

What do Ribozyme Engineering Experiments Really Tell Us About the Origin of Life?

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Are biochemists now routinely recreating the RNA World *in vitro*, and discovering the ease with which functional sequences arise from random pools of nucleotides? Many commentators appear to endorse this view. Ichiro Hirao and Andrew Ellington, for instance, two RNA World researchers at Indiana University, write:

The intersection of the discovery of ribozymes with the development of techniques for nucleic acid amplification allowed models of molecular evolution to be recapitulated in a test tube.[1](#)

On this view, *in vitro* ribozyme engineering provides a plausible and realistic model or experimental recapitulation of actual prebiotic processes. Thus, "selection" from "random pools of RNA sequences" in ribozyme engineering experiments is held to be *strongly analogous* to critical prebiotic conditions that led to RNA-based organisms. Ekland et al., for instance, argue that *in vitro* selection of novel ribozymes suggests

that even the most complex natural ribozymes, such as ribonuclease P and the group I and II self-splicing introns could have arisen in one step from long random sequences, and that complex ribozymes may have played an important role early in the RNA World.[2](#)

But are these experiments genuinely relevant to the prebiotic problems to be solved? We argue that they are not. *In vitro* RNA selection does *not* demonstrate that complex ribozymes could have arisen naturally in a prebiotic soup, because the *in vitro* experimental conditions are wholly unrealistic, revealing at every turn the fingerprints of intervening intelligence. RNA World researchers have taken their own engineering of ribozymes as analogous to plausible prebiotic processes, when in fact the two situations are profoundly different. Indeed, aspects of ribozyme engineering, together with other lines of evidence, support a very different view of biological origins from that advocated by RNA World researchers.

Ribozyme engineering involves two broad experimental strategies.[3](#) The "rational design" approach modifies existing types of ribozymes to produce better or even novel RNA catalysts. The "irrational design" approach, on the other hand, uses pools of partially randomized RNA molecules, which are screened -- "selected" -- for functional activity of a desired sort. Those molecules catalyzing the desired reaction are then used as the basis for the next round of "evolution." This randomization-selection process may be repeated several times, to yield increasingly faster RNA catalysts.

These experiments certainly add to our knowledge of RNA chemistry. A simple question directly illuminates the doubtful relevance of these experiments to prebiotic chemistry, however. How did pools of 10¹⁵ RNA molecules (to cite a value from a recent ribozyme engineering experiment⁴) accumulate on the early earth? How, for that matter, did any RNA accumulate?

Here an analogy may be helpful. Suppose you learn about a blackjack player who routinely beats the casinos in Las Vegas. You would not be impressed to find that the casinos had inexplicably made an exception for this person. They allowed him to fill parking lots, stadiums, and indeed the open desert around Las Vegas with millions of dealers who each dealt thousands of hands. The player monitored these millions of dealers electronically. Whenever a good hand turned up, he would play that hand, and ignore all the others.

Is that winning at blackjack? Not at all. The player contrives to "win" only by violating the actual rules of the game. In the case of prebiotic chemistry, the actual rules of the game govern the formation of RNA molecules *without the help of biochemists*. And, according to those rules (see discussion of postulates 1-4, main text, and below), RNA does not arise from its chemical constituents except (a) in organisms, and (b) in laboratories where intelligent organisms synthesize it.

The "randomization" and "selection" steps in ribozyme engineering, therefore, have no realistic prebiotic analogues. Ribozyme engineering, where RNA is necessarily synthesized by intelligent agents is, truly, *engineering* -- in the full, "intelligent design required" sense of that term.

Nothing conveys this better than reading the Methods and Materials section of any ribozyme engineering paper. There, one will encounter biologically-derived reagents such as DNA and RNA polymerases, automated DNA synthesizing machines -- e.g., the Biosearch 8750 programmable DNA synthesizer⁵ -- purified ribonucleoside triphosphates, and various experimental tricks needed to help reactions along. In one notable experiment, for instance, done by RNA World researchers David Bartel and Jack Szostak, it was found that the pools of randomized RNA *precipitated* -- that is, formed large, tangled, useless networks of molecules:

Incubation of the pool RNA...led to rapid and extensive aggregation; more than half of the pool RNA precipitated when incubated for 90 minutes at 37° C in high concentrations of Mg²⁺ and monovalent ions...and precipitation was even more rapid at higher temperatures. It appears that conditions that favor RNA intramolecular structure also stabilize intermolecular interactions; as molecules find regions of complementarity with more than one other molecule, RNA networks form and eventually become too large to remain in solution.⁶

How to solve the problem? Tie the RNA molecules down to something:

To minimize the problem of RNA aggregation, we immobilized the pool of RNA molecules on agarose beads before the addition of Mg²⁺.⁷

A clever move -- "once tethered to the agarose," Bartel and Szostak report, "the pool molecules could not diffuse and form intermolecular reactions, and could therefore be safely incubated"⁸ -- but this is not a trick the primordial soup would be likely to discover on its own. And, on occasion, the prebiotic unreality of ribozyme engineering breaks through even to its supporters. "It is difficult to believe," writes RNA World researcher Steven Benner, of ETH Zurich, "that larger pools of random RNA emerged spontaneously without the gentle coaxing of a graduate student desiring a completed dissertation."⁹

Indeed, an important parallel exists between these procedures and prebiotic simulation (i.e., chemical evolution) experiments. As Thaxton et al. have pointed out, the degree of investigator interference in chemical evolution experiments increases as the subject of the experiments gets closer to the molecular genetic system.¹⁰ In experiments simulating the primitive atmosphere the interference is minimal. The apparatus is filled with the starting gases, turned on, and left alone until products are analyzed.

But in experiments designed to simulate the prebiotic formation of biopolymers the investigator may use unrealistic reaction conditions (e.g., high concentrations of a few pure reagents) and/or change the conditions during the experiment to enhance the yields of desired products. This intelligent manipulation of the experimental conditions (to guide the reactions to the desired results) is most apparent in simulations of prebiotic polynucleotide synthesis. The reason why increasingly large doses of investigator influence are required is that if the chemical processes are "left to themselves" they would not produce the desired result, in fact, would go away from the living state, not toward it.

The continuity between the increasing role of investigator influence in prebiotic simulation experiments and the recent use of rational design and *in vitro* selection procedures to produce novel ribozymes lends support, not to the naturalistic but to the intelligent design view of biological origins. The powerful selection processes used in ribozyme engineering are of course unlikely to have occurred spontaneously (unassisted, without the input of intelligent design) on the prebiotic Earth. It will be argued that similar though much weaker selective processes must have occurred in the evolving RNA World. Over the hundreds of millions of years thought to have been available, these chemical selective processes resulted in a rich variety of ribozymes (most of which have long since disappeared from even the simplest organisms although a few survive in eukaryotes).

But it is very difficult to see how the mere extension of time would render a chemical evolution process more probable when every presumed stage of the process (assuming it

could spontaneously advance at all beyond its earliest stages) would have been powerfully hindered or suppressed by the natural chemical tendencies of the reacting substances and by the preponderance of destructive forces in the natural environment.

We can see that research in one context (ribozyme engineering) with its particular presuppositions and goals in mind and with results presumably providing insights into one putative process of origins has in fact provided powerful additional evidence for a very different view. This has occurred precisely because the procedures used in research on RNA catalysis reinforce the notion that intelligent design is required to produce molecular species that would not form due to the natural chemical tendencies of the reacting substances themselves, even over vast stretches of time.

Notes

- [1.](#) Ichiro Hirao and Andrew D. Ellington, "Re-creating the RNA World," *Current Biology* 5 (1995): 1017-1022; p. 1017.
- [2.](#) Eric H. Eklund, Jack W. Szostak, David P. Bartel, "Structurally Complex and Highly Active RNA Ligases Derived from Random RNA Sequences," *Science* 269 (1995): 364-370; p. 369.
- [3.](#) R.R. Breaker and G.F. Joyce, "Inventing and improving ribozyme function: rational design versus iterative selective methods," *Trends in Biochemistry* 12 (1994): 268-275.
- [4.](#) Eklund et al., p. 364.
- [5.](#) Rachel Green and Jack W. Szostak, "Selection of a Ribozyme That Functions as a Superior Template in a Self-Copying Reaction," *Science* 258 (1992): 1910-1915; p. 1914; see also Niles Lehman and Gerald F. Joyce, "Evolution *in vitro*: analysis of a lineage of ribozymes," *Current Biology* 3 (1993): 723-734 (oligodeoxynucleotides "were prepared on an automated DNA synthesizer" [p. 732]).
- [6.](#) David P. Bartel and Jack W. Szostak, "Isolation of New Ribozymes from a Large Pool of Random Sequences," *Science* 261 (1993): 1411-1418; p. 1412.
- [7.](#) Ibid.
- [8.](#) Ibid.
- [9.](#) Steven A. Benner, "Catalysis: Design Versus Selection," *Science* 261 (1993): 1402-1403; p. 1403.
- [10.](#) Charles Thaxton, Walter Bradley, and Roger Olsen, *The Mystery of Life's Origin* (New York: Philosophical Library, 1984), pp. 99-112.