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Question 5: On the Chemical Reality of the RNA World

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Abstract The discovery of catalytic RNA has revolutionised modern molecular biology and bears important implications for the origin of Life research. Catalytic RNA, in particular self-replicating RNA, prompted the hypothesis of an early "RNA world" where RNA molecules played all major roles such information storage and catalysis. The actual role of RNA as primary actor in the origin of life has been under debate for a long time, with a particular emphasis on possible pathways to the prebiotic synthesis of mononucleotides; their polymerization and the possibility of spontaneous emergence of catalytic RNAs synthesised under plausible prebiotic conditions. However, little emphasis has been put on the chemical reality of an RNA world; in particular concerning the chemical constrains that such scenario should have met to be feasible. This paper intends to address those concerns with regard to the achievement of high local RNA molecules concentration and the aetiology of unique sequence under plausible prebiotic conditions.

Keywords Origin of life · RNA world · Prebiotic synthesis of mononucleotides · RNA sequence space · RNA compartmentation

Introduction

The discovery of the catalytic properties of RNA molecules (Cech et al. 1981; Guerrier-Takada et al. 1983; Zaug and Cech 1980) has prompted the idea that at same stage in early evolution of life, RNA might have played the role of both information storage molecule and catalysts (Gilbert 1986; Zaug and Cech 1980). Scientists have speculated about plausible

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RNA systems capable of self-replication, heritable variations and increase complexity which would synthesise all the major feature of "living" systems in one molecule.

The debate about the reality of the RNA world hypothesis has stirred a tough debate over the last twenty years with a particular emphasis on:

- possible pathways to the prebiotic synthesis of reactive mononucleotides;
- the oligomerization or polymerization of reactive monomers;
- the possibility of spontaneous emergence of catalytic RNAs synthesised under plausible prebiotic conditions,
- the possibility of spontaneous emergence of catalytic network capable of sustain a life-like system

However, little effort has been done to put the RNA world hypothesis under the light of chemical constrains. Indeed, as it is stated in the editor question: "when one puts chemical constraints to this view [the RNA world, author's note], one realizes that self-replication cannot be achieved by one single molecule (it needs at least two), and generally for any workable chemical system one needs RNA local concentrations of at least femtomoles – which still means billions of identical copies of this compounds (and larger concentrations of the mono-nucleotides)."

The straightforward consequence of this statement is that RNA world has to be considered a product of a previous cellular metabolism capable of synthesising substantial amount of mononucleotide and to lead their polymerization in an ordered fashion.

Our thesis is that the RNA world hypothesis suffers a number of bottlenecks mainly concerning the robustness of plausible prebiotic synthetic routes rather than the achievement of high local concentration of monomers or the synthesis of identical copies of a given sequence.

In this paper we intend to address these issues reviewing scientific literature in order to shed light on the chemical reality of an RNA-based system in the prebiotic scenario.

Making Sense Out of the Soup

The first issue we would like to address concerns the local concentration of monomers required to set up an RNA-based world. An exhaustive review of mononucleotides synthetic routes is beyond the scope of this article, however local concentration of mononucleotides is intimately linked to their synthesis under prebiotic conditions.

Several different reactions have been proposed for the production of mononucleotides, ranging from the synthesis of nitrogen-bases (Oro 1960; Robertson and Miller 1995) to the synthesis of sugars (Reid and Orgel 1967; Socha et al. 1980) and their condensation to form reactive mononucleotides (Fuller et al. 1972). The vast majority of those reactions occur in aqueous solution, i.e. the "warm pond", and it is implicitly assumed that chemical products are slowly accumulated over thousands or millions of years, eventually reacting further to produce ancestral biological macromolecules. Although impressive progresses have been made in the field of prebiotic chemistry, current prebiotic synthetic reactions often suffer of low yield and produce complex mixture of products (Orgel 2004; Shapiro 1999). However, even assuming a robust and reliable synthetic route for the synthesis of mononucleotides were available, there would still be two conceptual bottlenecks undermining any reaction in an aqueous environment.

The first difficulty regards the slow accumulation of activated organic compounds, which is difficult to image due to the hydrolysis in aqueous solution. The second problem is the local concentration of reaction products which would have been far to low to sustain a workable condensation process.

Kanavarioti et al. (1990) investigated the conditions under which polymerization of selected imidazolide-activated nucleotides can effectively compete with the hydrolysis reaction. Results showed that initial concentration of activated monomer must be in the millimolar range which cannot be plausibly achieved with prebiotic synthetic reactions occurring in bulk unstructured aqueous solution.

Consequently, different scenarios from a simple unstructured "warm pond" have to be conceived in order to deal with those problems.

A possible solution was first proposed by Bernal (1949) who assumed that prebiotic organic molecules concentrated by adsorptions onto minerals. This scenario was experimentally investigated by Lahav and coworkers who measured the adsorption rate of AMP and CMP onto different dispersed minerals under different conditions of pH and chemical strength (Lazard et al. 1988). Among the others, montmorillonite clay has been proved to efficiently bind nucleotides by means of van der Waals forces mediated by exchangeable cations (Franchi et al. 2003). In addition, organic molecules bound to montmorillonite clay tend to be more stable than in the aqueous phase (Franchi et al. 1999). Moreover, montmorillonite clay might have had a role in the polymerization process as discussed hereafter.

Alternatively, high local concentration of mononucleotides could have been achieved assuming that RNA monomers were directly synthesised on a mineral surface. The *in situ* synthesis of prebiotic organic compound represents the main assumption of local chemio-autotrophic theory first developed by Günter Wächterschäuser (Waechtershaeuser 1988). According to the autotrophic theory, the entire sequence of reactions needed to synthesise RNA molecules occurred on pyrite, thus bypassing the problem related to the local concentration of mononucleotides. However, Wächterschäuser's autotrophic theory has not been proved feasible in laboratory conditions (Keefe et al. 1995) and thus remains a speculative, yet fascinating, theory.

Finally, Orgel and coworkers proposed the concentration of hydrogen cyanide and its reaction product HCN-tetramer, a main intermediate molecule toward the synthesis of purines (Oro 1960), by means of freezing eutectic mixtures (Sanchez et al. 1966).

Under the aforementioned conditions, high concentration of mononucleotides may be obtained, which are compatible with the chemical constrain of the polymerization processes.

RNA Molecules Synthesis and Chemical Constrains

In this section we would like to deal with the first part of the editor's statement highlighting that "for any workable chemical system one needs RNA local concentrations of at least femtomoles – which still means billions of identical copies of this compounds". This statement consists of two independent claims: the first deals with the actual concentration of any chemical component in a realistic "living system", the second with the aetiology of macromolecular sequences and in particular with the synthesis of identical copies of a given one.

The latter pronouncement suggests the necessity of identical copies of a given RNA sequence in order to set up a feasible chemical system. This constraint poses serious

limitation to the onset of the RNA world. Indeed, a single RNA sequence is only a dot in the space of all possible 4^l sequences of a given length l (Luisi 2006; Schuster et al. 1994). If RNA molecules were due to come from a prebiotic chemistry, the possibility of synthesise repeatedly a certain sequence is unlikely due to inherent random nature of prebiotic RNA synthetic routes known to date. Within this framework the possibility of "billions of identical copies" of a certain RNA sequence would represents a major obstacle.

However, the necessity of "billions of identical copies" comes only if one assumes that a single metabolic function has to be exclusively performed by only one precise RNA sequence so that this RNA sequence has to be repeatedly synthesised with a high degree of precision. This is unlikely the case of RNA, since many theoretical works have pointed out as the sequence-to-structure map is highly redundant in the sense that many different and unrelated sequences may easily assume the same structure and perform the same function.

Several independent investigations showed as given a fixed chain length the number of sequences far exceeds the number of structures. Schuster et al. (1994) employed an inverse folding algorithm to compute the number of sequences folding into the same secondary structure. Results showed that the sequence-to-shape space is of the kind many-to-one and that RNA sequences sharing a common shape are evenly scattered through the sequence space so that they share little or no homology (Schuster 1995).

Those theoretical findings are experimentally proved by the observation that only seven nucleotides are strictly conserved among the group I self-splicing introns (Lisacek et al. 1994), yet the overall secondary structure of the ribozyme and its function is conserved. The case of group I self-splicing introns clearly exemplifies the fact that different sequences with little or no homology can easily assume the same fold and perform the same function despite of their sequence diversity.

The reason behind the redundancy of RNA sequence-to-shape map lies in the modular organization of RNA secondary structure. Knight and co-workers emphasised that RNA molecules are structured in independent functional domains connected by flexible spacers, whose sequences are irrelevant to the overall secondary and tertiary structure. They developed a method to calculate the abundance of those functional motifs in a random pools assuming of RNA molecules. Results showed that the RNA modularity increases even further the redundancy of the sequence-to-shape map since the number of ways a given fold may be obtained dramatically increases due to the modularity of RNA domains (Knight and Yarus 2003).

In addition, RNA secondary motifs – crucial to ribozyme activity – are the result of base-paired regions that have no sequence requirements other than the Watson–Crick complementarity which increase the probability of finding those domains in the sequence space and in completely unrelated RNA sequences (Knight et al. 2005).

Finally, one has to consider that RNAs seem to have an intrinsic tendency to fold into compact secondary structures even in absence of any evolutive pressure. In our previous work (De Lucrezia et al. 2006b), we reported the study of the folding properties of de novo totally random RNAs by means of the RNA Foster assay (De Lucrezia et al. 2006a). Results suggested that the fraction of folded RNAs is large and evenly scattered through the sequence space, so that the emergence of folded and potentially functional RNAs might have been a probable event in the prebiotic scenario.

The straightforward consequence of these findings is that RNA sequence space is densely packed with folded RNAs. In addition, RNA sequence-to-shape map seems to be characterised by a high degree of redundancy of poorly correlated sequences sharing the same structure and presumably the same function.

These data suggest that in principle there is no need for identical copies of a given RNA sequences to set up a workable RNA world, which in turn makes the RNA scenario a little more plausible than what it would have been if RNA sequences had to be synthesised with a degree of precision beyond the one attainable by prebiotic synthesis.

However, these results leave unanswered the question regarding the local concentration of RNA molecules needed to establish a workable RNA world.

Several different solutions may be prospected to solve this problem; worth of notice is the concentration of RNA molecules on gel-like structure (Chetverin et al. 1991; Samatov et al. 2005) or on charged surfaces like mineral particles (Ferris 1993, 1999) as mentioned in the previous section. All those possibilities lies on transient and non-covalent interactions between RNA molecules and the surface that ensures a certain degree of freedom and mobility required to perform any catalytic function. In addition, surface interaction might have been also beneficial to RNA integrity and preservation in the prebiotic setting as mentioned before (Franchi et al. 1999). Any of these scenarios might suffice to reach high local concentration of RNA molecules and might have played a crucial role to drag RNA world out of the prebiotic soup.

However, the adsorption of RNA molecules on charged surface suffers of some limitations that make them not suited to further lead the evolution of an RNA world. The major limitation of a surface-bound RNA world lies in the impossibility to define a boundary which is crucial to define the unit of evolution. A surface-bound RNA world is an open system in which RNA molecules embedded with catalytic properties shares their ability with the rest of RNAs present in solution. Evolution can only occur when positive properties confer a selective evolutive advantage to the system they belong to. Otherwise "parasitic" RNAs – sequences that have no activity but are excellent templates – would overtake the system (Johns and Joyce 2005; Muller 2006).

In addition, a surface-based open system does not allow the establishment of a metabolism since the synthesis of metabolites represents an evolutionary advantage only if the metabolites are prevented from diffusing away (Muller 2006).

A possible solution to the problem of the local concentration of RNA molecules that does not suffer from the drawbacks of surface-bound open systems is RNA compartmentation.

Compartmentation requires the encapsulation of catalysts and its encoding gene within a boundary that prevents macromolecules leaking from the compartment and guarantees at the same time the permeability to metabolic substrates that must be replenished from the surrounding medium (Muller 2006). Double layer membranes suit perfectly to this scope, since they readily form from fatty acids (Hargreaves and Deamer 1978), which can be regarded as prebiotic molecules because they can be synthesized under plausible prebiotic conditions (Allen and Ponnamperuma 1967; Mccollom et al. 1999) or delivered by meteorites (Deamer and Pashley 1989).

Another intriguing feature of vesicles extremely relevant for the issue of RNA evolvability is the ease with which they can encapsulate macromolecules dispersed in solution. When vesicles are dried in the presence of other solutes molecules, they tend to fuse into multilayers structure that sandwich the solutes. Solutes as large as proteins and nucleic acids are efficiently captured upon rehydration when the lipid bilayers reseal into vesicles (Deamer and Barchfeld 1982; Shew and Deamer 1985). It is easy to imagine dehydration-hydration cycle occurring under plausible prebiotic condition in intertidal zones.

All together, those results show that lipid vesicles might have been an effective means to encapsulate and concentrate RNA molecules and bear a huge potential to drive further the evolution of an RNA-based living system.

Conclusions

The results presented in this paper seem to support our thesis that high local concentration of RNA molecules and their precursors can be achieved under plausible prebiotic conditions. Consequently, we do not consider the issue of the "high local concentration" as a major obstacle to the RNA world hypothesis. In contrast, we are rather prone to recognise that RNA World major bottleneck lies in the lack of any robust and coherent prebiotic synthetic route.

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