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LETTER TO THE EDITOR

Does the Ribosome Challenge our Understanding of the RNA World?

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Abstract In a recent article published in these pages, Bowman and colleagues propose that the ribosome represents a challenge to the RNA world model, a long-standing framework to explain the origin of DNA and genetically encoded proteins from a hypothetical RNA-based system. Specifically, they outline a scenario for the emergence and subsequent coevolution of the peptidyl transferase centre (PTC) of the ribosome with non-templated peptide products of this RNA through *chemical evolution*. They also propose that the PTC would have predated the emergence of enzymatic RNA replication, and that this in turn indicates that the RNA world never existed. We and others have previously incorporated non-templated peptide production as an early stage in the evolution of protein synthesis, which we would count as a chemical process, in agreement with Bowman and colleagues' model. However, their model raises an important question: to what extent could early protein synthesis and its products have evolved in the absence of Darwinian processes? We argue that evolution of the early ribosome requires Darwinian evolution, and that, while chemical evolution could give rise to peptidyl transferase activity, it is insufficient for subsequent improvement of a proto-PTC, or for ongoing coevolution of the proto-PTC with its early non-templated peptide products. We conclude that it is difficult to preclude the involvement of replicative processes, themselves subject to Darwinian evolution, from the evolution of the PTC. Finally, Bowman et al. call into question current models for the RNA to protein transition. We show that the difficulty that Bowman et al. have with this scenario is down to a misreading of our previous work.

Keywords RNA world · Ribosome · Polymer transition · Darwinian evolution · Chemical evolution · Translation

We read with interest the piece by Bowman et al. (2015), who propose that the RNA world model is problematic in light of our current knowledge of the ribosome. They propose that the peptidyl transferase centre (PTC) of the ribosome arose through *chemical evolution*. They go on to suggest that features of the ribosome, particularly the non-specific activity of the PTC, indicate a subsequent period of coevolutionary *chemical evolution* between non-templated proteins and RNA, prior to the emergence of self-replicating RNA and that, consequently, there was no RNA world preceding templated protein synthesis. Finally, they call into question what they call the 'Poole Hypothesis', in reference to past work of ours, published in the Journal of Molecular Evolution (Jeffares et al. 1998; Poole et al. 1998), where we examined in detail the evolutionary transition from RNA to protein.

Chemical Versus Darwinian Evolution

Bowman et al. rightly point out that an RNA with peptidyl transferase activity could arise through entirely chemical processes. In agreement with their argument for the antiquity of translation, the unambiguous cellular 'relics'

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from the earliest stages in the evolution of life are all associated with ribosome function: tRNA, RNase P, signal recognition particle (SRP) RNA, rRNAs. This signal is consistent in comparative genomics analyses of both RNA- (Hoeppner et al. 2012) and protein-coding genes (Goldman et al. 2013; Harris et al. 2003). Other RNAs that could be ancient, but which are non-universal, such as snoRNAs (Gardner et al. 2010; Hoeppner and Poole 2012), are likewise associated with the translation machinery. In contrast, the argument in favour of replication by RNA-based enzymes is based on indirect arguments such as the similarity in the chemistry of RNA and DNA polymerisation (Steitz 1998), and the theoretical plausibility of ribozyme RNA polymerases prior to protein-based polymerases, bolstered by ongoing efforts to generate polymerase ribozymes via in vitro selection (Attwater et al. 2013; Lehman 2013). However, there is no direct evidence from biology for the past existence of polymerase ribozymes, and there is therefore a discontinuity between a presumed RNA world and an early origin of extant RNA polymerases (Poole and Logan 2005).

Thus, in contrast with other processes deemed integral to an early RNA world model, there is agreement from both biology and chemistry on the antiquity of translation. However, a key unresolved question, which we feel warrants further discussion, is whether chemical processes alone could permit the subsequent evolutionary refinement of translation via coevolution of the peptidyl transferase machinery with the products of its action.

We agree with the general point that, prior to the origin of templated protein synthesis, the peptidyl transferase may have produced short non-templated proteins, allowing some degree of improvement of the function of the former. In fact, that was a feature of our original *Darwinian* model for the origin of protein synthesis (Jeffares et al. 1998; Poole et al. 1998), which we and others extended in follow-up publications (Noller 2004; Penny et al. 2009; Penny and Zhong 2014; Poole et al. 1999). However, coevolution of the ribosome with early peptides requires evolution by the twin processes of descent with modification (i.e. some kind of imperfect replicative process) and natural selection (such that some variants proliferate at the expense of others). We would count these processes as biological evolution, not chemical evolution.

In our original model, we proposed that there would be benefit in the non-templated production of short peptides by peptidyl transferase—very much akin to the coevolutionary scenario that Bowman et al. propose. However, as the specific order of amino acid incorporation would be uncontrolled in a non-templated system, we argued that the initial value would have been in functions associated with general properties of such peptides, such as stabilising catalytic RNA, including the PTC, via inclusion of basic amino acids. We agree that the production of such peptides could be a form of *chemical evolution*, as, without a template, there would be high stochasticity of production. Both we Poole et al. (1998, 1999), and, later, Bernhardt and Tate (2010), have proposed that the first mRNAs (and thus the first protein-coding genes) emerged from interactions between charged tRNAs and RNAs that served to favour the reaction by tethering the former in place, thus aiding peptide synthesis. It was from this beginning as a physical buttress that we envisaged the genetic code could start to evolve. So, we agree with a coevolutionary model (we called it a positive feedback loop, drawing from Hasegawa and colleagues' earlier work (Hasegawa et al. 1984)) where the products of early peptide synthesis improve the system.

However, attributing this solely to *chemical evolution* is problematic, because, without heredity, subsequent evolution is severely limited. We find the ongoing evolution of a non-genetically encoded PTC difficult to envisage for three reasons. First, without heritable templates (proto-mRNAs), useful peptides are the product of stochastic or environmental processes, so it is unlikely that multiple copies of a favourable peptide can be produced accurately or repeatedly. High fidelity production of these peptides becomes a greater problem as peptide chain length increases, for combinatoric reasons.

Secondly, peptides produced in a pre-genetic phase cannot improve (which requires selection between variants with preferential replication of favoured forms). Therefore, we expect RNA enzymes (which are genetically encoded in the RNA world model) to predominate because they *can* improve via Darwinian evolution. This does not exclude a role for peptides, but repeated accurate production of favourable peptides seems difficult under *chemical evolution*.

Finally, under *chemical evolution*, the genetic code cannot evolve because it does not exist—yet there is good evidence that modern translation is the result of an optimisation process (Ardell 1998; Freeland and Hurst 1998; Freeland et al. 2000; Vetsigian et al. 2006). The process of optimisation requires natural selection on the early ribosomal machinery, on the code itself, and on early genes. Coevolution can only occur if variation is not swamped by irreproducible randomness—some versions of the PTC must be better than others, some early genes must code more advantageous peptides, and some codes must be less prone to error. The only way for improved versions of the PTC to evolve is via some means of preferential copying of successful PTCs over less successful PTCs. There must be a feedback loop between genotype and phenotype.

We do agree that the emergence of a self-replicating RNA from chemical processes is a major difficulty for the RNA world. However, in the model presented by Bowman et al., it seems that the PTC still needs to be replicated

following its inception. Moreover, there must be some selective process by which replicated copies differentially proliferate or disappear. Thus, replacing the problematic self-replicating RNA polymerase with the PTC does not obviously escape the need for a polymerase activity. Whatever way we look at it, one still requires descent with modification and natural selection on the resulting variation to produce such an extensive RNA-based system. We submit that the excellent models presented by this team (Hud et al. 2013), wherein early polymers that readily self-assemble emerge through chemical processes, nevertheless require later refinement of these polymers through Darwinian evolution. We conclude that chemical processes would kickstart polymer evolution while Darwinian processes would help refine polymers. The chemical processes by which polymers first emerge because they are easy to assemble would not also select for better carriage of information—the latter is instead the product of Darwinian evolution via natural selection.

Catalytic Perfection Accounts for the Incomplete RNA to Protein Polymer Transition

A central part of the case that Bowman and colleagues make for their challenge to the RNA world model is to question the evidence for biological takeover of ribozyme function by protein enzymes. In particular, they question the RNA transition scenario presented previously by us (Jeffares et al. 1998). To give some context, we quote two passages from Bowman et al. that help to set the scene.

1. "The driver of the hypothetical Polymer Transition is the catalytic superiority of protein enzymes over RNA enzymes. Inexplicably, the ribosome was immune to the Polymer Transition".
2. "Poole proposed that the Polymer Transition was an incremental process in which ribozymes were replaced by ribonucleoprotein enzymes, which were then replaced by protein-based enzymes".

Regarding point 1, Bowman et al. are right to question this general statement concerning the supposed superiority of protein enzymes over ribozymes. Indeed, we did so too, and, in doing so, were able to provide a straightforward answer to the apparent 'immunity' of some catalytic RNAs to the polymer transition, including the ribosome. The critical point here is that catalytic superiority is not a trump card. We suspected this, since some catalytic RNAs clearly persist. Our explanation for the persistence of some apparent relics, despite the superiority of protein-based catalysts (Doudna and Lorsch 2005), is as follows.

If proteins are indeed superior catalysts to RNA, how is it that there are any catalytic RNAs remaining at all? For catalysis, we need to understand what natural selection can act on. As proteins are, overall, better catalysts than RNA, one might imagine that no RNAs should remain. However, it is well known that the overall rate of catalysis is influenced both by the speed of the chemical reaction (where protein may be expected to be superior) and the rate of diffusion of the substrate to the active site (which is a feature of the substrate, not the enzyme). In cases where diffusion is the rate-limiting step, an enzyme can be said to have reached 'catalytic perfection' (Albery and Knowles 1976), since no improvements to the speed of the reaction step will speed up the overall reaction. It is thus possible for a ribozyme to reach catalytic perfection where diffusion is the rate-limiting step in the reaction (Jeffares et al. 1998). All extant natural ribozymes that are strong candidates for being direct descendants of the 'RNA world' [on the basis of universal distribution—there are very few (Hoeppner et al. 2012)] have large substrates. Our application of Albery and Knowles' concept of catalytic perfection provides a model that can explain the incompleteness of the RNA to protein transition. To that end, we stand with Bowman and colleagues in challenging the naïve view that the catalytic superiority of protein enzymes is the complete explanation for the RNA to protein transition. If that were so, no RNA world relics should remain at all.

The second quote suggests that we view the polymer transition as incremental, which is a misreading of our model. Based on data showing that ribozymes seem to operate better in the presence of protein than in its absence, we argued that the earliest products of peptidyl transfer and of templated protein synthesis would have enabled improvements in ribozyme function via their capacity to stabilise catalytic RNAs (Jeffares et al. 1998). We predicted that a modest improvement in catalytic efficiency would be the outcome, owing to this stability. In this regard, the subsequent coevolution of catalytic RNAs and their non-catalytic protein 'chaperones' during the RNA to RNP (ribonucleoprotein) transition is not very different from the 'accretion' that Bowman and colleagues describe.

However, for the RNP to protein transition, where RNP enzymes are replaced by catalytic proteins, there is no requirement for this to follow accretion. We do not require that there is gradual evolutionary takeover of all the elements of catalysis by the RNA. That is one possible process, but it would be invisible to us in most instances. Thus, we agree with Bowman and colleagues' rejection of the statement, that the polymer transition, 'would require incremental changes from RNA to protein, while continuously preserving functionality'.

Instead, we expect it to be more likely that, for the RNP to protein transition, there was simply wholesale non-orthologous gene replacement (Koonin et al. 1996). In this

model, an unrelated polypeptide evolves catalytic activity that is superior to the RNP enzyme. For reactions involving small molecule substrates, diffusion is not rate-limiting and the chemical superiority of protein-based catalysis may result in loss of the original RNP and fixation of the protein enzyme (Albery and Knowles 1976; Jeffares et al. 1998). Thus, the RNP to protein transition does not require intramolecular takeover, which we would agree is exceedingly rare. In that regard, the example of RNase P takeover that Bowman and colleagues discuss is consistent with our model, not a challenge to it. Likewise, we do not view the ribosome as a challenge to the polymer transition, at least not as we envisage it.

In summary, we appreciate this valuable critique of our model for the RNA to protein transition. However, we find it difficult to imagine the evolution and refinement of a peptidyl transferase centre without the Darwinian processes of natural selection and descent with modification. In discussing these points with Bowman and colleagues it seems they broadly agree. The key to uniting 'top down' biological and 'bottom up' chemical perspectives lies in establishing how chemical evolutionary origins paved the way to early Darwinian evolution. We hope the above discussion goes some way to clarifying this.

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