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OPINION

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The potential versatility of RNA catalysis

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Abstract

It is commonly thought that in the early development of life on this planet RNA would have acted both as a store of genetic information and as a catalyst. While a number of RNA enzymes are known in contemporary cells, they are largely confined to phosphoryl transfer reactions, whereas an RNA based metabolism would have required a much greater chemical diversity of catalysis. Here we discuss how RNA might catalyze a wider variety of chemistries, and particularly how information gleaned from riboswitches could suggest how ribozymes might recruit coenzymes to expand their chemical range. We ask how we might seek such activities in modern biology.

This article is categorized under:

RNA-Based Catalysis > Miscellaneous RNA-Catalyzed Reactions
Regulatory RNAs/RNAi/Riboswitches > Riboswitches
RNA Structure and Dynamics > RNA Structure, Dynamics and Chemistry

KEYWORDS

coenzymes, riboswitches, ribozymes, the RNA world

1 | INTRODUCTION

For billions of years, protein enzymes have catalyzed the vast majority of chemical reactions that constitute the metabolism of cells. Yet a few reactions are catalyzed not by proteins but by RNA. Indeed, what is arguably the most important reaction in the cell, the condensation of amino acids to form polypeptides by the peptidyl transferase activity of the ribosome is catalyzed by RNA in the large subunit (Katunin et al., 2002; Nissen et al., 2000; Weinger et al., 2004). Another example is the splicing of mRNA, where the U2/U6 snRNA complex is a ribozyme. RNase P is a ribozyme that processes the 5′ end of tRNA in all domains of life (Guerrier-Takada et al., 1983). Some of the small nucleolytic ribozymes are widespread, such as the hammerhead (de la Pena & Garcia-Robles, 2010b) and twister ribozymes (Roth et al., 2014). Furthermore, hammerhead (de la Pena & Garcia-Robles, 2010a; Martick et al., 2008) and HDV-like ribozymes (Salehi-Ashtiani et al., 2006) are encoded in the human genome. With the exception of peptidyl transferase, all known natural ribozymes catalyze phosphoryl transfer reactions, transesterification reactions on a pentavalent phosphorus center. In this short review, we consider whether or not RNA might catalyze a wider variety of chemistries, and how we might seek such activities in modern biology. Breaker has recently discussed how a broader range of ribozymes might be conceived (Breaker, 2020). We also recommend an earlier review of Ellington and colleagues (Chen et al., 2007).

2 | CONTEMPORARY RIBOZYMES IN NATURE

Proteins are well suited to chemical catalysis in an aqueous environment, with 20 different amino acid side chains covering a wide chemical space. By contrast, the range of chemical functionalities in RNA is much more limited. It comprises four rather similar heterocyclic nucleobases, a 2′-hydroxyl group and an anionic phosphodiester with associated hydrated metal ions. Despite these limitations, RNA can accelerate phosphoryl transfer reactions by a million fold or more (Breaker et al., 2003). This is achieved by one or other of two main broad strategies. The group I self-splicing introns use divalent metal ions to organize the active center, activate the nucleophile and stabilize the transition state (Adams et al., 2004; Shan et al., 2001) and the group II introns and RNase P also appear to function as metalloenzymes (Gordon et al., 2007; Gordon & Piccirilli, 2001; Sigel et al., 2000). By contrast, the nucleolytic ribozymes use general acid–base catalysis most frequently utilizing nucleobases (Bevilacqua, 2003; Das & Piccirilli, 2005; Kath-Schorr et al., 2012; Liu et al., 2014; T. J. Wilson et al., 2019, 2006). Even though the natural pK_a values of the nucleobases are either low (adenine and cytosine) or high (guanine and uracil), generally resulting in a low fraction of active catalyst at physiological pH, a ribozyme like twister has evolved its active center to impose an in-line geometry for attack by the O2′ nucleophile, stabilize the phosphorane transition state and perform nucleobase-mediated general acid–base catalysis (T. J. Wilson et al., 2016) to achieve a substantial rate acceleration. Peptidyl transferase activity in the large ribosomal subunit does not use nucleobase-mediated catalysis, but the reaction appears to involve proton transfer mediated by a 2′-hydroxyl of tRNA (Schmeing et al., 2005).

According to the simplest version of the RNA world hypothesis (W. Gilbert, 1986) ribozymes would have catalyzed all cellular chemical reactions in a primitive metabolism. This would have required RNA to catalyze a far wider range of chemistry than we currently are aware of in nature, and it would have required relatively difficult reactions such as carbon–carbon bond formation. Many of the reactions available to the organic chemist for this purpose would be highly improbable for RNA catalysts, such as Grignard reagents and Pd cross-coupling reactions.

3 | SELECTING RIBOZYMES IN THE LABORATORY

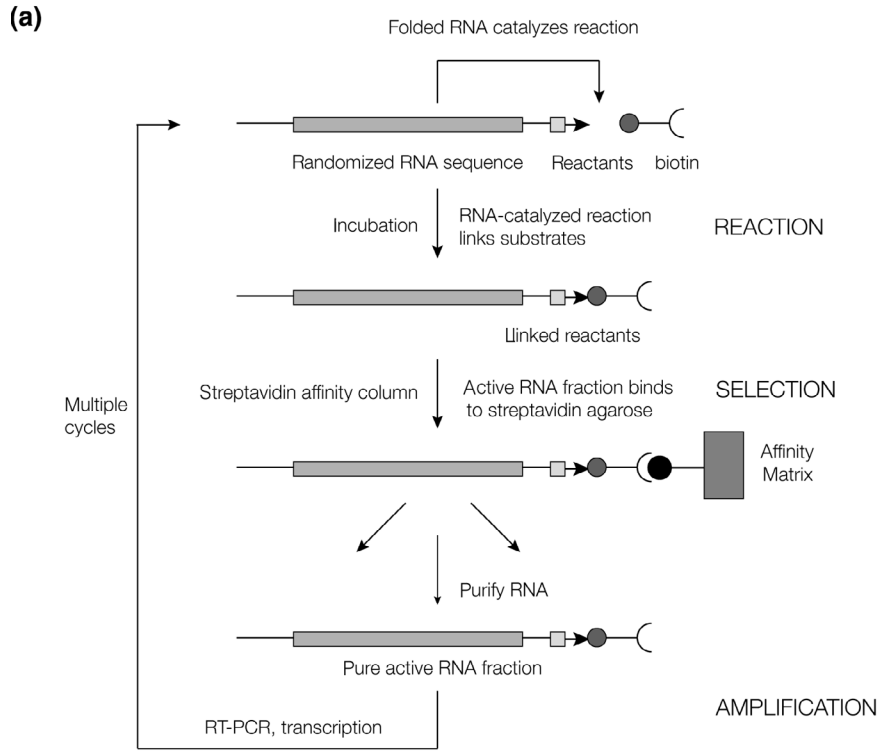
To explore what might be possible by way of RNA mediated catalysis of novel chemical reactions there have been many investigations in which in vitro evolution methods have been used to select RNA species that will accelerate a given reaction from a random pool of sequences. These experiments have generally been carried out in a similar manner in which one reactant is tethered to an RNA oligonucleotide whose sequence has been partially or totally randomized, while the other is linked to biotin. If an RNA within the pool can catalyze formation of a bond between the reactants this connects the RNA to the biotin, allowing it to be isolated by binding to streptavidin. This can then be amplified and a second round of selection performed. Something like 15–20 such cycles will be performed after which the reactant will be disconnected from the RNA to see if it will catalyze a reaction in *trans*. Clearly this strategy is limited to bond-forming reactions, and we can divide this into reactions leading to the formation of C—C, C—N, and C—S bonds (Figure 1).

Carbon–carbon bond formation. Ribozymes have been selected that can catalyze C—C bonds by the nonnatural Diels–Alder cycloaddition reaction (Seelig & Jäschke, 1999; Tarasow et al., 1997), the aldol reaction (Fusz et al., 2005) and related Claisen condensation (Ryu et al., 2006).

Carbon–nitrogen bond formation. Selected ribozymes catalyzing C—N bond formation include one that alkylates itself at a specific guanine N7 (C. Wilson & Szostak, 1995), amide (Wiegand et al., 1997) and peptide bond formation (Zhang & Cech, 1997) and glycosidic bond formation (Unrau & Bartel, 1998). Very recently Höbartner and colleagues (Scheitl et al., 2020) have selected an RNA that catalyzes methyl transfer from O^6-methylguanine to adenine N1.

Carbon–sulfur bond formation. C—S bond formation has been demonstrated by selected RNA species catalyzing Michael addition (Sengle et al., 2001) and CoA acylation (Jadhav & Yarus, 2002).

The activities of these ribozymes are typically characterized by k_{cat} in the range 0.01–0.1 min^{-1}. The estimated rate enhancements vary, being strongly dependent on the estimation of the uncatalyzed rate, but are frequently around 1000-fold. They are probably relatively unsophisticated catalysts. It is likely to be much easier to find an RNA that can



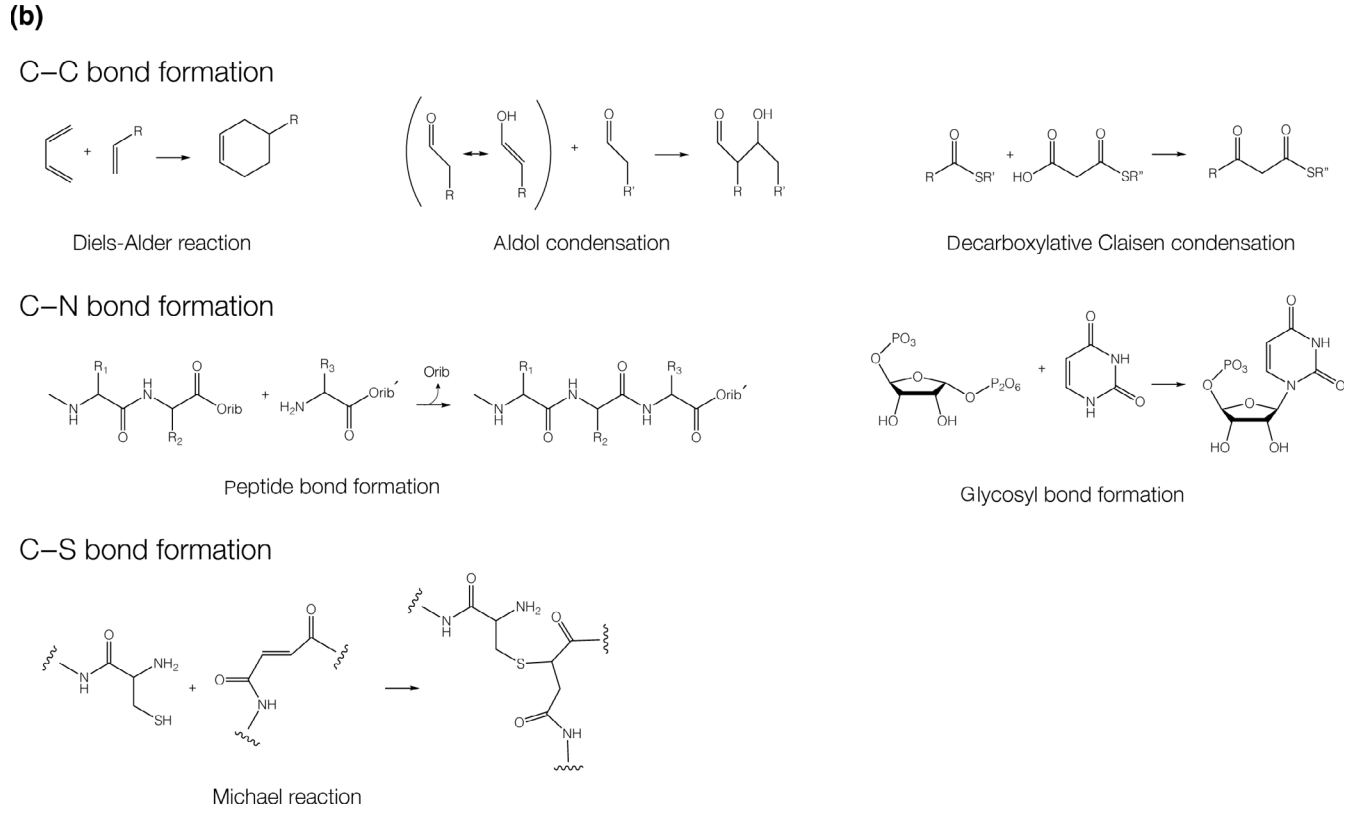


FIGURE 1 A summary of some selected ribozymes that catalyze synthetic chemistry. (a) A typical scheme for the selection of an RNA sequence that will catalyze a bond-forming reaction between two small-molecule species. (b) Some RNA-catalyzed bond-forming reactions. This shows C—C bond forming ribozymes, using Diels–Alder (Seelig & Jäschke, 1999; Tarasow et al., 1997), aldol (Fusz et al., 2005) and decarboxylative Claisen condensation reactions (Ryu et al., 2006), C—N bond forming ribozymes, showing peptide (Zhang & Cech, 1997) and glycosyl bond-forming reactions (Unrau & Bartel, 1998) and a C—S bond forming Michael reaction ribozyme (Sengle et al., 2001)

exploit metal ions in catalysis than one that uses nucleobases as chemical participants for example. Only a few have been subject to significant mechanistic investigation. One example is the aldolase ribozyme of Famulok and colleagues (Fusz et al., 2005) (Figure 1). In nature there are two classes of aldolase protein enzymes. One class uses a lysine to make a Schiff base to activate the carbonyl carbon, while the other uses a zinc ion as a Lewis acid in electrophilic catalysis. In RNA there is no equivalent of an aliphatic primary amine, while RNA can readily bind metal ions, so unsurprisingly the RNA aldolase uses the latter strategy. The two selected Diels–Alder ribozymes differ in that one uses copper ions (Tarasow et al., 1997) while the other has no identified cofactors (Seelig & Jäschke, 1999). These cycloaddition reactions likely proceed spontaneously when the diene and dienophile are juxtaposed correctly, and it is probable that propinquity and orientation are all that is required. The Diels–Alder ribozyme (Figure 1) isolated by Seelig and Jäschke (1999) is one of the few such catalytic RNAs that have had their structure determined by X-ray crystallography (Serganov et al., 2005), and this shows a binding cavity that accommodates the reactants with the required juxtaposition. This is consistent with a degree of stereoselectivity in the catalyzed reaction (Schlatterer et al., 2003).

While it is impressive that RNA can accelerate such reactions at all, these ribozymes are clearly not as efficient as the natural ribozymes. Nature would have had millions of years to evolve and refine ribozymes, and likely would have worked with rather longer segments of RNA or a related polymer. In the laboratory we are limited to rather short RNAs (<25 nt) for complete randomization (or a longer, partially randomized RNA) and a small number of cycles of selection. It is possible that previously-selected ribozymes could be further refined, following the example of the improvement of the polymerase activity selected by Bartel and colleagues (Johnston et al., 2001) by Holliger and co-workers (Wochner et al., 2011). Overall, selection of artificial ribozymes demonstrates that ribozymes can catalyze a broad range of chemical reactions, many of which are believed to have been essential in an RNA World.

4 | COENZYMES EXPAND THE RANGE OF CATALYTIC CHEMISTRY

While selection experiments provide some indications of a wider chemistry that might be RNA catalyzed, it seems likely that limitations in the breadth of chemical space would restrict the emergence of sophisticated catalysts. To expand the range of chemistry one possibility is to use chemically-activated substrates, like the imidazole-conjugated ligase substrates studied by Szostak and colleagues (Walton et al., 2020).

A much more widely adopted strategy in nature is the use of coenzymes, acting as "bolt-on chemical tools." Despite a far wider range of chemical functionality compared to RNA, around half of protein enzymes expand their catalytic repertoire in this way. Coenzymes might similarly greatly increase the catalytic range of RNA. For example, thiamine pyrophosphate (TPP) is an especially powerful coenzyme that can participate in C—C bond breaking and formation. The carbon of the thiazole ring between the two heteroatoms ionizes to form a carbanion that is a potent nucleophile, and can stabilize negative charge in a variety of adducts formed at that position. This can attack a carbonyl group, facilitating the cleavage of adjacent C—C bonds, such as in the decarboxylation of pyruvate. TPP readily binds RNA, and TPP-responsive riboswitches are extremely widespread. Pyridoxal phosphate is another highly versatile coenzyme. It forms a Schiff base with the amine of amino acids whereby its pyridine ring stabilizes negative charge, facilitating a variety of transformations around the α-carbon including deamination, decarboxylation, and racemization.

It is widely thought that many of the coenzymes evolved in the RNA world (Chen et al., 2007; White 3rd., 1976). Their ubiquity and the homology between classes of proteins that utilize them places many coenzymes in the last universal common ancestor (Weiss et al., 2016) and an earlier origin is suggested by their synthesis from components of RNA and the fact that a viable RNA world would have required an expanded chemical toolkit (Benner et al., 1989; White 3rd., 1976). Once a coenzyme had been adopted in the RNA World it is likely it would be retained due to the many-user syndrome, that is, being used by many macromolecules to catalyze different reactions would make it particularly difficult to replace (White 3rd., 1982).

There are also precedents for ribozymes utilizing coenzymes. RNA is unsuited to participate in redox reactions unaided, and the alcohol dehydrogenase ribozyme isolated by Suga and co-workers used nicotinamide adenine dinucleotide (NAD^+) as a coenzyme (Tsukiji et al., 2003, 2004). A precedent exists in nature too—the *glm*S riboswitch acts as a nucleolytic ribozyme to initiate the degradation of its mRNA, and uses the amine of its glucosamine-6-phosphate ligand as a general acid to stabilize the leaving group (Cochrane et al., 2007; Klein & Ferré-D'Amaré, 2006; W. C. Winkler et al., 2004).

5 | RNA IN THE BINDING OF LIGANDS

RNA is an excellent binding receptor for many classes of small molecules, and a number of the riboswitches bind contemporary coenzymes (Roth & Breaker, 2009; Serganov & Nudler, 2013; Sherwood & Henkin, 2016), including *S*-adenosylmethionine (SAM), thiamin pyrophosphate, tetrahydrofolate, flavin mononucleotide (FMN), cobalamin, and most recently a NAD^+-responsive riboswitch has been discovered (Malkowski et al., 2019). The TPP riboswitch (W. Winkler et al., 2002) is the most widely-occurring class of riboswitch, and multiple subclasses of SAM-responsive riboswitches have been found (Corbino et al., 2005; Fuchs et al., 2006; Grundy & Henkin, 1998; Poiata et al., 2009; Weinberg et al., 2008; W. C. Winkler et al., 2003) with varying structures and modes of binding (S. D. Gilbert et al., 2008; Huang & Lilley, 2018; Lu et al., 2008; Montange & Batey, 2006). A number of the coenzymes are based in part on adenosine (consistent with their origin in the RNA world), and the recent structure of the ADP-binding domain of the NAD^+-responsive riboswitch suggests a modularity in binding mode (Huang et al., 2020).

6 | HAVE RIBOSWITCHES EVOLVED FROM ANCIENT RIBOZYMES?

The discovery of coenzyme binding riboswitches has prompted speculation about whether they evolved from ancestral ribozymes extant in the RNA World (Breaker, 2012). We suggest three criteria for considering the plausibility of this hypothesis.

1. The more ancient the riboswitch the more likely it has evolved from a ribozyme. Many coenzymes utilizing enzymes can be reliably assigned to the last universal common ancestor (Weiss et al., 2016) so it is likely most if not all ribozymes had been replaced by enzymes by this stage of evolution. An ancient riboswitch is likely to be found in a broad range of bacterial phyla.
2. The riboswitch should bind the coenzyme in a potentially active conformation. An ancestral ribozyme must have bound its coenzyme in a reactive conformation whereas it might be advantageous for a riboswitch to bind a highly reactive coenzyme in a non-reactive conformation. An active conformation should be accessible without destabilizing coenzyme binding.
3. The riboswitch should have the potential to bind a substrate.

The SAM riboswitches illustrate these considerations. We consider the SAM-I/SAM-IV riboswitch family (which share a common SAM binding motif) to be a plausible candidate to have evolved from an RNA World ribozyme as they satisfy all three criteria. They are dispersed across the entire bacterial phylogenetic tree (Figure 2) so the family is clearly ancient. In order for SAM to donate a methyl group in an S_N2 reaction the nucleophile has to align with the sulfur–carbon bond. In the crystal structure of SAM-I the coenzyme sits between helices P1 and P3 in a pocket at the center of an elaborated four-way junction. The methyl group is not accessible for an in-line attack by a nucleophile because it is partially occluded by C8; however, it would require only a minor rotation to become fully exposed. Notably the two exchanging strands of the junction cap the SAM binding pocket and include nucleotides that are not well conserved (Figure 3a,b). We can envisage that these exchanging strands may have formed a substrate binding site in an ancient ribozyme. Very recently Breaker also proposed that SAM-I may have evolved from a ribozyme (Breaker, 2020).

The SAM-II/SAM-V family of riboswitches clearly fails the criteria. They are not as widely distributed as SAM-I (they are not found in the Firmicutes or Fusobacteria) (Figure 2) and more significantly SAM is bound deep in the major groove of the riboswitch so that the methyl group is not accessible (Figure 3c,d). The SAM-III riboswitch also fails our criteria, but for different reasons. The methyl group is orientated away from the RNA and is free to react (Figure 3e,f). However, it is found almost exclusively in the *Bacilli*, and solely in the Firmicutes (Figure 2), suggesting a more recent origin, and the compact structure of this riboswitch provides no obvious structure that could have bound a substrate.

The FMN riboswitch is another plausible candidate for having evolved from an ancestral ribozyme. It appears to be of ancient origin having a similar phylogenetic distribution to SAM-I (Figure 2). The active part of FMN is the isoalloxazine ring system where the protonation of nitrogen atoms N1 and N5 varies with the extent of oxidation (Figure 4a). N5 and the adjacent C4a are subject to nucleophilic attack when FMN is oxidized and participate in electron transfer in all three oxidation states. Thus, in a ribozyme N1, C4a, and N5 would need to be available to react. FMN is bound in a pocket in the core of the riboswitch with N1 exposed to solvent and able to exchange protons but with C4a and N5 packed in an unreactive conformation against the ribose of A49, which forms the first base pair of P4 (Figure 4b–d).

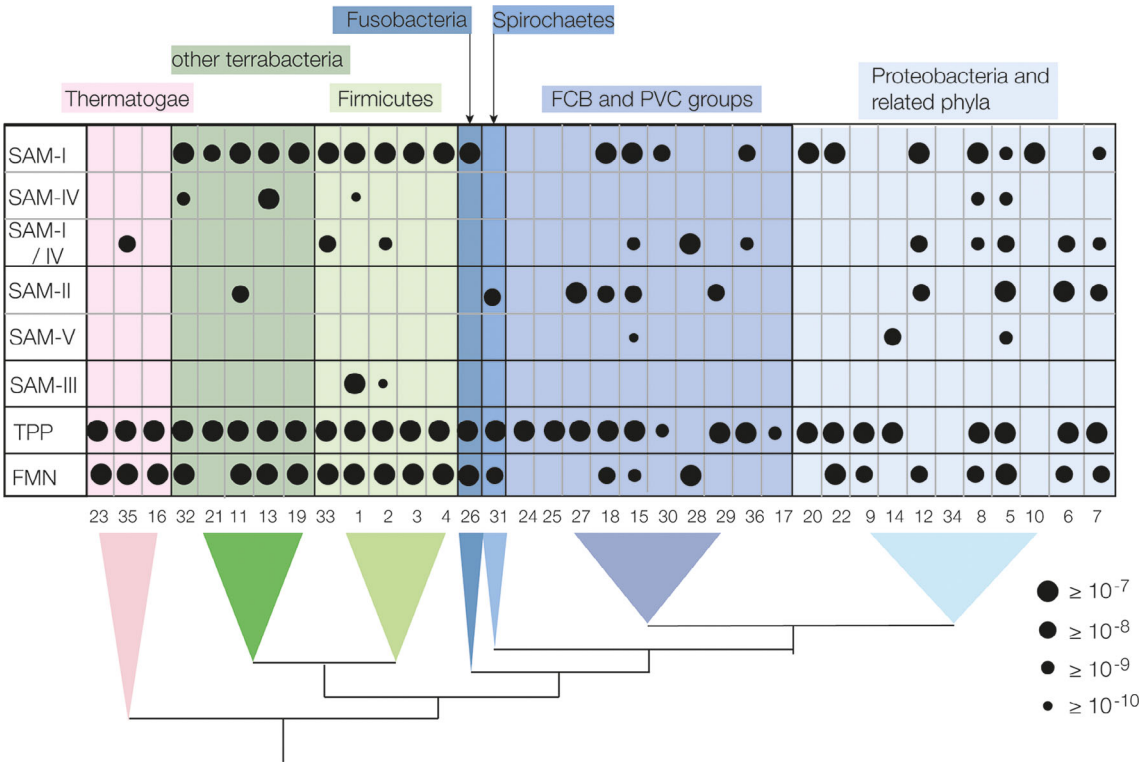


FIGURE 2 Phylogeny and distribution of selected SAM and FMN binding riboswitches. The occurrence of riboswitches in 36 bacterial divisions (phyla or orders) as given by McCown et al. (2017), arranged according to a recent phylogenetic tree for the bacteria (Zhu et al., 2019). Note that the time represented by the tree is not to scale. The size of the circle gives the abundance of riboswitches measured as the number of riboswitches per nucleotide of sequenced DNA, descending in decades from $\geq 10^{-7}$ (largest) to $\geq 10^{-10}$ (smallest). Organisms: 1 Bacilli, 2 Clostridia, 3 Erisipelotrichi, 4 Negativicutes, 5 Alphaproteobacteria, 6 Betaproteobacteria, 7 Gammaproteobacteria, 8 Deltaproteobacteria, 9 Epsilonproteobacteria, 10 Zetaproteobacteria, 11 Deinococcus-Thermus, 12 Acidobacteria, 13 Actinobacteria, 14 Aquificae, 15 Bacteroidetes, 16 Caldiserica, 17 Chlamydae, 18 Chlorobi, 19 Chloroflexi, 20 Chrysiogenetes, 21 Cyanobacteria, 22 Deferribacteres, 23 Dictyoglomi, 24 Elusimicrobia, 25 Fibrobacteres, 26 Fusobacteria, 27 Gemmatimonadetes, 28 Lentisphaerae, 29 Lentisphaerae, 30 Planctomycetes, 31 Spirochaetes, 32 Synergistetes, 33 Tenericutes, 34 Thermodesulfobacteria, 35 Thermotogae, 36 Verrucomicrobia

Hence the riboswitch does not appear to satisfy the other criteria. However, helix P4 is highly variable and makes no contact with the rest of the riboswitch, and if it is deleted the reactive edge of the isoalloxazine ring is seen to be fully exposed (Figure 4e). It is feasible that helix P4 is not required for coenzyme binding and that an ancestral ribozyme could have had a substrate binding domain that positioned substrate and coenzyme for reaction in place of that helix.

7 | MIGHT RIBOZYMES THAT CATALYZE A BROADER RANGE OF CHEMISTRY EXIST IN CONTEMPORARY CELLS, AND WHERE MIGHT THEY BE DISCOVERED?

The wealth of evidence supporting the RNA World hypothesis strongly suggests that a broader range of ribozymes existed at an early stage of evolution of life on the planet. But could ribozymes that catalyze reactions other than phosphoryl transfer exist in contemporary biology? Clearly the peptidyl transferase offers a precedent for this, although the ribosome is the exception. With many substrates and performing two distinct reactions it likely got trapped early in evolution in a similar manner to the coenzymes. Other ribozymes were presumably more readily replaced and the apparently nearly-total replacement indicates that the use of proteins as catalysts confers significant evolutionary advantage. The simplest explanation for other ribozymes being not discovered is that there is none to find.

Making proteins is energetically expensive, requiring hydrolysis of four ATP molecules per amino acid—a large fraction of the energy resources of bacteria go into protein synthesis because of regeneration of GTP cofactors, charging tRNAs and so on. In contrast, transcription of a ribozyme would require significantly fewer resources. Therefore, there

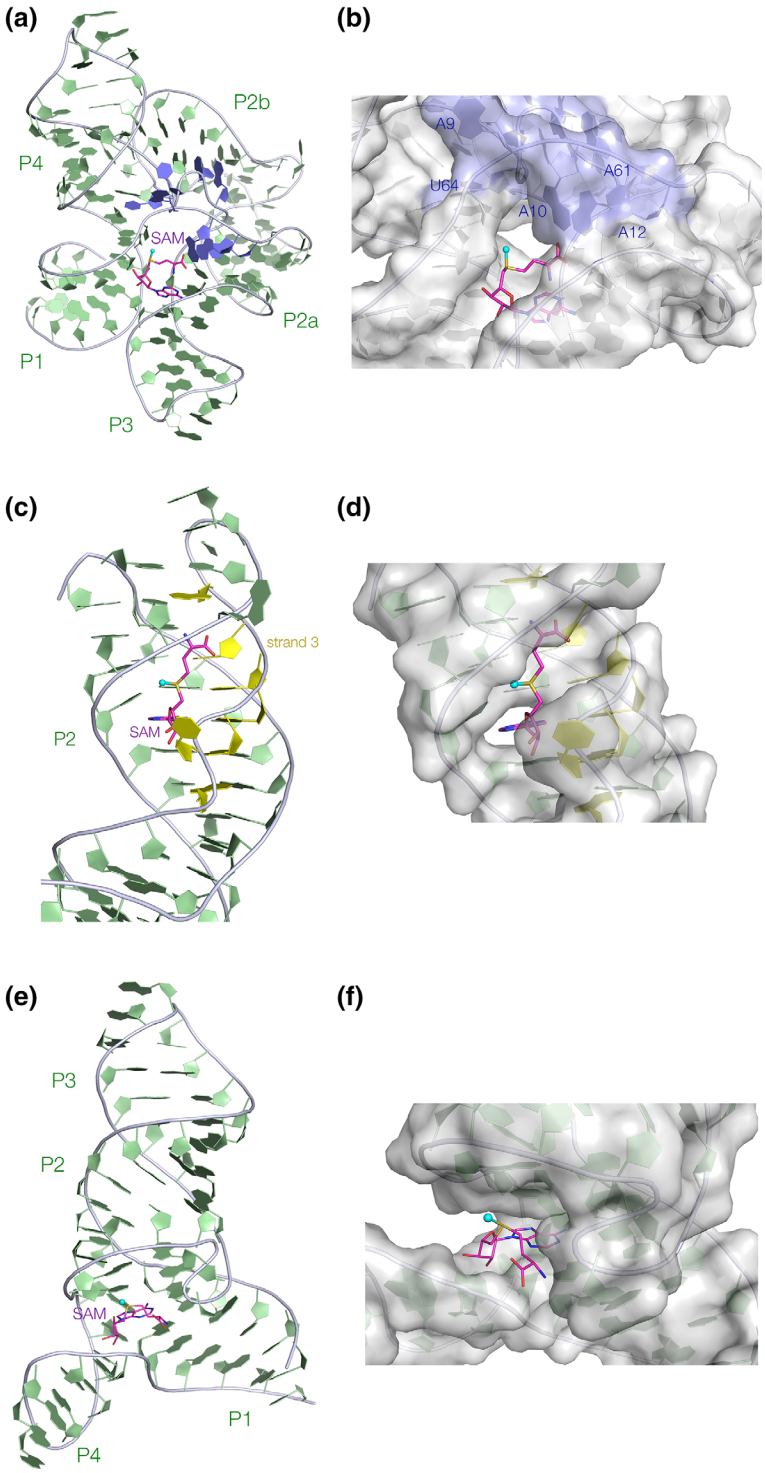


FIGURE 3 The differing environment of SAM in classes of SAM riboswitch. Each riboswitch is shown in cartoon form (left) and a closeup view of the SAM binding site (right) with a surface shown on the RNA. (a and b) The structure of the SAM-I riboswitch (PDB ID 3IQR) with SAM bound between helices P1 and P3 (Stoddard et al., 2010). The exchanging strands of the four-way junction are colored blue and SAM is shown in stick format with the labile methyl group represented as a cyan sphere. (c and d) The structure of the SAM-V riboswitch (PDB ID 6FZ0) with SAM bound in the deep groove of the triple helix so that the methyl group is inaccessible (Huang & Lilley, 2018). (e and f) The structure of the SAM-III riboswitch with SAM bound at the three-way junction with the methyl group exposed to solvent (Lu et al., 2008)

might be some advantage to using ribozymes in circumstances where resources are very limited (oligotrophic environments) so long as the ribozyme does not constrain the flux through its metabolic pathway. Such ribozymes might be ancient relics from an RNA world, or perhaps they might have arisen de novo more recently. It could be worthwhile

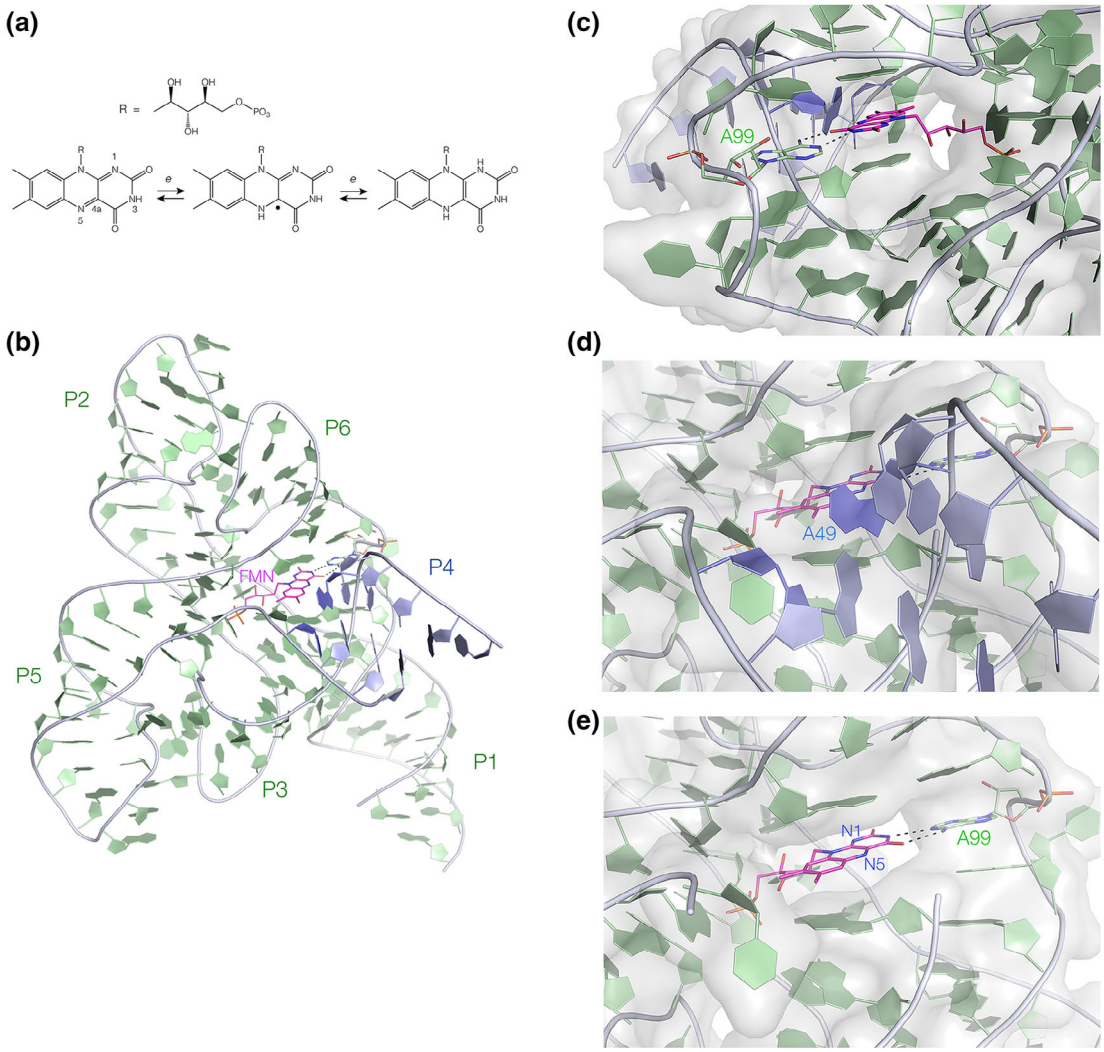


FIGURE 4 The FMN riboswitch. (a) The structure of FMN showing the oxidized, semiquinone and hydroquinone states of the isoalloxazine ring system. The protonation state of nitrogen atoms N1 and N5 vary between the three states. The unpaired electron of the semiquinone is delocalized; however, there is high spin density at C4a. (b) The structure of the FMN riboswitch (PDB ID 2YIE) with FMN shown in stick format and P4 shown in blue (Vicens et al., 2011). (c and d) Two views of FMN bound in the core of the riboswitch showing N1 exposed to solvent in the binding pocket and N5 packed against A49. (e) A view of the FMN binding pocket with helix P4 removed from the structure. In this hypothetical structure C4a and N5 are available to participate in reactions

investigating organisms from desert environments, or from a subglacial lake like Lake Vostok that has been isolated under kilometers of Antarctic ice for millions of years. Another possibility might be bacteria found in the oceans like *Pelagibacter ubique* (Rappe et al., 2002), particularly those that live below the photic zone. At the greatest depths perhaps organisms living in the reducing environment surrounding deep ocean hydrothermal vents might be interesting to explore from this point of view. Organisms living in oligotrophic environments sometimes undergo genomic streamlining, reducing the size of their genome significantly (*P. ubique* has one of the smallest genomes known; Giovannoni et al., 2005). Selection for small genome size might also favor ribozymes. The biology of oligotrophic environments is often poorly understood and their microorganisms are often difficult to culture so it would not be surprising if a ribozyme had so far escaped detection.

8 | HOW MIGHT WE SEEK RIBOZYMES WITH NOVEL CHEMISTRIES?

Although we have suggested ecological niches that might harbor ribozymes, an approach that focusses on individual organisms (such as a pull-down of RNA using a coenzyme) seems too restrictive. Here we suggest two bioinformatic

strategies for seeking new ribozymes, one based on sequence and structure and the other on a functional approach. A structure-based search would commence with a well characterized natural aptamer and here the obvious candidates are the coenzyme-binding riboswitches, especially those discussed above that might have evolved from ancient ribozymes. Weinberg et al. (2017) have developed a bioinformatics pipeline using conservation of primary sequence and secondary structure, identified by searching for covariation of base pairs, to identify riboswitches. We envisage extending this approach by first identifying a minimal core of the riboswitch necessary for binding and using this as a seed to search for aptamers with additional conserved structures unnecessary for function as a riboswitch. It should be relatively straightforward to demonstrate coenzyme binding by the aptamer but could the candidate be a ribozyme? This requires identification of a substrate and demonstration of activity. The function of the coenzyme will help to constrain the search for a substrate, as might the genomic context if known. The second approach described below might be applicable, as might traditional laboratory genetics. But none is certain to succeed and identification of a substrate could be very challenging.

A second approach could be to search for missing enzymes in complete genomes by using annotations to identify the presence of a particular metabolic pathway and then searching for all the enzymes of the pathway. A missing enzyme might have been replaced by a ribozyme, although it is more likely that the enzyme has not been identified by homology and so there is a high likelihood of false positives. Other information would be needed to evaluate whether or not to pursue the search. Such factors could include how readily the missing enzyme is identified in other genomes, the genomic context of the other genes of the pathway, and whether the reaction in question is likely to be regulated or constrain the flux through the pathway. If a plausible candidate could be identified then a pull-down technique using either a substrate analogue or coenzyme might isolate the ribozyme. Alternatively, genomic SELEX coupled with an appropriate assay might be successful.

9 | CONCLUSION

It seems highly probable to us that ribozymes would have catalyzed a broader range of reactions in the distant past, and it is very likely that coenzymes would have been important in expanding the range of possible chemistry. It remains an open question whether ribozymes with novel chemistries exist in modern biology and we have suggested ecological niches where ribozymes might be found and search strategies to identify them. Nevertheless, performing such a search would be a considerable undertaking and the question of whether the object of the search is a philosophers' stone or a needle in a haystack will only be resolved by a successful outcome.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Tmothy Wilson: Writing-original draft. **David Lilley:** Writing-original draft.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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