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The origin of the RNA world: Co-evolution of genes and metabolism

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Abstract

Discoveries demonstrating that RNA can serve genetic, catalytic, structural, and regulatory roles have provided strong support for the existence of an RNA World that preceded the origin of life as we know it. Despite the appeal of this idea, it has been difficult to explain how macromolecular RNAs emerged from small molecules available on the early Earth. We propose here a mechanism by which mutual catalysis in a pre-biotic network initiated a progression of stages characterized by ever larger and more effective catalysts supporting a proto-metabolic network, and the emergence of RNA as the dominant macromolecule due to its ability to both catalyze chemical reactions and to be copied in a template-directed manner. This model suggests that many features of modern life, including the biosynthetic pathways leading to simple metabolites, the structures of organic and metal ion cofactors, homochirality, and template-directed replication of nucleic acids, arose long before the RNA World and were retained as pre-biotic systems became more sophisticated.

Keywords: RNA World; Catalysis; Metabolism; Ribozyme; Pre-biotic chemistry; Evolution

1. Introduction

The proposal that life on Earth arose from an RNA World is widely accepted [1,2]. Many observations suggest that RNA could have served genetic, catalytic and regulatory

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roles before the advent of DNA and proteins. RNA is the genetic material for many viruses. The peptidyl transferase at the heart of the ribosome is a ribozyme [3], and the spliceosome may also be an RNA machine [4]. *In vitro* evolution experiments have demonstrated that ribozymes can catalyze a wide range of chemical reactions [5–9] and thus could have supported a complex metabolic network. RNA molecules serve various regulatory roles, as well [10].

The process by which the RNA World emerged remains a mystery. Much discussion has centered on the question of whether genes or metabolism arose first, a problem frequently likened to the classic puzzle of whether chickens or eggs arose first. The puzzle stems from the recognition that genes, whether constructed of DNA or RNA, could not have emerged without an underlying metabolism that supplied the necessary building blocks, and the seemingly contradictory assumption that metabolism could not have emerged without macromolecular catalysts encoded by genes. Framing the problem in this way obscures what is likely to be the correct answer—that genes and metabolism emerged together.

Here we propose a mechanism by which mutual catalysis in proto-metabolic reaction networks led, perhaps inexorably, to the emergence of RNA as the dominant macromolecule that supplied both catalysis and genetic information. We use the principles of physical organic chemistry to predict specific mechanisms for catalysis of proto-metabolic reactions. This perspective has been largely missing from discussions of the origin of the RNA World. An important feature of our model is that selection favored communities of molecules that collectively were best able to catalyze synthesis of their own constituents. Self-replication is viewed as a property of a metabolic network, rather than a property of individual molecules. We suggest that large RNA catalysts emerged beginning from a stage in which catalysis was performed by small molecules such as nucleotides, amino acids, and simple cofactors. Such catalysts would have been inefficient but critical for channeling the flux of organic molecules through productive pathways, as well as for accelerating the rates of reactions (see Fig. 1). Collections of catalysts that were able to increase the levels of monomeric building blocks would have favored formation of longer oligonucleotides and peptides, but only the oligonucleotides could have been replicated in a template-directed fashion. Thus, the system would have moved toward a collection of RNA catalysts that produced both the nucleotides necessary for their own replication, and the

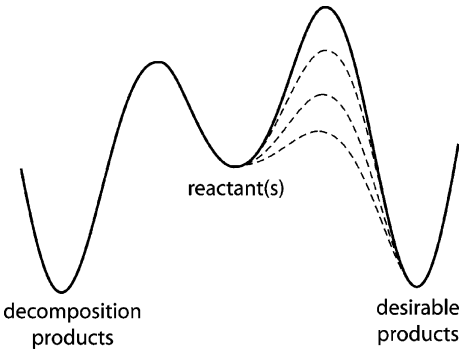

Fig. 1. By accelerating the rate of a desirable reaction, a catalyst can divert flux away from decomposition products.

amino acids and cofactors that could not be replicated directly, but that contributed to catalytic processes. These ideas build upon theoretical studies of the properties of self-sustaining and autocatalytic sets of molecules [11,12] by providing an explicit consideration of molecular reactivity and catalytic mechanisms.

2. The emergence of proto-metabolism

Hydrothermal vents, particularly those constructed of transition metal sulfides, are appealing sites for the emergence of proto-metabolism. At such sites, small molecules (including CO_2, H_2, H_2S and NH_3) are vented into porous structures lined with catalytic surfaces [13,14]. Notably, pyruvate can be synthesized under such conditions [15]. Molecules formed at high temperatures could have percolated through the porous walls into cooler chambers near the exterior, allowing synthesis of more fragile molecules, as well as compartmentalization essential for preventing dilution into the ocean [16]. Lagoons have also been proposed to be sites for the emergence of life. At such sites, organic molecules delivered to the ocean by comets and meteorites could be concentrated by evaporation, providing an organic soup. Lagoons lack three important features of hydrothermal vents: (1) a mechanism for continuous delivery of reactive small molecules; (2) abundant catalytic surfaces composed of transition metals; and (3) a mechanism for physical compartmentalization. Consequently, we favor hydrothermal vents as the site for the origin of life. However, the general principles that will be postulated below are likely to obtain regardless of the setting in which the RNA World emerged.

We hypothesize that extant metabolic pathways emerged out of less-ordered pre-biotic reaction networks by gradual acquisition of catalysts that favored flux through particular pathways. Fig. 2 shows a hypothetical example in which the availability of two catalysts would favor formation of desirable product at the expense of competing products. This illustrates the important principle that catalysts are critical for channeling flux of material through certain pathways in an otherwise very complex network, in addition to their more obvious function of accelerating reaction rates. Thus, catalysts contribute to the sparse-

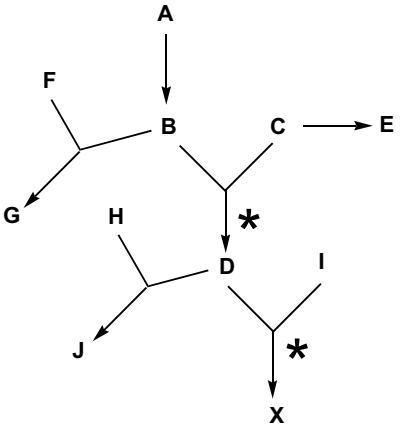

Fig. 2. A hypothetical example in which the presence of two catalysts (*) can favor the flux of material through the network to provide the desirable product X rather than the competing products E, G, and J.

ness characteristic of metabolic networks that allows accumulation of significant quantities of particular compounds, rather than miniscule amounts of every possible product.

Since acquisition of new catalysts probably occurred one at a time in a largely random fashion, many extant metabolic pathways likely resulted from reinforcement of pre-existing pathways, with gradual replacement of primordial catalysts by more efficient catalysts, beginning with metal ions and small molecules and progressing to macromolecular RNA and/or protein catalysts. Thus, extant metabolic pathways should provide strong clues about pre-biotic pathways, at least for the synthesis of the fundamental building blocks that must have been synthesized abiotically before life could emerge. We do not suppose a perfect correspondence between primordial and extant pathways. For example, steps in which NH_3 is delivered via glutamine and formate is delivered via N^{10}-formyltetrahydrofolate in modern metabolism likely involved NH_3 and formate, respectively, under prebiotic conditions. Furthermore, as cells (or proto-cells) accumulated a large number of catalysts, it would have become possible to patch together existing catalysts in novel ways to generate new metabolic pathways. Early catalysts likely had broad substrate specificity, and this feature would have allowed catalysts to facilitate similar chemical reactions in different pathways. If "patched-together" pathways were not as efficient as pre-existing pathways, they would not have persisted. However, if changes in availability of starting materials and catalysts rendered a novel pathway more effective, it might indeed replace a pre-existing pathway.

Even though the sequence of steps in a metabolic pathway may reflect primordial metabolism, the mechanisms of extant enzymes need not reflect the mechanisms of earlier catalysts. There are many ways to catalyze any reaction, and new modes of catalysis would have become available as catalysts became more sophisticated. However, ancient strategies that worked well were likely to have been maintained. For example, metal clusters [17] and/or cofactors [18] are found in the majority of extant enzymes, and are likely to be relics of a time when these clusters and cofactors performed similar roles in the absence of proteins. The substrate-assisted catalysis used by the ribosome is a second example. The 2′-OH of the terminal A of the tRNA substrate accelerates the rate of peptide bond formation by 10^6 [19]. This simple and effective strategy may have been preserved since the origin of the ribosome.

3. A metabolic module for synthesis of nucleotides

Extant autotrophs utilize a universal core of metabolic pathways (with some slight variations) for synthesis of nucleotides, the 20 amino acids found in proteins, and a number of cofactors. The earliest stages of proto-metabolism need not have involved all of these molecules. Complex amino acids and cofactors might have emerged later. The "core of the core" necessary to launch the process of evolution toward the RNA World must have consisted of a pathway for synthesis of organic compounds from CO_2 and H_2 (such as the reductive TCA cycle or the Wood-Ljungdahl pathway), and pathways for synthesis of ribose, purines, pyrimidines, and simple amino acids and cofactors. Additional "modules" for synthesis of complex amino acids, additional cofactors, and fatty acids likely accreted onto this "core of the core" at later times.

The metabolic module required to generate nucleotides is surprisingly small (Fig. 3). All of the components can be synthesized from pyruvate, with inputs of NH_3, formate, CO_2, ATP, and glyoxalate. Pyruvate can be synthesized under hydrothermal conditions [15].

Furthermore, the reactions that interconvert metabolites in this core are relatively simple. Notably, the only cofactor required is NADH. (In extant life, synthesis of iminoaspartate, which is required for synthesis of NADH, requires a flavin-dependent enzyme. Under prebiotic conditions, it could have been made by attack of ammonia on oxaloacetate.)

4. Emergence of the RNA World through a series of simpler stages

The nucleotide synthesis module necessary to generate the precursors for RNA is simpler that might be supposed. We now consider how a system of ever more complex catalysts might have led to a situation in which certain components of the network—i.e., RNA molecules—could have been replicated in a template-directed fashion. Fig. 4 illustrates a series of stages leading from simple monomers to the macromolecular RNA World. The characteristics of each stage will be discussed below.

4.1. The monomer stage

Critical components at the earliest stage would have included α-keto acids such as pyruvate, simple amino acids, NADH, ribose, purines, and pyrimidines. We postulate that these compounds were produced in or near hydrothermal vents as a result of catalysis by mineral surfaces and other small molecules in the network. Mineral surfaces would have helped concentrate and retain reactants. Furthermore, mineral surfaces might have catalyzed reactions by polarizing carbonyl groups and enhancing their electrophilicity, or by providing nucleophilic hydroxyl groups. General acid and general base catalysis

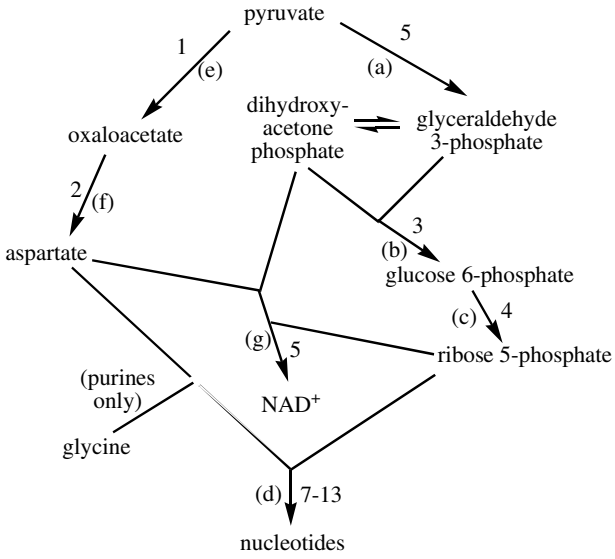

Fig. 3. The nucleotide synthesis module in the "core of the core". Numbers by arrows indicate the number of steps required to generate the product. Inputs for each reaction: (a) 2 ATP, NADH; (b) none; (c) 2 NAD^+; (d) variable amounts of ATP, CO_2, NH_3, formate, O_2 or an alternative oxidant, and NAD^+; (e) CO_2; (f) NH_3, NADH; (g) 3 ATP, NH_3, O_2 or an alternative oxidant.

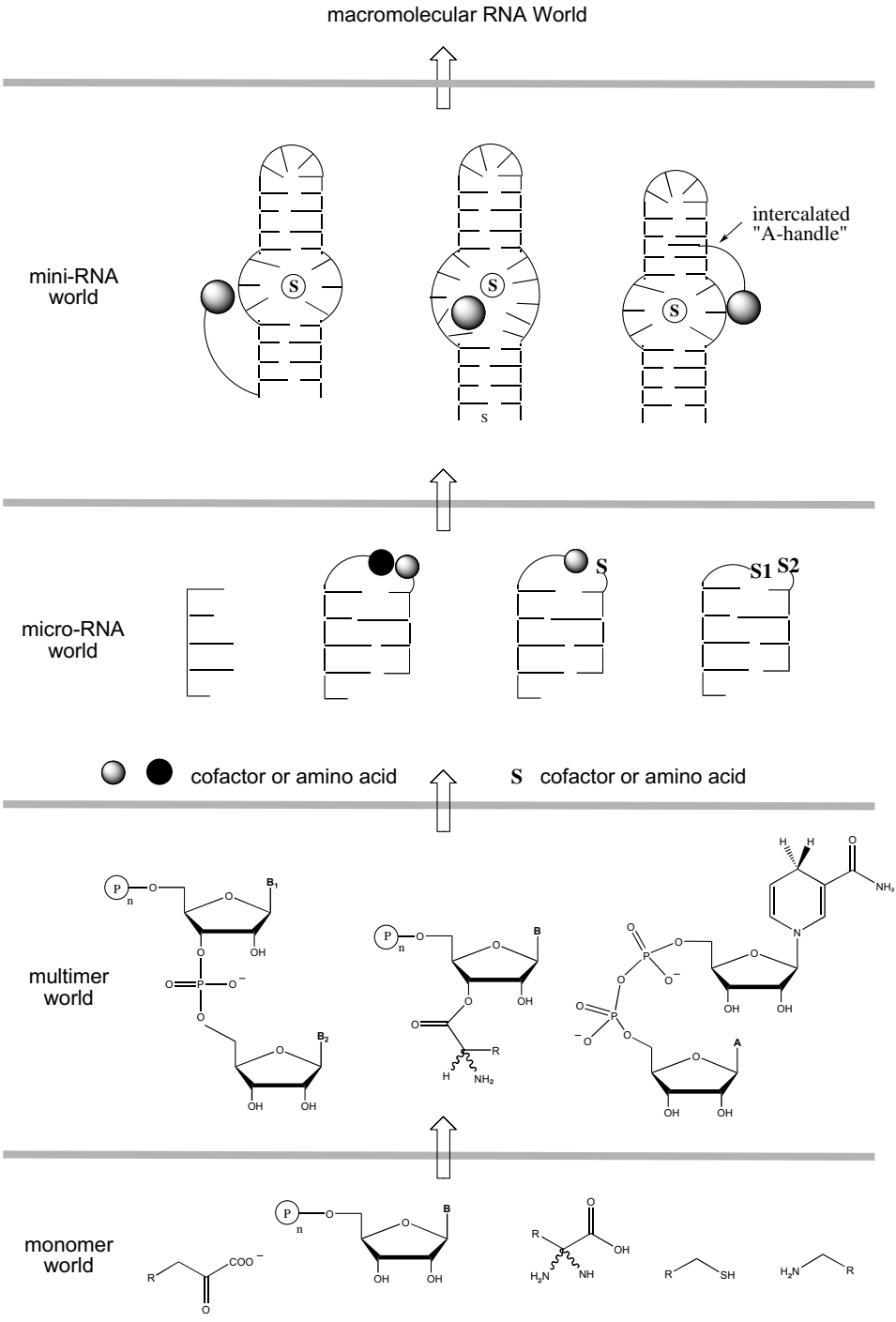

Fig. 4. Stages of emergence of the RNA World.

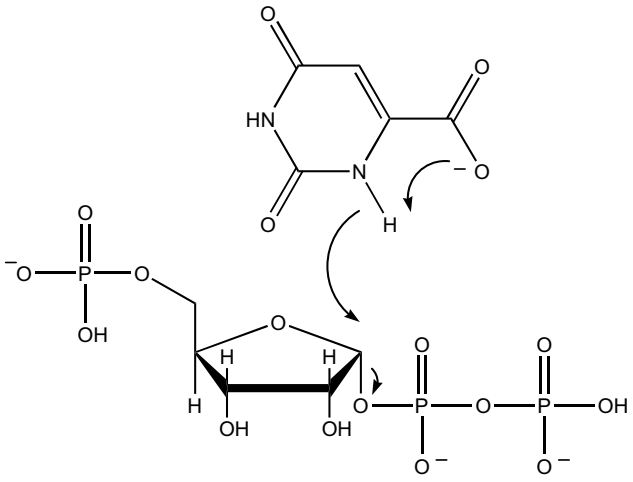

Fig. 5. The carboxylate group of orotate might facilitate attack on phosphoribosyl pyrophosphate (PRPP). This is an important step in the synthesis of pyrimidine nucleotides.

could have been provided by amino, carboxylate, and phosphate groups. Rate accelerations and control over regioselectivity and stereochemistry would likely have been modest, leading to small preferences for certain reactions and products. The catalytic capabilities of small molecules have not been explored systematically, but it is clear that significant rate accelerations can be achieved. For example, ammonia and amines catalyze aldol condensations [20], and proline catalyzes aldol, Mannich, and Michael reactions [21].

Substrate-assisted catalysis would have been an important source of rate acceleration at this stage. Fig. 5 shows a hypothetical example in which a carboxylate acts as a general base during attack of orotate on PRPP to form orotidine 5′-phosphate. Substrate-assisted catalysis can provide impressive rate enhancements. For example, the rate of hydrolysis of a simple amide is accelerated by 10^6-fold when a catalytic carboxylic acid is added to the molecule [22].

Because binding interactions between small molecules are weak and non-specific, mechanisms for enhancing the probability of productive encounters would have been important. Covalent tethering of substrates to catalytic molecules may have been helpful, particularly for bimolecular reactions for which involvement of a catalyst requires a third-order reaction. In essence, covalent tethering creates a situation in which substrate-assisted catalysis is possible. Box 1 describes the rate equations for a second-order reaction in the absence and presence of a catalyst to which one reactant can be covalently attached. Although we lack sufficient knowledge of concentrations and rate constants to make specific predictions, there are clearly circumstances in which covalent tethering will provide rate enhancement. Furthermore, covalent tethering can orient substrates to favor certain stereochemical outcomes. For example, reaction of glyceraldehyde 3-phosphate (GAP)[1] with dihydroxyacetone phosphate (DHAP) to form D-fructose-1,6-bisphosphate might be facilitated by tethering of GAP to the 2′hydroxyl of GMP and Schiff base forma-

[1] *Abbreviations used:* DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate.

$$A + B \xrightarrow{k_{uncat}} P$$

$$A\text{-l.g.} + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A\text{-}C + \text{l.g.} \qquad A\text{-}C + B \xrightarrow{k_{cat}} A\text{-}P \rightleftharpoons A + P$$

$$v_{uncat} = k_{uncat}(A)(B)$$

$$v_{cat} = \frac{k_1 k_{cat}(A)(B)(C)}{k_{-1}(\text{l.g.}) + k_{cat}(B)}$$

$$\frac{v_{cat}}{v_{uncat}} = \frac{k_1 k_{cat}(C)}{k_{uncat}[k_{-1}(\text{l.g.}) + k_{cat}(B)]}$$

$$\text{if } k_{cat}(B) >> k_{-1}(\text{l.g.}) \qquad \frac{v_{cat}}{v_{uncat}} = \frac{k_1(C)}{k_{uncat}(B)}$$

$$\text{if } k_{cat}(B) << k_{-1}(\text{l.g.}) \qquad \frac{v_{cat}}{v_{uncat}} = \frac{k_1 k_{cat}(C)}{k_{uncat}\, k_{-1}(\text{l.g.})}$$

Box 1. Comparison of the rates of conversion of A and B to P in the absence and presence of a catalyst to which A can be covalently attached. The leaving group that is displaced by attachment to A is shown explicitly only for the catalyzed reaction. Rate constants for the removal of P from the catalyst are disregarded because in most pathways, attachment of the product to the catalyst will not interfere with the next reaction, and indeed may facilitate the next reaction. "l.g." denotes a leaving group.

tion between DHAP and the exocyclic amino group of GMP (Fig. 6). The orientation shown would produce D-fructose-1,6,-bisphosphate.

Notably, every metabolite in the pathways for synthesis of simple amino acids and nucleotides has a carboxylate or phosphate group. In extant cells, these groups prevent loss of metabolites through the membrane and provide "handles" for binding to proteins.

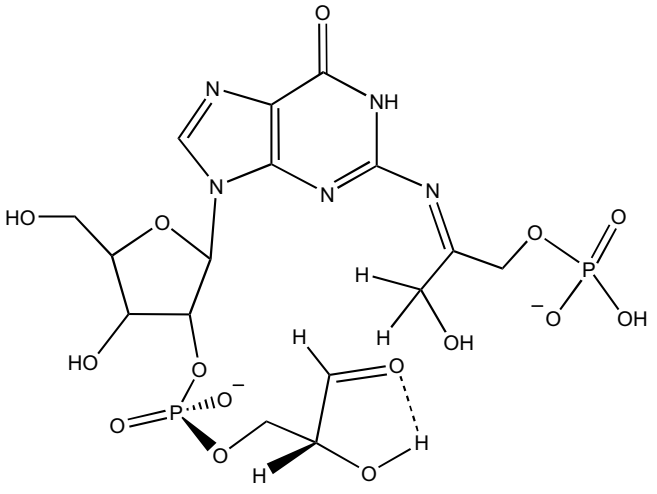

Fig. 6. Aldol condensation of DHAP and GAP might be facilitated by formation of a Schiff base between DHAP and the exocyclic amino group of GMP and tethering of GAP to the 2′-hydroxyl of GMP. The reaction should be accelerated due to formation of the Schiff base, and the stereochemistry of the reaction should be influenced by the structure of the chiral catalyst. The orientation shown would produce D-fructose-1,6,-bisphosphate.

However, they may have been favored early on because their ability to attach to catalytic small molecules promoted their use in proto-metabolic pathways, and/or because they participated in substrate-assisted catalysis. In many cases, these "handles" are not explicitly involved in the next reaction in a biosynthetic pathway, and therefore their covalent attachment to a catalyst would not interfere with the chemistry. Indeed, sequential reactions might have occurred with the intermediates tethered to a small molecule catalyst. Vestiges of the use of covalent tethering may be found in the tRNA-dependent amino acid transformations in which synthesis of the cognate amino acid is completed after a precursor is attached to the 3′-OH of a tRNA. Such processes occur for synthesis of glutaminyl-$tRNA^{Gln}$ in most bacteria and all Archaea, asparaginyl-$tRNA^{Asn}$ in some Archaea, and selenocysteinyl-$tRNA^{Sec}$ in all domains of life [23].

4.2. The multimer stage

Increased concentrations of activated monomers would have allowed formation of multimers [24] in which amino acids, nucleotides, and cofactors were linked in random assemblages. All of the combinatorial possibilities for attachments of monomers at various sites would have been explored. For example, nucleotides might have contained alternative sugars and bases, and dinucleotides might have been linked by 2′–5′ linkages rather than 3′–5′ linkages, or even by alternative backbone structures.

Multimers should have enhanced catalytic abilities compared to their monomeric precursors as a multimer can bring a greater number and variety of functional groups into proximity of the reacting atoms. We have proposed that dinucleotides might have catalyzed synthesis of reactions leading from α-keto acids to amino acids utilizing hydrogen bonding interactions to orient and polarize reactants, general base or nucleophilic catalysis provided by functional groups on the bases, and participation by the phosphate groups, either as general acid/general base catalysts or as sites for binding of catalytic Mg^{2+} ions [25]. We now suggest that such mechanisms might have been a general property of the small molecule catalysts available in the multimer world. A known case is the ability of the dipeptide seryl-histidine to cleave DNA, proteins, and esters [26]. An additional mechanism for catalysis that could have arisen at this stage may have been "peptide nests" that protected catalytic metal sulfide clusters [14]. Such "nests" would be pre-biotic precursors of the binding sites for metal ions in modern protein enzymes.

NADH is a multimer and may have been the first organic cofactor to appear in proto-metabolic networks. It is the only cofactor required in the nucleotide synthesis module shown in Fig. 3. Nicotinamide is easily synthesized non-enzymatically from DHAP and aspartate [27]. NADH contains a pyrophosphoryl linkage between the 5′ hydroxyl groups of AMP and nicotinate mononucleotide, only one of several ways in which these monomers could be joined. This structure may have been critical for its function in a pre-biotic chemical network. Protein dehydrogenases contain a metal ion or amino acid that polarizes the carbonyl of the substrate, and, in the latter case, acts as a proton donor, as well. Before the advent of macromolecular catalysts, a Mg^{2+} chelated to the pyrophosphate moiety of NADH might have served to polarize the carbonyl of a substrate. Reduction reactions might also have been facilitated by covalent attachment of substrates to the 2′ or 3′ hydroxyl of the ribose of the adenosine moiety. These structural features of NADH may have been retained later even when more sophisticated catalysts obviated the need for the metal–ion chelating pyrophosphoryl linkage and the hydroxyl substituents of the

ribose ring to which substrates could be tethered. The same pyrophosphoryl linkage is found in FAD and CoA, which probably appeared somewhat later. In these cases, the pyrophosphoryl linkage may have contributed to catalysis before the advent of macromolecules, or may simply have been preserved due to promiscuous use of a catalyst capable of synthesizing NAD. Indeed, formation of the pyrophosphoryl linkages in NADH, FAD, and CoA can be catalyzed by a single non-specific ribozyme [8].

4.3. The micro-RNA stage

As concentrations of activated nucleotides increased within a compartment occupied by a community of catalytic monomers and multimers, random polymerization would have yielded longer oligonucleotides. We define the "micro-RNA stage" as consisting of oligonucleotides of length 3–10. Such RNAs are too small to adopt secondary structures, but can form duplexes with complementary strands. Three important innovations with far-reaching consequences would have become possible at this stage. First, longer oligomers would have been more efficient catalysts. Second, base pairing between complementary oligomers would have allowed sequence-specific replication of components of the network. Third, replication of catalytic oligomers by template-specific ligation would have provided the first impetus for the emergence of a homochiral system.

Micro RNAs should have novel mechanisms for catalysis due to their increased size and ability to form duplexes (Fig. 4). If a dinucleotide provides a minimal "active site", then a longer oligomer is a concatenated set of several active sites. If these active sites catalyzed successive reactions in a pathway, a reactant might undergo several reactions catalyzed by a single-stranded oligomer. Numerous novel catalytic strategies are available to RNA duplexes. A cofactor or amino acid could be incorporated at the end of one strand, and a substrate tethered in close proximity at the end of the opposite strand. Alternatively, two catalytic auxiliaries (cofactors or amino acids) might be attached to the ends of opposite strands to form a multifunctional active site capable of, for example, simultaneous general acid and general base catalysis. Tethering of two substrates to the ends of opposing strands might favor condensation reactions. New possibilities for interaction of cofactors with "A-handles" with micro-RNA catalysts would have emerged, as well. The adenosine could have been incorporated at the end of an RNA strand, stacked between bases in a helix, or docked into the minor groove by an A-minor interaction. Finally, transient associations could bring together a catalytic strand carrying a cofactor or amino acid and a substrate-binding strand, allowing catalysis and then departure of the product. Strategies involving duplexes may have little dependence on sequence if the duplex primarily provides a scaffold for supporting catalytic groups. However, catalysis by single-stranded RNA molecules would likely be sequence-dependent, so the linkage between sequence and function that we propose to have emerged at the multimer stage would have persisted at this stage. The discovery that GAAA complexed with UUU undergoes a Mn^{2+}-promoted cleavage after the G [28] provides a striking example of the capacity for catalysis in small RNAs. Notably, this reaction depends on substrate-assisted catalysis, as two Mn^{2+} ions required for structure and catalysis are coordinated to the GAAA tetranucleotide [29].

A second major innovation would have been the direct replication of the RNA molecules that participated in catalysis. While cofactors, amino acids, and peptides undoubtedly contributed to catalysis, they could not have been replicated directly. Their

continued participation would have depended upon replication of the RNA catalysts that promoted their synthesis. Replication of RNA at this stage would likely have proceeded by template-directed ligation. Template-directed ligation favors 5′–3′ linkages due to formation of a double helical structure on both sides of the gap to be ligated [30]. Primer extension, in contrast, yields a high percentage of 5′–2′ linkages in the absence of an enzyme because the structure adopted by the incoming nucleotide places the 5′ phosphate in a position to react with the 2′-hydroxyl of the primer.

Non-enzymatic template-directed ligation is slow, occurring with a $t_{1/2}$ of 15–30 years at pH 7.4 and 100 mM Mg^{2+} [30]. Emergence of a catalyst that promoted ligation would have enhanced the function of the entire network, and set the stage for the emergence of larger and more effective RNA catalysts. Ribozymes such as the Group I introns use a two metal ion mechanism to catalyze formation of a phosphodiester bond, much like protein enzymes, and this strategy could have arisen very early [31]. Such an early RNA replicase should not be considered as solely ''self-replicating''; its ''success'' would have been intimately tied to its ability to replicate all of the catalytic RNA molecules required to maintain the proto-metabolic network.

The third major innovation would have been selection for homochirality. Homochiral dinucleotides are more stable to hydrolysis than are heterochiral dinucleotides [32]. Further, duplex RNA is more resistant to hydrolysis than single-stranded RNA [33] and homochiral duplexes are more stable than heterochiral duplexes [34]. To the extent that duplex formation is required for efficient catalysis, stability, and replication, there will be selection for a system in which only monomers of one chirality are produced, and monomers of the opposing chirality are disfavored. Chiral catalysts bias the stereochemistry of the products formed at their active sites, so the most effective set of catalysts will produce monomers with the chirality characteristic of the catalysts. The choice of chirality may have occurred by chance as a small excess of D-ribose and/or L-amino acids led to a positive feedback for increasing levels of those stereoisomers. The choice of stereochemistry in ribose and in amino acids may have been linked, such that a choice with respect to one biased the choice for the other.

This would have been the stage at which the die was cast, and emergence of RNA as the dominant macromolecule became inevitable. Although catalysis likely involved many different types of molecules, only RNA could have been replicated in a sequence-dependent fashion, so the communities of molecules with the best RNA catalysts would have been favored. Furthermore, the homochirality that characterizes extant life likely originated at this stage as a result of selection for RNA molecules that could form duplexes that both enhanced catalysis and allowed replication.

4.4. The mini-RNA stage

Continued selection for communities of catalysts that increased the levels of monomers would have favored emergence of larger RNA molecules. RNA molecules in the range of 11-40-mers can form secondary structures. This is a useful innovation for catalysis because it allows formation of pockets for binding of small molecules (Fig. 4). *In vitro* evolution of ''aptamers'' has demonstrated that molecules of this size can bind a variety of structurally diverse ligands. RNAs in this size range can be catalytic. A 29-mer catalyzes aminoacyl-RNA and peptidyl RNA synthesis [35], and 38-mers catalyze a Diels-Alder reaction [36] and hydrolysis of an ester [37].

At this stage, many catalytic strategies used by modern protein enzymes would have become available. Non-covalent binding of substrates or cofactors would have allowed more rapid turnover, as well as more precise orientation of reacting groups and provision of multiple interactions to promote reactivity, regioselectivity, and stereospecificity. Catalytic abilities could have been expanded by use of amino acid, metal, or organic cofactors. The use of an amino acid as a cofactor is exemplified by a histidine-dependent deoxyribozome [38]. Peptides could have contributed functional groups to active sites, or stabilized conformations of RNA molecules required for function [39,40].

Replication of RNA molecules would have become more challenging, as a processive enzyme that could unfold and copy a large RNA would have become critical. The earliest processive replicase might still have utilized template-directed ligation. It has been suggested that the first RNA replicase was a "triplicase" that utilized trimers and had a ratcheting mechanism for pulling a template through the catalytic machinery, and that later this machine was co-opted to serve as the ribosome when translation emerged [41]. Ultimately, this process was replaced by a primer-extension strategy. Ribozymes that catalyze primer extension reactions have been generated by *in vitro* evolution [42], but ribozymes that can copy long RNA molecules have not yet been found.

4.5. The macro-RNA stage

The RNA World eventually reached a stage in which very large RNA molecules formed complex tertiary structures. The ribosome, the spliceosome, and the RNA component of RnaseP are likely relics of that time. Based upon the wide range of catalytic activities that have been generated using *in vitro* evolution, it is likely that every reaction needed to sustain metabolism in an RNA World could have been catalyzed by a ribozyme. We emphasize the importance of three points. First, the RNA World must have been supported by a metabolic network whose biosynthetic pathways may have been quite similar to those seen in extant life. Second, catalysis likely did not rely upon RNA alone; the catalytic abilities of amino acids, metals, and organic cofactors were probably fully exploited in ways similar to those in modern metabolism. Third, replication would have been catalyzed by a ribozyme that copied all of the RNAs present in the population. Only the RNA(s) comprising this machine would be "self-replicating". At this stage, as at the earlier stages, catalysts supporting both the underlying metabolism and the machinery for replication would be essential for success of the community of molecules.

5. Summary

The RNA World was a world of significant complexity. We describe here a mechanism by which macromolecular RNAs might have emerged from an early chemical reaction network. Our model describes a continuous path for emergence of this sophisticated system from a simpler reaction network fueled by geochemical processes. The processes involved in metabolism and replication were intertwined from the very beginning, a concept that neatly eliminates the chicken/egg problem. Further, this model suggests that many features of the RNA World, and indeed modern life, arose long before the RNA World and were retained as pre-biotic systems became more sophisticated. These include the biosynthetic pathways for simple metabolites, the use of metal ion and organic cofactors, the structure of NADH, homochirality, and heritability of information carried by nucleic

acids. At all stages, replication was a function of a community of molecules that collectively generated monomers necessary to create larger molecules, rather than a property of a particular self-replicating RNA molecule.

The hypothesis developed here suggests new avenues for research into the origin of the RNA World. Studies of the catalytic abilities of small RNAs focused upon reactions in the nucleotide synthesis module shown in Fig. 3 will allow us to better understand how the RNA World emerged from a proto-metabolic network fueled by geochemical processes.

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