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Peer-Review Record:

How Amino Acids and Peptides Shaped the RNA World

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Reviewer 1: Charles Carter
Reviewer 2: Anonymous
Editor: Niles Lehman (Guest Editor of Special Issue "The Origins and Early Evolution of RNA")

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First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

This manuscript affords an excellent summary of a broad range of recent results relating to the origin of genetic coding and the transition from chemical to biological evolution. It appears not to report any original research of the authors, excepting their general synthesis of the literature, which is most useful in its own right.

Response: We thank the reviewer for these comments.

The authors' conclusion—"We argue that an RNA world completely independent of amino acids never existed"—expresses a very useful viewpoint, especially in light of the overwhelming support given uncritically to the "RNA World" hypothesis. Examples are drawn from diverse sources to bolster this argument. I found the following to be most useful and convincing: (i) the relevance of acidic peptides for their ability to coordinate Mg^{2+} ions, limiting metal-catalysed hydrolysis of oligoribonucleotides; (ii) experiments such as those of Yarus and Shimizu on the chemical biology and catalytic properties of short oligonucleotides, and importantly their dependence on catalytic peptides; (iii) the resume of the work of Williams and Fox on the evolution of the peptidyl-transferase center; (iv) the important possibility that short peptides served as the first ancestral RNA polymerases; and (v) the clarity of their scenario in §11 on the gradual incorporation of pairs of amino acids into the emerging genetic code according to the proposal of Rodin and Ohno that the two classes arose on opposite strands of the same gene. The way in which all of this happened appears now to be unfolding rapidly, and the authors contribute substantively with their synthesis.

Response: Again, we thank the reviewer for these comments.

The paper would benefit from minor revisions to improve clarity; proper attribution; and to eliminate redundancy. Specific examples include:

(i) Line 64: the citations at the end of the sentence would be more helpful toward the beginning of the sentence after the first reference to Wong's coevolution theory, in order to clarify their precise implications of their shift in meaning from that originally proposed. It took me several readings to get their point.

Response: We get the problem, and changed the text and the position of the references (changes in red everywhere).

(ii) Lines 45–46; 90–100: the authors suggest a rather important point in these two disparate sentences: a key determinant of the direction of pre-biological evolution was the selection of molecular functionality. It is a shame that this idea isn't developed further, and especially with greater focus.

Response: We thought this an excellent suggestion. We extended the introduction, setting out this strategy explicitly, so it can function as a backdrop for the reader in the remainder of the article.

(iii) Lines 161–163: the authors appear not to be aware that the earliest version of the proposal that RNA and peptides coevolved from very short precursors was published by Carter and Kraut in 1974: A Proposed Model for Interaction of Polypeptides with RNA, Proceedings of the National Academy of Sciences, USA 71, 283–287. It is important to acknowledge that precedent here, as this paragraph presents specific recent suggestions as to how such a world might have been implemented.

*Response: We now refer to this publication. We refer to it earlier than the reviewer suggested, however. This is because it is a very early (!) instance of a "co-evolutionary theory" which clearly deserves mention. However, it is based on modelling a much more advanced stage of protein evolution: the beta sheet. See the text under "**3. What Do We Mean by 'Coevolutionary' Theory Here?**".*

(iv) Lines 239–240: Citation here of a paper by Schimmel, Giegé, Moras, and Yokoyama (1993) "An operational RNA code for amino acids and possible relationship to genetic code", Proc. Nat. Acad. Sci. USA 90, 8763–8768) would strengthen both the proposal of Di Giulio and of the authors by relating the dual nature of tRNA molecules to the duality evident also in the aminoacyl-tRNA synthetases.

Response: The reviewer cannot know this, but we discussed the implications of this article by Schimmel et al. extensively among ourselves before writing up the final version of our article. It is indeed highly tempting to link the proposal of Di Giulio, the dual nature of tRNAs as envisaged by Schimmel and the duality evident in the aminoacyl-tRNA synthetases. However, upon careful analysis we came to the conclusion we had to leave it out. Your point (iv) shows this to have been unwise. We added this text to our manuscript: "(3) This scenario should be distinguished from another popular model regarding tRNA evolution: the one in which 'tRNA' started out as a small CCA acceptor only, which would be aminoacylated according to the second genetic code, with the current genetic code appearing at a later stage upon extension of the acceptor minihelix with an anticodon stem-loop (see e.g., [52]). It should be stressed that the two domains (acceptor-TψC stem-loop and anticodon-D stem-Biloop, which is taken as a later addition) do not coincide with the two original regions of the 'Di Giulio dimer', as can be clearly seen in Figure 1. In our model the acceptor stem is not 'older' than the anticodon loop and the link between the genetic code and the second genetic code 'read' by the aminoacylating domain of the

synthetases is not tagged on afterwards, but present because they are sterically together from the beginning. This explains the specificity of the the different aaRSs even though they must have started out too small to bridge the distance to the anticodon loop."

Round 1: Reviewer 2 Report and Author Response

This review is an attempt to imagine how short peptides and ribonucleotide molecules might have collaborated during the origin of biological systems on the Earth. This is a meritorious topic, and certainly not sufficiently explored, so I am well-disposed toward anything that clarifies the topic. However, the present ms needs considerable editing to be readable and publishable, along the following lines.

There are many places where crucial references are omitted; the manuscript needs very close reading to find them all. But for example, Lines 82, 86, 87, 89, 98, 213, 241, 283 and 293 are examples.

Response: Crucial references have been added at the indicated lines (and others); changes in red everywhere.

There are no figures at all, particularly noticed in many places where text is simply not specific enough to make an unambiguous point and a graphic would clarify the exposition; for example at Lines 250 and 381.

Response: The reviewer makes a valuable point. Both passages refer to the same description and now contain a referral to "Figure 1". We have added this figure and agree that "a figure is worth a thousand words".

There are essential junctures at which the argument just seems to jump into space with uncertain empirical basis. For example, how much protection might be afforded to RNA by the peptides being considered at about Line 170?

Response: We refer here to the protection by ASP containing peptides via the chelation of Mg^{2+} ions, according to ideas as e.g., expressed by Jack Szostak. Calculating how much protection that will actually give is of course rather difficult.

What are the "storage functions" being discussed at Line 172?

Response: Carbon storage, as we now make clear in the text.

For example, how would peptides protect the RNAs responsible for them *in trans*, as is apparently suggested at Line 179?

Response: We have extended and clarified the argument, thus:

A few important complicating aspects have to be mentioned, as we have only described selection functioning at the level of individual molecules at this stage. Presumably "collective evolution" [37] could also start functioning at the level of communities of molecules. A possible example of influence "in trans": we start out with the GlyGly producing RNA benefiting from the protection by Asp-containing oligopeptides that are able to sequester Mg^{2+} ions. These Asp-containing oligopeptides are in turn produced by GlyGly.

37. Vetsigian, K.; Woese, C.; Goldenfeld, N. Collective evolution and the genetic code. Proc. Natl. Acad. Sci. USA 2006, 103, 10696–10701.

What is a "soak it in asp" strategy (Line 301)?

Response: This again refers to the protection by ASP containing peptides via the chelation of Mg^{2+} ions, according to ideas as e.g., expressed by Jack Szostak. We have replaced this (much too colloquial) expression and clarified the text.

Peptides "become able to replicate RNA" in a startling and possibly unsupported fashion at Line 347.

Response: We made the mistake of not clarifying that part of the argument for this statement would come up in the next section. We changed the text, thus:

This could even imply that peptides made by this protoribosomal core may have become able to replicate RNA (see the next section) before possible RNA-based RNA replicases ever evolved.

Line 388 is a, likely ungrammatical, assertion that I cannot understand at all.

Response: It was not ungrammatical, but it was unclear. We changed the text.

The net effect of all this acrobatic argument is that this reader is left with the nagging feeling that an argument of the usual kind may not be present. This is devastating to the reader's ability to distinguish what is known from what is, for example, being suggested as a good experiment to do now.

Response: In the original manuscript we tried to make this clear by referencing explicitly the things that were known/shown and using expressions such as the following when they are not:

... only speculate about. A possible scenario will ... (Section 7)

Possibly a conformational change allows ... (Section 8)

... on montmorillonite, with a hypothetical community of 4 different tRNAs, ... (Section 11)

Etc., etc., etc.

However, we have improved our manuscript along the lines suggested by the reviewer and also brought in more references at appropriate places.

Lastly, the presentation is credulous in the extreme. For example, heavy emphasis is placed on Shimizu's experiments with peptides (e.g., Line 236), even to the extent of lamenting that these have not been repeated. I believe that these experiments were simply not reproducible, but would be happy to be proven wrong. The experiments are among the few potential data in the area of the review, and so play a disproportionate role in the argument. The authors might contact one of the original authors and do everyone a service by clarifying this matter.

Response: This is the only point that we really have to completely disagree with the reviewer.

Shimuzu's experiments are not more important than those of Yarus or the findings of Di Giulio.

The experiments of Shimuzu belong to the published record (they have not been retracted or publicly "denounced"). Because these experiments have important evolutionary implications, we went through the paper carefully and could not find obvious mistakes.

However, because we consider these experiments so important we think it necessary for them to be repeated. Thus we state: "It is strange to notice that follow-up experiments (even at the level of reproducing the results obtained, let alone extending them) seem not to have been performed, as far as

we know. This becomes even more puzzling when we consider the momentous step the Shimuzu experiments seem to illuminate: the possible origin of coded synthesis." And again (!) in the discussion: "However, one has to think in terms of 'Shimizu interactions' as described above (and of which the model studies should be urgently revisited and extended) …".

We do not think this shows us to be "credulous in the extreme" (quite the opposite), as it is a polite way of saying we want these experiments to be repeated!

The original paper was authored by Shimizu on his own (no "authors" here), 20 years ago. We think it highly unlikely that he would be in a position to repeat the experiments.

In Google Scholar, we find that the paper has been referred to 33 times. This does not mean in any way that the experiments are OK (that would indeed be "credulous in the extreme"). It does indicate, however, that just hypothesizing that the experiments are "simply not reproducible" is not the way forward. This is precisely the reason why we wrote that we would very much like to see these experiments repeated.

If above gaps in the exiting argument can be closed, or even if the gaps can only be better defined in order to stimulate a new generation of experiments, I would certainly be willing to take another look at this review.

Response: We hope that our additions to the article concerning these and other aspects as well as the introduction of the figure will satisfy the reviewer, and thank him/her for the comments which allowed us to improve the manuscript.

Round 2: Reviewer 2 Report

While I am not in complete agreement with the revision, I believe that the suggestions for research on RNA-small peptide interactions that might come from circulation of this speculation justify its publication. Certainly the intelligibility of the argument has been improved.