively large molecules of RNA and small ones encoding reverse complementary copies of themselves and not encoding any proteins, i.e., in the strict sense of the term, carrying no genetic information. Already, because of a much smaller length, the latter RNAs were capable to replicate with greater accuracy (Eigen and Schuster 1979) and were much closer to the treasured replication fidelity than others. The one and only molecule that was replicated so accurately that it kept its basic properties—primarily the ability to replicate faithfully—over successive generations could occur among them. It may have been formed as a result of a rare coincidence of huge numbers of random events subject to the immutable laws of Nature. Thus, Eigen's paradox (Maynard Smith 1983) that "no enzymes without a large genome and no large genome without enzymes" could be circumvented to a certain extent. It is necessary to agree that the formation of the molecule and proteic fragments with weak replicase-like properties by the mechanism of conformal evolution (Kolomiytsev and Poddubnaya 2007) is not by far the same as the repeated reproduction of translational apparatuses instructed by a large genome.

As the fidelity of replication of such a molecule was determined mainly by the structure of its sequence as a whole, a lesser differentiation of the structural information (i.e., a substantial reduction of the contribution of separate structural elements in comparison with that in the sequence carrying genetic information) was allowed. Furthermore, the recent study of real ribozymes by Kun et al. (2005) has shown that even a minor increase in replication fidelity may have had a large effect on the size of the accurately replicated RNAs. Therefore, if the peculiar bioorganic–inorganic cooperative catalysts mentioned above were present in the environment, they could lead to an additional increase in RNA replication fidelity, thereby increasing the size of these molecules. Finally, it is not necessary to write off the fact that many of the mutations that arise in RNA sequences will be neutral or compensatory and have no effect on fitness (Holmes 2005; Kun et al. 2005). The combination of all these factors could raise the error threshold for switching on adaptive evolution ("repeated formation," according to Eigen) compared to what has been calculated (Eigen 1971; Eigen and Schuster 1979) for the sequences of nucleotides carrying the information about the order of amino acids in the sequences of certain proteins and replicating in the absence of enzymes. Hence, the length of the faithfully replicated RNA molecule could exceed 100 nucleotides. We will

call this an "accurate replicator" by analogy with Dawkins's "replicator." Spontaneity of its formation combined with a very low probability of recurrence of a similar event in a foreseeable time frame has defined both the sense of homochirality and the enantiomerical homogeneity of biological macromolecules.

If the replication rate of the accurate replicator was larger than its destruction rate, the replicator could quickly produce a clone of its not identical copies. Each of the copies was replicated in turn, and the structural variety of the molecules in the clone increased more and more due to relatively inaccurate replication. After some time, the individual and group diversity of such a clone did not have to differ substantially from the ones of the populations of asexual and unisexual cellular organisms. Within the limits of the clone, the favorable conditions for biological selection to operate were established. Owing to that, the opportunity to increase any of the accurate replicators' useful features cumulatively (no matter how poorly they were developed) appeared for the first time. Later, both separate accurate replicators and their groups could be transferred by currents in a liquid to other places and, if there was a suitable environment, produce new clones.

The Diffuse Organism

Only a very specific environment could be "suitable" for the first accurate replicators. It had to contain the "assembly lines" of all the precursors of RNAs and polypeptides, and also primitive replicative and translational apparatuses. Besides, the sources of available energy, even in the form of inorganic pyrophosphate (de Duve 1991), which is used as an alternative to adenosine triphosphate (ATP) in one of the metabolic reactions even by some current cellular organisms (Morgan and Ronimus 1998), had to be present in the environment in sufficient quantity. But that is not yet all. As is known, the synthesis of various amino acids, purinic and pyrimidinic nitrogen bases, ribose, nucleotides, and polypeptides, as well as RNA replication, require specific media, which, with rare exceptions, are not compatible with one another. Therefore, Darwin's "warm little pond" as well as a pond filled with self-copying RNA molecules and concentrated solutions of all the biochemical precursors of RNA (Gilbert and de Souza 1999) could scarcely exist. And because the different media cannot be combined in one area, the only imaginable way to solve the problem is the isolation by spatial separation of biochemical processes demanding a specific composition of local environments. Various bottom-dwelling mineral surfaces, submarine hydrothermal vents, and volcanoes (Ferris 1992; Wächtershäuser 1992; Leman et al. 2004; Washington 2004), the hydrocarbon layer that presumably covered the surface of the primordial ocean (Morchio and Traverso 1999), and many other factors could contribute to create the necessary environmental heterogeneity. Then the RNA building blocks and polypeptides that are synthesized at a different distance from a place of replication should be brought together by diffusion and a continual convection flux of fluid, as well as by water currents induced by actions of winds, tides, and the Coriolis force. Of course, the transfer of hydrophilic molecules through the aqueous parts of an environment could be possible only after formation by them of hydrophobic complexes with other organic or mineral substances. Only all these factors when combined would provide both the existence and the periodic self-reproduction of the first accurate replicators or, in Dawkins's (1989) terms, be their "survival machine." Actually, we are speaking here about a very rough analog of the structural–functional organization of the biosynthetic apparatus of a cell, the imperfection of which is compensated by its huge dimensions. This is similar to what we often see in products of industry. It is clear that such a survival machine had to be formed prior to the formation of the first accurate replicator, and, since the appearance of this entity, it had acquired a new quality, having become (together with the clone of accurate replicators) the first living system.

The presence of selective permeability barriers separating the specific local environments within which certain biochemical processes are performed, and some boundary coat, would only prevent water-soluble substances from freely passing between different regions of the system. This would thereby endanger the entire process of reproduction of accurate replicators, and without that, greatly depend on random circumstances. The compartmentalization of such a system could begin only simultaneously with the development of biochemical mechanisms of transport of small and particularly large molecules through compartments. But neither the one nor the other was necessary at this stage. As biological selection took place only among non-protein-coding templates, favoring their resistance to destruction and capacity to replicate more and more accurately and quickly, there was no necessity to link the templates with the products of their translation in some boundary coat, as was believed before (Gilbert and de Souza 1999).

Regarding the necessary concentration of chemicals needed for biochemical reactions, it can be provided not only by means of compartmentalization (Oparin 1957) but also, say, by evaporation in lagoons or seawater tidal pools (Robertson and Miller 1995; Nelson et al. 2001), or by the selective absorption of organic molecules from fluid onto mineral surfaces (Bernal 1951; Paecht-Horowitz et al. 1970; Dashman and Stotzky 1982; Wächtershäuser 1988; Ferris et al. 1996; Sowerby et al. 1998; Zubay 2000).

So, the first living system might be formed in some area of the open sea, a lagoon, or a surface, or an underground reservoir; but the size of a tiny cavity in a mineral, or closed membranous vesicle, or microsphere would be insufficient for that purpose. Because the system has no fixed boundaries and

its parts are diffused in space, we will call it "diffuse organism." This term has been used earlier by Ehrensvärd (1962) for the hypothetical initial stage of life, but we give it a somewhat different content.

The Simplest Modern Diffuse Organisms

Today, systems very similar to the first diffuse organism are formed by subviral, autonomously replicating entities-viroids (Diener 1971) when infecting the cells of some plants. Diener (1989), the discoverer of viroids, himself has assumed that, alongside other small plant pathogenic RNAs, they may be remnants of a precellular RNA world. Some other authors hold very similar views on viroids (Maizels and Weiner 1994; Semancik and Duran-Vila 1999; Flores 2001; Lehto and Karetnikov 2005; Koonin et al. 2006). And although many researchers doubt that ribozymes can catalyze the replication of RNA matrixes without the help of enzymes—a cornerstone of the RNA world concept—the idea of precellular life based on a low molecular weight RNA as the carrier of information looks most reasonable. Several reasons speak in favor of this hypothesis (Eigen and Schuster 1979; Jeffares et al. 1998; Gilbert and de Souza 1999; Joyce 2002; Gesteland et al. 2006; Koonin et al. 2006). The existence of viroids also points to this assumption.

Viroids have a particularly simple structure, do not possess protein-coding capacity (Davies et al. 1974; Hall et al. 1974), and some of them incorporate very simple ribozymes (Hutchins et al. 1986; Hernández and Flores 1992; Navarro and Flores 1997). Viroid nucleotide sequences are characterized by a high (commonly higher than 50%) content of guanine and cytosine (Trivedi et al. 2007), forming the most stable base-pairing structures (Eigen and Schuster 1979). Moreover, viroids are able to replicate autonomously in susceptible cells without any helper viruses (Diener 1979), are not capable of being integrated in the genomes of other organisms, have no ability to form a dormant stage, and are transmitted from diseased to healthy plants by contact, contaminated cultivating equipment, aphids, and through seed and pollen (Diener 1987; Elena et al. 1991). All this may point to the antiquity of viroid-like entities and their primary origin. Phylogenetic researches of viroids and viroid-like RNAs also revealed their very old origin from a common ancestor, most likely from "free-living" molecules (Elena et al. 1991, 2001). The most plausible explanation of viroid-like entities coming into existence today comes from this very hypothesis of their independent origin (Diener 2001; Trivedi et al. 2007).

After adaptation to an intracellular existence and the usurpation of a part of the very complicated biosynthetic machinery and highly effective biochemical transport mechanism of plant cells, modern viroidal diffuse organisms became much more compact. A living cell thus has replaced for them possibly a whole primordial ocean bay with its submarine hydrothermal vents, mineral surfaces, hydrocarbon layer, and so on, though has not confined them within cellular boundaries forever. Viroidal diffuse organisms, just like their ancestors, have no fixed systemic space. They can occupy both a part of a cell volume and, passing through the plasmodesmata, a number of neighboring cells, and, moving via the phloem vascular channels, a large part of a plant or an infected plant in its entirety (Palukaitis 1987; Ding et al. 1997; Zhu et al. 2002; Daròs et al. 2006).

Modern viroid RNAs are only 246–401 nucleotides in length (Flores 2001). The first accurate replicators should be even shorter, probably not more than 200 nucleotides, for the free-living diffuse organisms could not have effective proofreading mechanisms, and there is no doubt that their primitive polymerases copied RNAs with much lower fidelity than cellular DNA-dependent RNA polymerases. But even at such a small size, the resistance of the first diffuse organism RNAs to premature degradation could be provided, as well as the resistance of the modern viroid RNAs (Sänger et al. 1976; Gross et al. 1978; Diener 1989; Sanjuań et al. 2006), only by the high ratio of the highly stable G–C base pairs and the formation of single-stranded covalently closed (with a high degree of intramolecular base-pairing) circular structures. Besides, as the hydrophilic, accurate replicators were immersed in an aqueous environment, where most of the chemical and biochemical reactions took place, they had to be able to form ribonucleoprotein complexes in which protein-like molecules would give them a hydrophobic coat. There are data (Gómez and Pallás 2001, 2004; Owens et al. 2001; Ding et al. 2005) indicating that the viroid RNAs are moving in the phloem vascular system in such complexes, and presumably the complexes are the main form of existence of viroids, for water is the predominant substance in the nucleuses and chloroplasts.

The replication of present-day viroids entirely depends on the host enzyme systems, and it is known that the reproduction of members of the Avsunviroidae family can be carried out only by one nuclear-encoded chloroplastic RNA polymerase (Navarro et al. 2000; Daròs et al. 2006). But perhaps it was not always so. Like all modern organisms, viroids are products of about four billion years of adaptive evolution, during which they have inevitably changed greatly. Their ambience, it is believed, has radically changed as well. If this is true, diffuse organisms, at the onset of their evolution, might have been quite free-living; and their RNAs might have reproduced themselves (though not as stably and fast as the viroid RNAs), but in a less suitable environment, with the help of more simple and less specific catalysts, and via fewer steps. So, if the complementary copies of the first accurate replicators had very close functionality, the replication intermediate ("negative strand")

and processing might be missing. At the same time, at low concentration and poor diversification of protein-like molecules in the "primordial soup," some catalytic activity of accurate replicators could come in rather usefully. Probably, we found the relics of this activity in the ability of members of the Avsunviroidae family to cleave the multimeric replication products being formed by means of the rolling circle mechanism (Branch and Robertson 1981, 1984) into monomers through a hammerhead ribozyme contained in their RNA (Hutchins et al. 1986; Hernández and Flores 1992; Navarro and Flores 1997) and then to ligate the monomers without host plant enzymes, albeit with low efficiency, to yield mature circular viroid RNAs (Lafontaine et al. 1995; Baumstark et al. 1997; Côté et al. 2001). These viroids can be considered as very close to the common ancestor of all viroids and viroid-like satellite RNAs (Elena et al. 1991; Diener 1995).

During the formation of new diffuse organisms, the information molecules of several preexisting ones might sometimes occur in the same area and jointly undergo natural selection. Thus, long before the appearance of a sexual process, this has probably allowed the diffuse organisms inhabiting in the vicinity to reach a high degree of unification of the structure of accurate replicators and increase considerably the rate of their evolution. Today, such events must take place every time a plant organism is simultaneously infected with two or more viroidal RNAs. Something similar has been shown in site-directed mutagenesis experiments in which the clones of viroids were subjected to selective pressure (Diener 1995). The intense processes of interclonal and intermolecular recombination of viroidal RNAs, combined with the lack of proofreading mechanisms, establishes viroids as the most rapidly evolving biological system known (Elena et al. 1991; Diener 1995).

The Possible Evolution of Diffuse Organisms

Since, from the very beginning, the necessary properties of accurate replicators could be defined mainly by their structure, an increase in fidelity and rate of their self-replication on the one hand and an increase in the accurate replicators' resistance to degradation on the other could be among few possible directions of adaptive evolution of the first diffuse organisms. Essential progress in the first direction could not be achieved without an additional expansion of the information content of the replicating molecules (Eigen 1971). If so, first the very small size of the faithfully replicated RNAs would require the loss of functional equivalence of the complementary polynucleotide strands, and then their progressive specialization. The one complementary strand (so-called positive strand) might increase its own resistance to environmental changes, while maintaining the ability to replicate accurately, thereby becoming a more stable repository for information and simultaneously expanding the clone capability to spread in space. At the same time, the other complementary strand (also called negative strand), being involved in this process to a lesser degree, might increase its rate of transcription. Such a functional differentiation of the complementary chains of accurate replicators, which has begun at viroidal diffuse organisms, has to be considered as the first "major transition" in biological evolution. A subsequent increase in the specialization of the complementary RNA strands to perform certain functions (hence an expansion of their information content) could be achieved only by means of their elongation. It needs to take into account an increase in structural complexity of a linear polymer, which due to its elongation has the feature that it leads not only to an increase in the intrinsic energy of the system but also to an increase in its conformational possibilities. Quite often, some of the new conformations of longer polynucleotide chains are thermodynamically more stable than those of shorter ones. This effect can be enhanced by complex formation of RNA with oligopeptides and proteins as it takes place in viroids. However, the possibilities of thermodynamic optimization of a polymeric molecule are not boundless, and therefore the adaptive evolution of the rather small accurate replicators had to run into insuperable limitations very early. As we can see, for billions of years, the structural and functional organization of viroids has undergone very small changes in comparison with the ones of other organisms. By means of biological selection, the RNAs of viroids have probably only increased a little the fidelity and rate of self-replication and adapted to the intracellular environment of some plants. Hardly probable, it is possible to imagine something simpler and at the same time capable to adaptive evolution. In comparison with viroids, the viruses and simplest cellular life forms seem too complex to arise spontaneously, even in a very favorable environment.

It is clear that not only the formation of a viroidal diffuse organism but also its long existence depends on many random circumstances. Therefore, from the beginning of adaptive evolution, the dependence of the accurate replicators' reproduction on unpredictable changes of the remaining part of the diffuse organism had to decrease progressively. This might maintain the trend toward the formation of the accurate replicators' own (instructed by them) biosynthetic machinery, first in addition to the one already available in the environment and then in replacement of them. This sequence of events seems more plausible than the stage-by-stage transition of the somatic part under the control of the information part, the mechanism of which is scarcely imaginable. Thus, the following stage of the diffuse organism's evolution was apparently connected with the appearance *de novo* of potentially continuous "true genetic information" and its carriers, i.e., genes. This could occur only through the significant elongation of the accurate replicators' chains that in turn could be achieved, for example, as a result of disturbance of the cleavage of the multimeric

Table 1. The major transitions on early stages of life's evolution.

Accurate replicators (RNAs) the complementary chains of those have very close functionality	→	Accurate replicators (RNAs) the complementary chains of those have different functionality
RNAs encoding reverse complementary copies of themselves and not encoding any proteins	→	RNAs encoding proteins (genetic code)
RNA as a main repository for genetic information	→	DNA as a main repository for genetic information
Diffuse organisms	→	Prokaryotes

product of rolling circle transcription, resulting in tandem repeats of the unit length of the replicator sequence (Diener 1989, 1991). During the first stages of their evolution, the elongated accurate replicators might have encoded polypeptides without enzymatic activities (Poole et al. 1998) but able to interact with the replicators relatively easily. Such an interaction would provide some mutual stability to both macromolecules against hydrolysis (Wicken 1987). It would be quite enough that the encoding of polypeptides might be favored by biological selection, and hence receive further development. As soon as the incorporation of the information and somatic parts of a diffuse organism into the common unit of natural selection has begun, the structural and functional features of the protein component began to mediate the selective value of accurate replicators more and more. Using Maynard Smith and Szathmáry's (1997) terminology, it was the second "major transition" in the evolution of living matter, whose importance far exceeds that of many other evolutionary events. We tend to believe that the size limitation of genotypes set by the error rate during replication (the "Eigen limit") could be overcome only during the adaptive evolution of the replicators, viz., when life had already begun. When accurate replicators became the carriers of the true genetic information, they had the possibility to accumulate and correct that information during biological selection. It was the beginning of the era of viral diffuse organisms with their already nearly unlimited evolutionary opportunities. The polynucleotide sequences of mimivirus (Raoult et al. 2004), which in many respects may be considered the apex of the evolution of diffuse organisms, contain numerous genes encoding central protein translation components, six tRNAs, both type I and type II topoisomerases, components of all DNA repair pathways, many polysaccharide synthesis enzymes, and one intein-containing gene. It is the encoding of the data on the structure of enzymes and tRNA-like adapters in the order of ribonucleotide bases in the accurate replicators that enabled the coordinated adaptive evolution of all components of the organism. From this moment on, the transition to DNA as a main repository for genetic information and, later, the emergence of the first cellular organism became only a matter of time.

So, taking into account our hypothesis of the origin of life, the beginning of the list of the major stages in the evolution of complexity, and the transitions between them will be as given in Table 1.

Viruses seem to play a critical role in several major evolutionary transitions (Forterre 2006); however, their limited genome capabilities have not allowed viral diffuse organisms to evolve complex enough biosynthetic apparatuses and start a compartmentalization. The preservation of the structural and functional organization of these organisms was probably determined in many respects by their adaptation to parasitizing on the biosynthetic apparatuses of cellular life forms, which has essentially limited their evolutionary requirements.

Information–Somatic Structural Duality of Diffuse Organisms

From the moment of their appearance on, diffuse organisms had to consist of an information and somatic part that is characteristic of all living things. The first of them is the clone of self-replicating, low molecular weight RNAs in the nucleotide sequences of which the information on structural features required for the faithful replication (and probably some other, not numerous properties of these entities) is stored. Many researchers believe that the nucleotidic sequences of modern viroids contain some more information on some functions linked with infection—in particular, with the cell-to-cell movement of viroidal RNAs (Ding et al. 1997; Zhu et al. 2001, 2002; Qi et al. 2004).

The somatic part of the diffuse organisms consists of a set of specific local environments integrated by a common circulating liquid medium. Each of the local environments in turn involves the "assembly lines" of one or several substances needed for the existence and sufficiently accurate self-reproduction of the information molecules.

The structural and functional organization of the diffuse organisms is in conformity with the common principle of organization of all living things and at the same time has a distinguishing feature: The information part of these organisms depends still one-sidedly on the somatic, which acts in relation to accurate replicators essentially as a part of their environment common for all members of the clone. Hence, from the moment of their appearance, the accurate replicators parasitized on the somatic part of diffuse organism. But, such a broad interpretation of "parasitism" can be applied to the relationships between the population of any cellular organism and the abiotic component of its environmental niche (relationships

between the population and the biotic components are more diverse owing to coevolution).

Organism–Population Duality of Diffuse Organisms

The accurate replicators of a diffuse organism reproduce themselves independently from each other. During this process they inevitably vie for the same resources in the somatic part. Thus, we find here intra-organismic (intermolecular) selection, the units of which are RNA molecules capable of self-replicating with high fidelity or later, at viral stage of evolution, nucleic acid molecules together with their translation products that begin to mediate the selective value of these molecules. Hence, each clone of accurate replicators is an elementary evolutionary system, the analog of an ecological population. Various other nucleic acids molecules that are present in a diffuse organism, as well as the products of their translation, self-assembled polypeptides, carriers of chemical energy, and so on, do not depend on accurate replicators in their evolutionary changes. However, all these substances provide the survival and successful self-reproduction of accurate replicators and, together with them, carry out organismal functions. Thus, the diffuse organism is an organismal and evolving system at the same time. It seems that only such a system that has emerged in the only specimen as a result of a set of chance events operating under a system of universal physical and chemical laws was able to give rise to life and evolution by means of biological selection.

Later during adaptive evolution, the accurate replicators of diffuse organisms could evolve into individuals within which the synthesis of all substances necessary for metabolism, which were formed in the nonliving nature with difficulty, or not at all, began to occur. However, even the most perfect organisms, like their predecessors, need water, minerals, energy, and so on, which are supplied from outside. Since some of these resources can be very scarce in the environment, the individuals of the same populations also often enter into a competition for them. This competition additionally increases the pressure of natural selection on the organisms. Besides, the individuals form symbiotic relationships with other organisms that have become absolutely necessary (obligate) for many of them to provide the certain processes of vital activity. Thus, the populations of cellular organisms and parts of their environments form systems in which, at a new level of living matter organization, the principle of the diffuse organism's organization is repeated.

The Diffuse Organism Among Other Hypotheses About the Origin of Life

Most of the modern hypotheses of the origin of life and variations on these hypotheses fall into one of the following three major groups: "metabolism first," "replicator-first," and "double-origin hypothesis" (Table 2).

The hypotheses of the first group follow Oparin's coacervate theory. They postulate that the origin of life was connected with the emergence of a primitive metabolism, and the metabolic processes assembled prior to the existence of replicators. The network of biosynthetic and metabolic pathways is obligatorily enclosed in coacervate droplets, mineral compartments, or lipid or protein microspheres (therefore the most of the "cell first" hypotheses belongs to this group). It is supposed that such entities have the capability to grow, reproduce, and even evolve.

In contrast with the "metabolism first" model, the "replicator-first" scenario, known as the RNA world hypothesis (Gilbert 1986), proposes a single biopolymer, probably RNA, acting as both a replicator (therefore providing the continuity of structural information) and a catalyst for its own assembly and replication. By now, researchers have constructed several biosynthetic pathways of synthesis of all the biochemical precursors of RNA and offered various hypothetical scenarios of the RNA world (see, for example, Orgel 2004; Gesteland et al. 2006).

The "double-origin hypothesis," traceable to Oparin's ideas, tries to combine eclectically the "metabolism first" scenario for the emergence of life with the elements of the RNA world. According to this model, life began with protein-based systems, proto-cells reproducing by statistical division, which were later "infected" by parasitic nucleic acids (Dyson 2000). Experiments have shown that when liposomes are dried in the presence of macromolecules, the latter are captured upon rehydration (Deamer 1997). The problem is that the mechanism of gradual transition of the metabolic processes and the synthesis of the vesicle membrane under the control of the information molecules seems scarcely imaginable.

Our conception of the "diffuse organism" is of course near to the RNA world model, but unlike the latter it takes into consideration the following facts: (1) polypeptides are synthesized easier than polyribonucleotides; (2) only relatively large RNAs with complex tertiary structure, the formation of which in turn requires complex catalysts, can be ribozymes with high catalytic activity; (3) only very small RNA molecules could be formed spontaneously or with the help of very simple catalysts; (4) pools of such molecules could exist only within the limits of a certain system providing their regular reproduction and resistance to premature degradation; (5) the synthesis of various organic compounds requires specific media, which, with rare exception, are not compatible with one another; and (6) the structural organization of the first living systems should be analogous to the structure of the simplest modern living beings.

All the scenarios had to have starts, of course, from simple organic molecules formed by prebiotic processes.

Table 2. Comparison of hypotheses about the origin of life.

Hypotheses	The Initial Stage of Life	Supporting Reasons	Problems and Difficulties
"Metabolism first"	The network of biosynthetic and metabolic pathways operating without needing any information molecules, which are enclosed in any compartments.	The proteic fragments can be formed easier than nucleotides. Proteins are the most efficient catalysts known. Membranes and walls protect complex molecules from dispersion in dilute solutions.	Without the high fidelity of the entities' reproduction there is no ensuring adaptive evolution. Membranes and mineral walls prevent water-soluble substances from freely passing between living system and the environment.
"Replicator-first"	Pools of a single biopolymer, which have served both informational and catalytic roles, adsorbed on a mineral surface or enclosed in mineral compartments.	The discovery of RNA enzymic activity and the creation of artificial ribozymes. There is no need for protein enzymes and a large genome. Currently, various RNAs play key roles in the maintenance of vital functions of all living things without exception.	RNA is difficult to synthesize abiotically. The chemical fragility of RNA.
"Double-origin hypothesis"	A protein-based proto-cell reproducing by statistical division, which was later "infected" by parasitic nucleic acids.	The capability of incorporation of macromolecules into liposomes has been experimentally shown.	The mechanism of gradual transition of metabolic processes under the control of information molecules seems scarcely imaginable.
"Diffuse organism"	A particular material system representing a set of specific local environments integrated by a common circulating liquid medium where relatively short RNAs are replicated with great accuracy, and evolve.	Eigen's paradox, "no enzymes without a large genome and no large genome without enzymes," could be, to a certain extent, circumvented. Very similar systems are formed by viroids at infecting the cells of some plants.	As yet, we know of no example of a viroid able to live and evolve on its own.

Conclusion

There are good reasons to believe that life may have arisen within the limits of a particular material system representing a set of specific local environments integrated by a common circulating liquid medium where a clone of relatively short RNAs (about 200 nucleotides), which are replicated with great accuracy and encoding no proteins, existed. In the clone, the conditions required for adaptive evolution to operate were established for the first time, and in each of the local environments, the synthesis of certain substances, which are required for accurate replication and survival of the RNAs, are carried out. That is to say, in such a system, the complexes of functions that today are considered as organismic are performed. Such a system, which can be called "diffuse organism," is, in essence, a very rough and bulky analog of the structural–functional organization of the cell's biosynthetic machinery. The diffuse organism may have originated by conformal evolution (Kolomiytsev and Poddubnaya 2007) somewhere in the unique environment of our planet. For this to happen, an extremely rare coincidence of very many favorable events that are a rare occurrence but real nonetheless was certainly required. Such a single system was able to give rise to life and adaptive evolution because of its organism–population duality. During adaptive evolution, diffuse organisms may have changed noticeably, and in viruses, even began the formation of components of the biosynthetic apparatus.

The colonization of the cellular forms of life that have emerged by then and the usurpation of the cells' biosynthetic machinery (accompanied by the likely secondary loss of some primordial components) could allow these living systems to become much more compact and carry out organismal functions more effectively. As a result, diffuse organisms have not disappeared from the face of the Earth and quite successfully coexist with their more perfect progenies and demonstrate a surprising persistence in various environments and extensive adaptive radiation (Breitbart and Rohwer 2005). Today they bring to us the echo of the initial stages of the animation of matter again and again during their formation in viroid and virus infections. Thus, the diffuse organisms are, in many respects, so peculiar among life forms that they should be assigned to the highest taxonomic category, which ought to be placed in the root of a comprehensive phylogenetic tree of life.

This hypothesis, as well as all the other origin of life concepts without exception, remains beyond the reach of

experimental verification and, as well as the others, involves speculation. As yet, we know of no example of a viroid able to live and evolve on its own, but any samples of protobiont, hypercycle, or RNA world have never been found in nature and have never been produced artificially as well.

At the same time, the individual components of our hypothesis can be considered as tested. So, all the ideas of RNA world incorporated in the hypothesis either have been tested experimentally or there is some real hope of deciding whether they are true or false of real experience (Orgel 2004; Gesteland et al. 2006). Reproduction in the "diffuse organism" model of the principle of the structural organization of the systems that are formed by viroids and viruses at infecting the cells can, to a certain extent, also be considered as the test of the hypothesis.

The hardest problem all researchers face is the abiotic synthesis of RNA molecules and their monomers. In order to achieve its solution, it will likely be required to form a maximum realistic idea of how the great diversity of precursors of biological macromolecules could come about naturally from nonliving matter and how the first accurate replicators and enzymes were generated.

The scenario of the origin of life outlined here allows us to narrow down the still insuperable gap between prebiological chemistry and the first accurate replicators without devising conceptions unrelated to the realities of life. Our hypothesis also suggests a possible direction to solve the chicken-and-egg dilemma of how the first life carriers could come into existence in the absence of complex catalytic machineries by lowering the level of complexity of self-replicating molecules and, so doing, bring together the concepts already in existence.

Acknowledgments

We are grateful to the members of the Department of Biology, Cherepovets State University, for informative discussions, and to our students for asking good questions and inspiring us to write this article. We would also like to thank two anonymous reviewers who suggested very useful structural changes, which have allowed us to improve the manuscript substantially.

References

Baumstark T, Schroder ARW, Riesner D (1997) Viroid processing: Switch from cleavage to ligation is driven by a change from a tetraloop to a loop E conformation. EMBO Journal 16: 599–610.

Bernal JD (1951) The Physical Basis of Life. London: Routledge and Kegan Paul.

Branch AD, Robertson HD (1981) Longer-than-unit-length viroid minus strands are present in RNA from infected plants. Proceedings of the National Academy of Sciences of the USA 78: 6381–6385.

Branch AD, Robertson HD (1984) A replication cycle for viroids and other small infectious RNAs. Science 223: 450–454.

Breitbart M, Rohwer F (2005) Here a virus, there a virus, everywhere the same virus? Trends in Microbiology 13: 278–284.

Côté F, Lévesque D, Perreault JP (2001) Natural 2′, 5′-phosphodiester bonds found at the ligation sites of peach latent mosaic Viroid. Journal of Virology 75: 19–25.

Daròs J-A, Elena SF, Flores R (2006) Viroids: An Ariadne's thread into the RNA labyrinth. EMBO Reports 7: 593–598.

Dashman T, Stotzky G (1982) Adsorption and binding of amino acids on homoionic montmorillonite and kaolinite. Soil Biology and Biochemistry 14: 447–456.

Davies JW, Kaesberg P, Diener TO (1974) Potato spindle tuber viroid. XII. An investigation of viroid RNA as a messenger for protein synthesis. Virology 61: 281–286.

Dawkins R (1989) The Selfish Gene. 2nd ed. New York: Oxford University Press.

Deamer DW (1997) The first living systems: A bioenergetic perspective. Microbiology and Molecular Biology Reviews 61: 239–261.

de Duve C (1991) Blueprint for a Cell: The Nature and Origin of Life. Burlington, NC: Neil Patterson Publishers.

de Duve C (1997) RNA without protein or protein without RNA? In: What is Life? The Next Fifty Years: Speculations on the Future of Biology (Murphy MP, O'Neill LAJ, eds), 79–82. Cambridge, UK: Cambridge University Press.

Diener TO (1971) Potato spindle tuber "virus" IV. A replicating low molecular weight RNA. Virology 45: 411–428.

Diener TO (1979) Viroids: Structure and function. Science 205: 859–866.

Diener TO (1987) Potato spindle tuber. In: The Viroids (Diener TO, ed), 221–223. New York: Plenum Press.

Diener TO (1989) Circular RNAs: Relics of recellular evolution? Proceedings of the National Academy of Sciences of the USA 86: 9370–9374.

Diener TO (1991) Subviral pathogens of plants: Viroids and viroid-like satellite RNAs. FASEB Journal 5: 2808–2813.

Diener TO (1995) Origin and evolution of viroids and viroid-like satellite RNAs. Virus Genes 11: 119–131.

Diener TO (2001) The viroid: Biological oddity or evolutionary fossil? Advances in Virus Research 57: 137–184.

Ding B, Itaya A, Zhong X (2005) Viroid trafficking: A small RNA makes a big move. Current Opinion in Plant Biology 8: 606–612.

Ding B, Kwon M-O, Hammond R, Owens R (1997) Cell-to-cell movement of potato spindle tuber viroid. Plant Journal 12: 931–936.

Dyson F (2000) The Origins of Life, 2nd ed. Cambridge, UK: Cambridge University Press.

Ehrensvärd G (1962) Life: Origin and Development. Chicago, IL: University of Chicago Press.

Eigen M (1971) Self-organization of matter and the evolution of biological macromolecules. Naturwissenschaften 58: 465–523.

Eigen M, Schuster P (1979) The Hypercycle: A Principle of Natural Self-Organization. New York: Springer.

Elena SF, Dopazo J, de la Penã M, Flores R, Diener TO, Moya A (2001) Phylogenetic analysis of viroid and viroid-like satellite RNAs from plants: A reassessment. Journal of Molecular Evolution 53: 155–159.

Elena SF, Dopazo J, Flores R, Diener TO, Moya A (1991) Phylogeny of viroids, viroid-like satellite RNAs, and the viroid-like domain of hepatitis δ virus RNA. Proceedings of the National Academy of Sciences of the USA 88: 5631–5634.

Ferris JP (1992) Marine hydrothermal systems and the origin of life: Chemical markers of prebiotic chemistry in hydrothermal systems. Origins of Life and Evolution of the Biosphere 22: 109–134.

Ferris JP (1993) Catalysis and prebiotic RNA synthesis. Origins of Life and Evolution of the Biosphere 23: 307–315.

Ferris JP (2002) Montmorillonite catalysis of 30–50 mer oligonucleotides: Laboratory demonstration of potential steps in the origin of the RNA world. Origins of Life and Evolution of the Biosphere 32: 311–332.

Ferris JP, Hill AR, Liu R, Orgel LE (1996) Synthesis of long prebiotic oligomers on mineral surfaces. Nature 381: 59–61.

Flores R (2001) A naked plant-specific RNA ten-fold smaller than the smallest viral RNA: The Viroid. Comptes Rendus de l'Académie des Sciences de Paris. Sciences de la vie/Life Sciences 324: 943–952.

Forterre P (2006) The origin of viruses and their possible roles in major evolutionary transitions. Virus Research 117: 5–16.

Gesteland RF, Cech TR, Atkins JF, eds (2006) The RNA World: The Nature of Modern RNA Suggests a Prebiotic RNA World, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Gilbert W (1986) The RNA world. Nature 319: 618.

Gilbert W, de Souza SJ (1999) Introns and the RNA world. In: The RNA World, 2nd ed (Gesteland RF, Cech TR, Atkins JF, eds), 221–231. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Gómez G, Pallás V (2001) Identification of an in vitro ribonucleoprotein complex between a viroid RNA and a phloem protein from cucumber plants. Molecular Plant-Microbe Interactions 14: 910–913.

Gómez G, Pallás V (2004) A long-distance translocatable phloem protein from cucumber forms a ribonucleoprotein complex *in vivo* with hop stunt viroid RNA. Journal of Virology 78: 10104–10110.

Gross HJ, Domdey H, Lossow Ch, Jank P, Raba M, Alberty H, Sänger HL (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. Nature 273: 203–208.

Hall TC, Wepprich RK, Davies JW, Weathers LG, Semancik JS (1974) Functional distinctions between the ribonucleic acids from citrus exocortis viroid and plant viruses: Cell-free translation and aminoacylation reactions. Virology 61: 486–492.

Hernández C, Flores R (1992) Plus and minus RNAs of peach latent mosaic viroid self-cleave *in vitro* via hammerhead structures. Proceedings of the National Academy of Sciences of the USA 89: 3711–3715.

Holmes HC (2005) On being the right size. Nature Genetics 37: 923–924.

Hutchins CJ, Rathjen PD, Forster AC, Symons RH (1986) Self-cleavage of plus and minus RNA transcripts of avocado sunblotch viroid. Nucleic Acids Research 14: 3627–3640.

James KD, Ellington AD (1998) Catalysis in the RNA world. In: The Molecular Origins of Life (Brack A, ed), 269–294. Cambridge, UK: Cambridge University Press.

Jeffares DC, Poole AM, Penny D (1998) Relics from the RNA world. Journal of Molecular Evolution 46: 18–36.

Joyce GF (1989) RNA evolution and the origins of life. Nature 338: 217–224.

Joyce GF (2002) The antiquity of RNA-based evolution. Nature 418: 214–221.

Kolomiytsev NP, Poddubnaya NY (2007) The origin of life as a result of changing the evolutionary mechanism. Rivista di Biologia/Biology Forum 100: 11–16.

Koonin EV, Senkevich TG, Dolja VV (2006) The ancient virus world and evolution of cells. Biology Direct 1: 29.

Kun A, Santos M, Szathmary E (2005) Real ribozymes suggest a relaxed error threshold. Nature Genetics 37: 1008–1011.

Lafontaine D, Beaudry D, Marquis P, Perreault J-P (1995) Intra- and intermolecular nonenzymatic ligations occur within transcripts derived from the peach latent mosaic viroid. Virology 212: 705–709.

Lehto K, Karetnikov A (2005) Relicts and models of the RNA world. International Journal of Astrobiology 4: 33–41.

Leman L, Orgel L, Ghadiri MR (2004) Carbonyl sulfide-mediated prebiotic formation of peptides. Science 306: 283–286.

Lohrmann R (1977) Formation of nucleoside-50-phosphoramidates under potentially prebiological conditions. Journal of Molecular Evolution 10: 137–154.

Maizels N, Weiner AM (1994) Phylogeny from function: Evidence from the molecular fossil record that tRNA originated in replication, not translation. Proceedings of the National Academy of Sciences of the USA 91: 6729–6734.

Martin W, Russell MJ (2003) On the origin of cells: A hypothesis for the evolutionary transformation from abiotic geochemistry to chemoauthotrophic prokaryotes, and from prokaryotes to nucleated cells. Philosophical Transactions of the Royal Society B: Biological Sciences 358: 59–85.

Maynard Smith J (1983) Models of evolution. Proceedings of the Royal Society B: Biological Sciences 219: 315–325.

Maynard Smith J, Szathmáry E (1997) The Major Transitions in Evolution. New York: Oxford University Press.

Morchio R, Redaelli A, Traverso S (2001) Proteins, nucleic acids and genetic codes. Rivista di Biologia/Biology Forum 94: 7–57.

Morchio R, Traverso S (1999) The hydrophobic superficial layer: The primordial cradle of life? Rivista di Biologia/Biology Forum 92: 105–118.

Morgan HW, Ronimus RS (1998) Pyrophosphate-dependent phosphofructokinase in thermophilic and non-thermophilic microorganisms. In: Thermophiles: The Key to Molecular Evolution and the Origin of Life (Adams MW, Wiegel JW, eds), 269–278. London: Taylor and Francis.

Navarro B, Flores R (1997) Chrysanthemum chlorotic mottle viroid: Unusual structural properties of a subgroup of viroids with hammerhead ribozymes. Proceedings of the National Academy of Sciences of the USA 94: 11262–11267.

Navarro J-A, Vera A, Flores R (2000) A chloroplastic RNA polymerase resistant to tagetitoxin is involved in replication of avocado sunblotch viroid. Virology 268: 218–225.

Nelson KE, Robertson MP, Levy M, Miller SL (2001) Concentration by evaporation and the prebiotic synthesis of cytosine. Origins of Life and Evolution of the Biosphere 31: 221–229.

Oparin AI (1957) The Origin of Life on the Earth, 3rd ed. New York: Academic Press.

Orgel LE (2004) Prebiotic chemistry and the origin of the RNA world. Critical Reviews in Biochemistry and Molecular Biology 39: 99–123.

Owens RA, Blackburn M, Ding B (2001) Possible involvement of the phloem lectin in long-distance viroid movement. Molecular Plant-Microbe Interactions 14: 905–909.

Paecht-Horowitz M, Berger J, Katchalsky A (1970) Prebiotic synthesis of polypeptides by heterogeneous polycondensation of amino-acid adenylates. Nature 228: 636.

Palukaitis P (1987) Potato spindle tuber viroid: Investigation of the long-distance, intra-plant transport route. Virology 158: 239–241.

Poole AM, Jeffares D, Penny D (1998) The path from the RNA world. Journal of Molecular Evolution 46: 1–17.

Qi Y, Pélissier T, Itaya A, Hunt E, Wassenegger M, Ding B (2004) Direct role of a viroid RNA motif in mediating directional RNA trafficking across a specific cellular boundary. Plant Cell 16: 1741–1752.

Rao M, Odom DG, Orò J (1980) Clays in prebiological chemistry. Journal of Molecular Evolution 5: 317–331.

Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Suzan M, Claverie J-M (2004) The 1.2-megabase genome sequence of Mimivirus. Science 306: 1344–1350.

Robertson MP, Miller SL (1995) An efficient prebiotic synthesis of cytosine and uracil. Nature 375: 772–774.

Sänger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK (1976) Viroids are single-stranded covalently closed circular RNA molecules existing as highly basepaired rod-like structures. Proceedings of the National Academy of Sciences of the USA 73: 3852–3856.

Sanjuań R, Forment J, Elena SF (2006) *In silico* predicted robustness of viroids RNA secondary structures. I. The effect of single mutations. Molecular Biology and Evolution 23: 1427–1436.

Semancik JS, Duran-Vila N (1999) Viroids in plants: Shadows and footprints of a primitive RNA. In: Origin and Evolution of Viruses (Domingo E, Webster R, Holland J, eds), 37–64. London: Academic Press.

Smith JV (1998) Biochemical evolution. I. Polymerization on internal, organophilic silica surfaces of dealuminated zeolites and feldspars. Proceedings of the National Academy of Sciences of the USA 95: 3370–3375.

Sowerby SJ, Edelwirth M, Heckl WM (1998) Self-assembly at the prebiotic solid-liquid interface: Structure of self-assembled monolayers of adenine and guanine bases formed on inorganic surfaces. Journal of Physical Chemistry 102: 5914–5922.

Spirin AS (2002) Omnipotent RNA. FEBS Letters 530: 4–8.

Trivedi S, Gunwant S, Swati P (2007) Analysis of similarities between viroid, prokaryote and eukaryote genomes to revisit theories of origin of viroids. Journal of Cell and Molecular Biology 6: 9–18.

Washington J (2004) The possible role of volcanic aquifers in prebiologic genesis of organic compounds and RNA. Origins of Life and Evolution of the Biosphere 30: 53–79.

Wächtershäuser G (1988) Before enzymes and templates: Theory of surface metabolism. Microbiological Reviews 52: 452–484.

Wächtershäuser G (1992) Groundworks for an evolutionary biochemistry: The iron-sulfur world. Progress in Biophysics and Molecular Biology 58: 85–201.

Wicken JS (1987) Evolution, Information and Thermodynamics: Extending the Darwinian Program. New York: Oxford University Press.

Zhu Y, Green L, Woo Y-M, Owens R, Ding B (2001) Cellular basis of potato spindle tuber viroid systemic movement. Virology 279: 69–77.

Zhu Y, Qi Y, Xunm Y, Owens R, Ding B (2002) Movement of potato spindle tuber viroid reveals regulatory points of phloem-mediated RNA traffic. Plant Physiology 130: 138–146.

Zubay G (2000) Origin of Life on the Earth and in the Cosmos, 2nd ed. San Diego, CA: Academic Press.

Zubay G, Mui T (2001) Prebiotic synthesis of nucleotides. Origins of Life and Evolution of the Biosphere 31: 87–102.