

Review Article

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The RNA World: A Critique

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Introduction

One of the earliest published suggestions that RNA-catalyzed RNA replication preceded and gave rise to the first DNA-based living cells was made by Carl Woese in 1967, in his book *The Genetic Code*[1](#). Similar suggestions were made by Crick and Orgel[2](#), for reasons that are not difficult to grasp. Prior to the discovery of catalytic RNAs, proteins were considered by many to be the only organic molecules in living matter that could function as catalysts. DNA carries the genetic information required for the synthesis of proteins. The replication and transcription of DNA require a complex set of enzymes and other proteins. How then could the first living cells with DNA-based molecular biology have originated by spontaneous chemical processes on the prebiotic Earth? Primordial DNA synthesis would have required the presence of specific enzymes, but how could these enzymes be synthesized without the genetic information in DNA and without RNA for translating that information into the amino acid sequence of the protein enzymes? In other words, proteins are required for DNA synthesis and DNA is required for protein synthesis.

This classic "chicken-and-egg" problem made it immensely difficult to conceive of any plausible prebiotic chemical pathway to the molecular biological system. Certainly no such chemical pathway had been demonstrated experimentally by the early 1960s. So the suggestion that RNA molecules might have formed the first self-replicating chemical systems on the primitive Earth seemed a natural one, given the unique properties of these substances.

They carry genetic information and (unlike DNA) occur primarily as single-stranded molecules that can assume a great variety of tertiary structures, and might therefore be capable of catalysis, in a manner similar to that of proteins. The problem of which came first, DNA or proteins, would then be resolved.

Self-replicating RNA-based systems would have arisen first, and DNA and proteins would have been added later. But in the absence of any direct demonstration of RNA catalysis, this suggestion remained only an interesting possibility.

Then, in the early 1980s[3](#), the discovery of self-splicing, catalytic RNA molecules (in the ciliated protozoan *Tetrahymena thermophila*), put molecular flesh on the speculative bones of the idea of an early evolutionary stage dominated by RNA. These catalytic RNA molecules have subsequently been termed "ribozymes." "One can contemplate an RNA World," wrote Walter Gilbert in 1986, "containing only RNA molecules that serve

to catalyze the synthesis of themselves."[4](#)

The phrase "RNA World" stuck to the general hypothesis, and has since come to denote the RNA-first, DNA-and-proteins-later scenario depicted in [Figure 1](#). The long-standing "chicken-and egg" puzzle at the origin of life indeed appeared amenable to a solution:

The primordial...conundrum -- which came first, informational polynucleotides or functional polypeptides -- was obviated by the simple but elegant compaction of both genetic information and catalytic function into the same molecule.[5](#)

A second impetus to the RNA world hypothesis came from the cluster of technical innovations now known generally as *ribozyme engineering*. Naturally occurring RNA catalytic activities are actually restricted to a small set of highly specialized reactions, e.g., the processing of RNA transcripts primarily in eukaryotic cells. However, ribozyme engineering, made possible by techniques such as DNA sequencing, *in vitro* transcription and the polymerase chain reaction [PCR][6](#), allow molecular biologists to manipulate RNA to whatever extent the molecule will allow. Thus, the catalytic repertoire of RNA can be expanded beyond the naturally occurring activities -- in the main, by two broad strategies of ribozyme engineering.

One strategy involves the direct modification of existing species of ribozymes, to produce better or even novel catalysts. This has been called the "rational design" approach. The other strategy employs pools of short (often 50-100 nucleotide units) randomized RNA molecules, which are subjected repeatedly to a selection process designed to enhance the concentration of RNA molecules with the desired functional activity. The few selected molecules are then multiplied a million-fold or more by using the polymerase chain reaction, which uses activated nucleotide precursors and enzymes. This has been termed the "irrational design" method.

Judging from the progress in ribozyme engineering in recent years, it seems likely that new and improved types of RNA catalysts will be produced in years ahead. Moreover, molecular biologists may discover additional catalytic roles of RNA in living cells, although the variety of such roles is not expected to rival that of the protein enzymes. Thus, one might expect that the RNA World hypothesis will continue to have supporters.

Yet beyond the immediate foreground of RNA World excitement lies a disquieting landscape of chemical problems, largely ignored in the recent literature on ribozyme engineering. As researchers broaden their focus to include the chemical plausibility of the RNA World itself, however[7](#), these difficulties cannot be avoided.

Furthermore, the relevance of ribozyme engineering to naturalistic theories of the origin of life is doubtful at best, primarily because of the necessity for intelligent intervention in the synthesis of the randomized RNA; then again in the selection of a few functional RNA molecules out of that mixture; then, finally, in the amplification of those few functional RNA molecules [[see box](#), "What Do Ribozyme Engineering Experiments Really Tell Us About the Origin of Life?"].

Hubert Yockey, borrowing a metaphor from Jonathan Swift, suggests that current origin-of-life research, including the RNA World hypothesis, floats improbably in mid-air like the roof of a house built by an architect of the Grand Academy of Lagado. This savant had contrived a method of building houses by beginning at the roof and working downwards. "The architect pointed out that among the advantages of this procedure," Yockey notes[8](#), "was that once the roof was in place [before the walls or foundation] the rest of the construction could proceed quickly and without interruption by weather." That "roof" -- consisting in this instance of tiles which represent the catalytic activities of RNA -- may look solid to those believers in the

existence of a prebiotic RNA World. But is the roof really solid? Is it supported by walls and a foundation?

Once one peers over the edge of the roof to look beneath, we shall argue, the implausibility of the theoretical structure as a whole is inescapable. In what follows, we present the key postulates or presuppositions on which the RNA World hypothesis must rest (see [Figure 2](#)). Each represents an unsolved chemical problem, in every case well-known to origin-of-life researchers. Unfortunately, in many articles on the RNA World, these problems are often collapsed into the "prebiotic soup" and "self-assembly" phases of the scenario, and receive no discussion. We suggest that new discoveries about the catalytic activities of RNA should be seen for what they really are: *not* elucidating prebiotic processes on the early Earth, but rather as extending our knowledge of the molecular biology of the cell in important ways (see below).

The relevance of catalytic RNA to the problem of the naturalistic origin of life is, however, a different matter entirely.

We take heart in noting that, despite the frequent neglect in much of the popular literature of the chemical difficulties of the RNA World scenario, many of the scientists involved with that hypothesis are quite candid in their assessment of the problems associated with it. These are represented for instance by the numerous contributors to *The RNA World*⁷. Since the RNA World hypotheses are so broad, we will attempt to break them down into somewhat narrower postulates. In this way one may see more clearly some of the presuppositions that are involved.

Problematic Chemical Postulates of the RNA World Scenario

Postulate 1: There was a prebiotic pool of beta-D-ribonucleotides.

Beta-D-ribonucleotides (see [Figure 2](#)) are compounds made up of a purine (adenine or guanine) or a pyrimidine (uracil or cytosine) linked to the 1'-position of ribose in the beta-configuration.

There is, in addition, a phosphate group attached to the 5'-position of the ribose. For the four different ribonucleotides in this prebiotic scenario, there would be hundreds of other possible isomers.

But each of these four ribonucleotides is built up of three components: a purine or pyrimidine, a sugar (ribose), and phosphate. It is highly unlikely that any of the necessary subunits would have accumulated in any more than trace amounts on the primitive Earth. Consider ribose. The proposed prebiotic pathway leading to this sugar, the formose reaction, is especially problematic⁹. If various nitrogenous substances thought to have been present in the primitive ocean are included in the reaction mixture, the reaction would not proceed. The nitrogenous substances react with formaldehyde, the intermediates in the pathways to sugars, and with sugars themselves to form non-biological materials¹⁰. Furthermore, as Stanley Miller and his colleagues recently reported, "ribose and other sugars have surprisingly short half-lives for decomposition at neutral pH, making it very unlikely that sugars were available as prebiotic reagents."¹¹

Or consider adenine. Reaction pathways proposed for the prebiotic synthesis of this building block start with HCN in alkaline (pH 9.2) solutions of NH₄OH.¹² These reactions give small yields of adenine (e.g., 0.04%) and other nitrogenous bases provided the HCN concentration is greater than 0.01 M. However, the reaction mixtures contain a great variety of nitrogenous substances that would interfere with the formose reaction. Therefore, the conditions proposed for the prebiotic synthesis of purines and pyrimidines are clearly incompatible with those proposed for the synthesis of ribose. Moreover, adenine is susceptible to deamination and ring-opening reactions (with half-lives of about 80 years and 200 years respectively at 37° C and neutral

pH), making its prebiotic accumulation highly improbable¹³. This makes it difficult to see how any appreciable quantities of nucleosides and nucleotides could have accumulated on the primitive Earth. If the key components of nucleotides (the correct purines and pyrimidines, ribose, and phosphate) were not present, the possibility of obtaining a pool of the four beta-D-ribonucleotides with correct linkages would be remote indeed.

If this postulate, the first and most crucial assumption, is not valid, however, then the entire hypothesis of an RNA World formed by natural processes becomes meaningless.

Postulate 2: Beta-D ribonucleotides spontaneously form polymers linked together by 3', 5'-phosphodiester linkages (i.e., they link to form molecules of RNA; see figure 2).

Joyce and Orgel discuss candidly the problems with this postulate¹⁴. They note that nucleotides do not link unless there is some type of activation of the phosphate group. The only effective activating groups for the nucleotide phosphate group (imidazolides, etc.), however, are those that are totally implausible in any prebiotic scenario. In living organisms today, adenosine-5'-triphosphate (ATP) is used for activation of nucleoside phosphate groups, but ATP would not be available for prebiotic syntheses. Joyce and Orgel note the possible use of minerals for polymerization reactions, but then express their doubts about this possibility¹⁵:

Whenever a problem in prebiotic synthesis seems intractable, it is possible to postulate the existence of a mineral that catalyzes the reaction...such claims cannot easily be refuted.

In other words, if one postulates an unknown mineral catalyst that is not readily testable, it is difficult to refute the hypothesis.

Joyce and Orgel then note that if there were activation of the phosphate group, the primary polymer product would have 5', 5'-pyrophosphate linkages; secondarily 2', 5'-phosphodiester linkages -- while the desired 3',5'-phosphodiester linkages would be much less abundant. However, all RNA known today has only 3',5'-phosphodiester linkages, and any other linkages would alter the three-dimensional structure and possibilities for function as a template or a catalyst.

Even waiving these obstacles, and allowing for minute amounts of oligoribonucleotides, these molecules would have been rendered ineffective at various stages in their growth by adding incorrect nucleotides, or by reacting with the myriads of other substances likely to have been present. Moreover, the RNA molecules would have been continuously degraded by spontaneous hydrolysis and other destructive processes operating on the primitive Earth¹⁶.

In brief, any movement in the direction of an RNA World on a realistically-modeled early Earth would have been continuously suppressed by destructive cross-reactions.

Postulate 3: A polyribonucleotide (i.e. RNA molecule), once formed, would have the catalytic activity to replicate itself, and a population of such self-replicating molecules could arise.

The difficulty with this postulate is evident in the following quotation from Joyce and Orgel:

...it is assumed...that a magic catalyst existed to convert the activated nucleotides to a random ensemble of polynucleotide sequences, a subset of which had the ability to replicate. It seems to be implicit that such sequences replicate themselves but, for whatever reason, do not replicate

unrelated neighbors.[17](#)

They refer to this as a component of "The Molecular Biologists Dream," and discuss the difficulties inherent in such a view. In order for a *stable population* of self-replicating RNA molecules to arise -- a prerequisite for further evolution -- the RNA molecules must be able to replicate themselves with high fidelity, or the sequence specificity which makes self-replication possible at all will be lost. While "it is difficult to state with certainty the minimum possible size of an RNA replicase ribozyme," Joyce and Orgel note, it seems unlikely that a structure with fewer than 40 nucleotides would be sufficient. Suppose, then, that "there is some 50-mer [RNA molecule of 50 nucleotides length]," Joyce and Orgel speculate, that "replicates with 90% fidelity. ... Would such a molecule be expected to occur within a population of random RNAs?"

Perhaps: but one such self-replicating molecule will not suffice.

"Unless the molecule can literally copy itself," Joyce and Orgel note, "that is, act simultaneously as both template and catalyst, it must encounter another copy of itself that it can use as a template." Copying any given RNA in its vicinity will lead to an error catastrophe, as the population of RNAs will decay into a collection of random sequences. But to find another copy of itself, the self-replicating RNA would need (Joyce and Orgel calculate) a library of RNA that "far exceeds the mass of the earth."[18](#)

In the face of these difficulties, they advise, one must reject

the myth of a self-replicating RNA molecule that arose *de novo* from a soup of random polynucleotides. Not only is such a notion unrealistic in light of our current understanding of prebiotic chemistry, but it should strain the credulity of even an optimist's view of RNA's catalytic potential. If you doubt this, ask yourself whether you believe that a replicase ribozyme would arise in a solution containing nucleoside 5'-diphosphates and polynucleotide phosphorylase![19](#)

Postulate 4: Self-replicating RNA molecules would have all of the catalytic activities necessary to sustain a ribo-organism.

S.A. Benner et al. note[20](#):

...one is forced to conclude that the last ribo-organism had a relatively complex metabolism that included oxidation and reduction reactions, aldol and Claisen condensations, transmethyations, porphyrin biosynthesis, and an energy metabolism based on nucleoside phosphates, all catalyzed by riboenzymes...It should be noted that this reconstruction cannot be weakened without losing much of the logical and explanatory force of the RNA World model.

Although Benner et al. speak of the last "ribo-organism," surely the *first* ribo-organism would have required nearly all of the same metabolic capabilities in order to survive. It is also apparent that the scenario of Benner et al. would surely include enclosing the ribozymes within a membrane with the ability to transport ions and organic molecules across that membrane.

Anyone who is familiar with biochemistry would recognize that it would take hundreds of different ribozymes, each with a particular catalytic activity, to carry out the metabolic processes described above. It should also be apparent that most of these metabolic capabilities would have to be functional within a short period of time (certainly not hundreds of years), in the same microscopic region, or the ribo-organism would never survive.

When one recognizes that catalytic activities of RNA are just as dependent upon specific sequences of nucleotides in RNA²¹ as protein enzymes are of amino acid sequences, then the probability of postulate 4 being valid is seen to be vanishingly small.

Benner et al. note that the diverse catalytic properties of enzymes often require coenzymes or prosthetic groups. They mention particularly the iron-porphyrin, heme, and pyridoxal, but have no suggestion how these (and other co-enzymes) could have functioned in the catalytic activities of early RNA molecules.

The other unproven assumption of postulate 4 is that RNA molecules initially had all of these suggested catalytic activities, but nearly all of these activities have been subsequently lost. RNA molecules with catalytic activity that are known today predominantly have nuclease or nucleotidyl transferase activity with some minimal esterase activity²². There is no solid evidence that RNA molecules ever had the broad range of catalytic activities suggested by Benner et al., even though a number of the authors of *The RNA World* speak of present-day RNA molecules as being vestiges of that early RNA World.

Conclusion

We have more to learn about RNA, both *in vivo* (as used by organisms) and *in vitro*, in terms of its chemistry generally and functional properties in particular. RNA is a remarkable molecule.

The RNA World hypothesis is another matter. We see no grounds for considering it established, or even promising, except perhaps on the objectionable philosophical grounds of philosophical naturalism (and its operational offspring, methodological naturalism), according to which the best naturalistic hypothesis is perforce the hypothesis to be accepted. We consider that historical biology should be open to all empirical possibilities, including design -- and see the molecular biological system of organisms, of which RNA is so stunning a part, as exemplars of design.

We find ourselves, however, distinctly in the minority of biologists. If design exists at all, it is a matter of subjective intuition, the majority of our colleagues would claim, asserting with science writer George Johnson that "the point of science is...to explain the world through natural law."²³

We would put the point rather differently. The point of science is to *explain* the world, through natural laws *or* whatever other causes best account for the phenomena at hand.

Philosopher of science Stephen Meyer captures the point well:

The (historical) question that must be asked about biological origins is not "Which materialistic scenario will prove adequate?" but "How did life as we know it actually arise on earth?" Since one of the logically appropriate answers to this latter question is that "Life was designed by an intelligent agent that existed before the advent of humans," I believe it is anti-intellectual to exclude the "design hypothesis" without consideration of all the evidence, including the most current evidence, that would support it.²⁴

Detecting design is not a matter of subjective intuition.²⁵ To see design as a real causal possibility, however, one must break free of the constraints of naturalism.

[What do Ribosome Engineering Experiments Tell Us About the Origin of Life?](#)

Notes

- [1.](#) Carl Woese, *The Genetic Code* (New York: Harper and Row, 1967).
- [2.](#) F.H.C. Crick, "The origin of the genetic code," *J. Mol. Biol.* 38 (1968): 367-379; L.E. Orgel, "Evolution of the genetic apparatus," *J. Mol. Biol.* 38 (1968): 381-393.
- [3.](#) K. Kruger, P.J. Grabowski, A.J. Zaug, J. Sands, D.E. Gottschling, and T.R. Cech, "Self-Splicing RNA: Autoexcision and Autocyclization of the Ribosomal RNA Intervening Sequence of Tetrahymena," *Cell* 31 (1982): 147-157.
- [4.](#) Walter Gilbert, "The RNA World," *Nature* 319 (1986): 618.
- [5.](#) I. Hirao and A.D. Ellington, "Re-creating the RNA World," *Current Biology* 5 (1995): 1017-1022; p. 1017.
- [6.](#) Mullis, K.B. and Faloona, "Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction," *Methods Enzymol* 155 (1987): 335-350.
- [7.](#) G. Joyce, "RNA evolution and the origins of life," *Nature* 338 (1989): 217-224; T.J. Gibson and A.I. Lamond, "Metabolic complexity in the RNA World and implications for the origin of protein synthesis," *J. Mol. Evol.* 30 (1990): 7-15; G.F. Joyce and L.E. Orgel, "Prospects for understanding the origin of the RNA World," in *The RNA World*, eds. R.F. Gesteland and J.F. Atkins (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1993), pp. 1-25.
- [8.](#) H.P. Yockey, "Information in bits and bytes: reply to Lifson's Review of *Information Theory and Molecular Biology*," *BioEssays* 17 (1995): 85-88; p. 87.
- [9.](#) R. Shapiro, "The improbability of prebiotic nucleic acid synthesis," *Origins of Life* 14 (1984): 565-570; R. Shapiro, "Prebiotic ribose synthesis: a critical analysis," *Origins of Life* 18 (1988): 71-85.
- [10.](#) Recently it has been shown that reaction mixtures containing dilute glycoaldehyde phosphate and formaldehyde or glyceraldehyde-2-phosphate will generate reasonably high yields of ribose 2,4-diphosphate and a few other sugar phosphates in less amounts. See D. Muller, S. Pitsch, A. Kittaka, E. Wagner, C.E. Wintner, and A. Eschenmoser, "Chemie von alpha-aminonitrilen. Aldomerisierung von glykoaldehydphosphat zu *racemischen* hexose- 2,4,6-triphosphaten und (in gegenwart von formaldehyd) *racemischen* pentose 2,4-diphophaten: *rac.*allose-2,4,6-triphosphat und *rac.*-ribose-2,4,-diphosphat sind die reaktionshauptproducte. *Helv. Chim. Acta* 73 (1990): 1410-1468; Joyce and Orgel, *ibid.* However, if these reactions are not also run in the presence of amines and other nitrogenous compounds (i.e., in chemical mixtures of the complexity proposed for the "prebiotic soup"), their relevancy to the origin of life is problematical.
- [11.](#) Rosa Larralde, Michael P. Robertson, and Stanley L. Miller, "Rates of decomposition of ribose and other sugars: Implications for chemical evolution," *Proc. Natl. Acad. Sci. USA* 92 (1995): 8158-8160. The ribose half-lives are very short, Larralde et al. report: 73 minutes at pH 7.0 and 100° C and 44 years at pH 7.0 and 0° C.
- [12.](#) J.P. Ferris, P.C. Joshi, E.H. Edelson, and J.G. Lawless, "HCN: a plausible source of purines, pyrimidines and amino acids on the primitive Earth," *J. Mol. Evol.* 11 (1978): 293-311.
- [13.](#) R. Shapiro, "The prebiotic role of adenine: a critical analysis," *Origins of Life and the Evolution of the Biosphere* 25 (1995): 83-98.
- [14.](#) Joyce and Orgel, *ibid.*
- [15.](#) *Ibid.*, p.4
- [16.](#) C. Thaxton, W. Bradley, and R. Olsen, *The Mystery of Life's Origin* (New York: Philosophical Library, 1984).
- [17.](#) Joyce and Orgel, *ibid.*, p.7.
- [18.](#) *Ibid.*, p.11.
- [19.](#) *Ibid.*, p.13.

- [20.](#) S.A. Benner, M.A. Cohen, G.H. Gonnert, D.B. Berkowitz, and K.P. Johnsson, "Reading the Palimpsest: Contemporary Biochemical Data and the RNA World," in *The RNA World*, eds. R.F. Gesteland and J.F. Atkins (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1993), pp. 27-70; p. 57.
- [21.](#) T.R. Cech, "Mechanism and Structure of a Catalytic RNA Molecule," in *40 Years of the Double Helix*, The Robert A. Welch Foundation 37th Conference on Chemical Research, 1993, pp. 91-110; see also T.R. Cech, "Structure and Mechanism of the Large Catalytic RNAs: Group I and Group II Introns and Ribonuclease P," in *The RNA World*, eds. R.F. Gesteland and J.F. Atkins (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1993), pp. 239-269.
- [22.](#) Ibid.
- [23.](#) George Johnson, *Fire in the Mind: Science, Faith, and the Search for Order* (New York: Alfred A. Knopf, 1995), p. 314.
- [24.](#) Stephen C. Meyer, "Laws, Causes, and Facts," in *Darwinism: Science or Philosophy*, eds. J. Buell and V. Hearn (Richardson, Texas: Foundation for Thought and Ethics, 1994), p.34.
- [25.](#) See William A. Dembski, "The Design Inference: Eliminating Chance Through Small Probabilities," unpublished Ph.D. dissertation, 1995, Department of Philosophy, University of Illinois-Chicago Circle.

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