ISSN 2075-1729
www.mdpi.com/journal/life

Peer-Review Record:

The Origin and Evolution of Ribonucleotide Reduction

Daniel Lundin, Gustav Berggren, Derek T. Logan and Britt-Marie Sjöberg

***Life* 2015, *5*, 604-636, doi:10.3390/life5010604**

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

Received: 15 January 2015 / Accepted: 6 February 2015 / Published: 27 February 2015

First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

This is a thoughtful and well reasoned discussion of plausible pathways for the origin and evolution of ribonucleotide reductase. The paper should be of broad general interest because it pertains directly to the origin of DNA-based genomes, following the so-called RNA world. With a few minor revisions this paper should be acceptable for publication.

It seems likely that RNR has undergone more evolutionary change than any other protein. Why all the tinkering? The authors make the good point that the chemistry of the reaction is difficult, so the variation may involve trying different paths to find a more efficient mechanism. And of course, there is the appearance of oxygen to deal with. I wonder whether catalytic efficiencies (Km/Vmax) improved as evolution progressed. Of course kinetic data cannot be available for the proposed urRNR and protoRNR, but I wonder whether sufficient published data would point toward the idea that evolution toward class I RNR involved improvements in catalytic efficiency. The point is addressed on lines 738–739: "....which of the two RNRs is most (should be more) effective...." But effectiveness is not defined, and maybe class III should be included in this discussion.

Response: We are deliberately somewhat imprecise when discussing potential differences in enzyme effectiveness since it, to our knowledge, has proven difficult to directly compare the catalytic efficiencies of different RNRs. As a rough estimate, the E. coli diiron class I, the L. leichmannii class II and the T4 class III all have turnover rates of the same magnitude (between 3 and 10 turnovers per second), providing the to our knowledge best estimate of relative effectiveness between RNRs. Within that same magnitude lies, however, a threefold difference, certainly sufficient for selective advantages under the right circumstances. Moreover, it appears more just to compare enzymes encoded in the

same genome. However, if we had numbers from all three classes from e.g. Pseudomonas aeruginosa or from class I and II from any genome encoding both classes, the comparison would still not take into account the specific environment in which the organism in which the duplication and eventual specialisation occurred. We have therefore avoided discussing effectiveness as much as possible in the manuscript, although the question remains highly interesting. If new data is made available, we would be happy to bring up the question again.

Clarity of the paper would be improved with a table of nonstandard abbreviations. Here are a few that I noted: TIM, SAM, dAdoH, GREs, Pfam, HMMER, SCOP, PFL. The paper uses two different abbreviations for S-adenosylmethionine: SAM and AdoMet. Be consistent.

Response: Good suggestion. We have added a paragraph right after the abstract and keywords paragraphs explaining abbreviations. We chose not to include ECOD, HMMER, Pfam or SCOP since they are all established names. We chose to do the same with TIM and SAM, as we only use the abbreviation as part of the name of a protein superfamily and family respectively. Furthermore, we prefer to use AdoMet when referring to S-adenosylmethionine since this is established practice in the field, and use SAM only when referring to the protein family "radical-SAM enzymes", again since this is a name.

The proposed evolution of the presumably monomeric protoRNR molecule to the dimeric urRNR needs a bit more justification, or at least, emphasis, than the "inferences based on modern proteins" (line 219).

Response: The evolutionary path from the protoRNR to the urRNR is one which we unfortunately cannot say more about than which changes must have occurred from a hypothetical protein to a reconstructed model. We have added two sentences to the end of the paragraph to clarify this.

Line 425 "...uracil deoxynucleotides are subsequently converted to thymidine deoxynucleotides by thymidylate synthase....." is a bit of an oversimplification. As pointed out by Reichard in his 1988 Fed. Proc. review, most dTTP in animal cells arises as a result of CDP reduction followed by dCMP deaminase.

Response: We have added a short clause describing this, referring to Mathews et al. 2014.

Line 634 is garbled in my printout.

Response: The review pdf is indeed garbled, but we can't find the problem in our word processor original. The line contains only the end of paragraph "superfamily".

Line 659. Table 3, not chapter 3

Response: We do intend to refer to chapter 3 here and not table 3.

Line 834, 2-ketobutyrate (one word)
Response: Corrected.

Round 1: Reviewer 2 Report and Author Response

This is an excellent, thorough, well-written, and well-presented review of RNR mechanisms and the possible evolutionary relationships among existing and prior forms of this important enzyme class. It was a pleasure to read, and I would like to commend the authors for such a nice job. Although I cannot tell which, if any, of these ideas have been published previously by the current authors, the review is so comprehensive that I can recommend its ultimate publication in Life.

I have only one suggestion for the authors to consider prior to final acceptance and publication. On the top of page 7, they claim that "...the potenital to activate substrates via H-atom abstraction to perform reactions that were likely unreachable in a pure RNA-based world." I would like some discussion of why this statement is made so strongly. The argument made here is that the protoRNR achieved catalytic prowess by exploiting the redox potential of a metal ion directly. Why cannot "pure" RNA have done the same? RNA can perform exquisite substrate positioning (especially when the substrates are nucleotides, as they are here), and can use metals to assist in catalysis. While redox-active catalytic RNAs are rare, and perhaps absent from Nature, Yarus' "Cheshire Cat Hypothesis" (FASEB J, 7:31–39; 1993) forces us to consider the possibility that early RNAs were quite metal dependent. Given the authors' appeal to parsimony as a reasoning agent (e.g., later on p. 7), it behooves them to defend their denial of primitive RNA-metal-driven nucleotide reduction a little more completely.

Response: This is indeed a difficult question, and we did try to find a route between all potential pitfalls. Apparently, we did not convey the clear message we wished. We have updated the paragraph in question to allow more room for RNA alternatives, while still suggesting that a protein scaffold is the most likely candidate for the strong metal catalyzed radical chemistry required for ribonucleotide reduction.