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Review

The RNA World as a Model System to Study the Origin of Life

Abe Pressman[1,2,#], Celia Blanco[1,#], and Irene A. Chen[1,3,*]
[1]Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA, USA
[2]Program in Chemical Engineering, University of California, Santa Barbara, CA, USA
[3]Program in Biomolecular Sciences and Engineering, University of California, Santa Barbara, CA, USA
[#]These authors contributed equally to this manuscript
*Correspondence: chen@chem.ucsb.edu
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Understanding how life arose is a fundamental problem of biology. Much progress has been made by adopting a synthetic and mechanistic perspective on originating life. We present a current view of the biochemistry of the origin of life, focusing on issues surrounding the emergence of an RNA World in which RNA dominated informational and functional roles. There is cause for optimism on this difficult problem: the prebiotic chemical inventory may not have been as nightmarishly complex as previously thought; the catalytic repertoire of ribozymes continues to expand, approaching the goal of self-replicating RNA; encapsulation in protocells provides evolutionary and biophysical advantages. Nevertheless, major issues remain unsolved, such as the origin of a genetic code. Attention to this field is particularly timely given the accelerating discovery and characterization of exoplanets.

Introduction

How might life arise from a chemical soup? This is one of the most fundamental questions of science, requiring input from nearly every discipline. Although hampered early on by some doubts about its propriety as a field of science, research on the origin of life within the broader field of astrobiology has accelerated, becoming a major endeavor and attracting new sources of funding, in addition to the long-standing NASA programs, in the last decade. From a biological perspective, one of the drivers of this progress has been the synthetic view toward this problem [1], which shifts the emphasis from 'how did life arise?' to 'how *might* life arise?' The distinction is subtle but important. Instead of focusing on the specific historical emergence of life on Earth, the synthetic view focuses on possible mechanisms for the chemical invention of life. In other words, we care less about how our particular life arose and more about the possible ways life could arise under a variety of conditions. This shift will serve us well for understanding the surfeit of exoplanets being rapidly discovered by astronomers.

Here we review the biochemistry of the origin of life, with an emphasis on an intermediate stage of life called the RNA World [2]. Life today generally follows the central dogma of molecular biology, with DNA encoding genetic information that is copied into complementary RNA, which is then translated into protein sequence according to the genetic code. Thus even the simplest life based on our current biochemistry requires at least three major biopolymers (DNA, RNA and protein), as well as the corresponding machinery for replication, transcription, and translation. The complexity and interdependence of this biochemistry strongly implies that a simpler system must have preceded it. The RNA World is a proposed primitive biochemistry dominated genetically and functionally by RNA (Figure 1). Its existence was proposed in the 1960s [3–5], based on the centrality of RNA and RNA-like cofactors in modern metabolism, as well as the observation that RNA could fold into complex three-dimensional structures reminiscent of proteins. The discovery of catalytic RNAs and the revelation that the ribosome is in fact a ribozyme added strong circumstantial evidence for this theory [6]. While the RNA World is an area under very active study, its name should not be taken too literally. As we discuss, the RNA World was probably somewhat untidy and included other molecules, such as lipids and simple peptides. Moreover, it will be difficult to establish the historical accuracy of a specific RNA World, since its greatest invention, the genetic code, ultimately led to its replacement. Instead, in the synthetic view, we see the RNA World as a particularly tractable model system for studying the emergence of biological complexity during an origin of life.

The Prebiotic Chemical Inventory

To understand what chemical compounds would be available prebiotically, researchers have undertaken various organic syntheses simulating prebiotically plausible conditions [7]. A few classic examples of these experiments include the alkaline formose reaction [8], which yields dozens of sugars; the Miller-Urey spark discharge experiment [9], which yields dozens of diverse compounds including many amino acids; and Oro's experiments that lead to the synthesis of purine nucleobases [10–12]. In many cases, amino acids are produced in good yield relative to other classes of organic compounds. In addition, the relative abundances of amino acids produced in these experiments mirror those found in the Murchison and other carbonaceous chondrite meteorites [13,14], which are believed to reflect the composition of the early Earth. The recent detection of complex cyanides in the protoplanetary disk around a young star suggests a rich prebiotic inventory similar to that of our own solar system [15], supporting the astrobiological relevance of experiments simulating the Earth's prebiotic chemistry.

In the last few years, our understanding of the prebiotic inventory has advanced substantially. After decades of effort devoted to the prebiotic synthesis of RNA, many had come to believe that

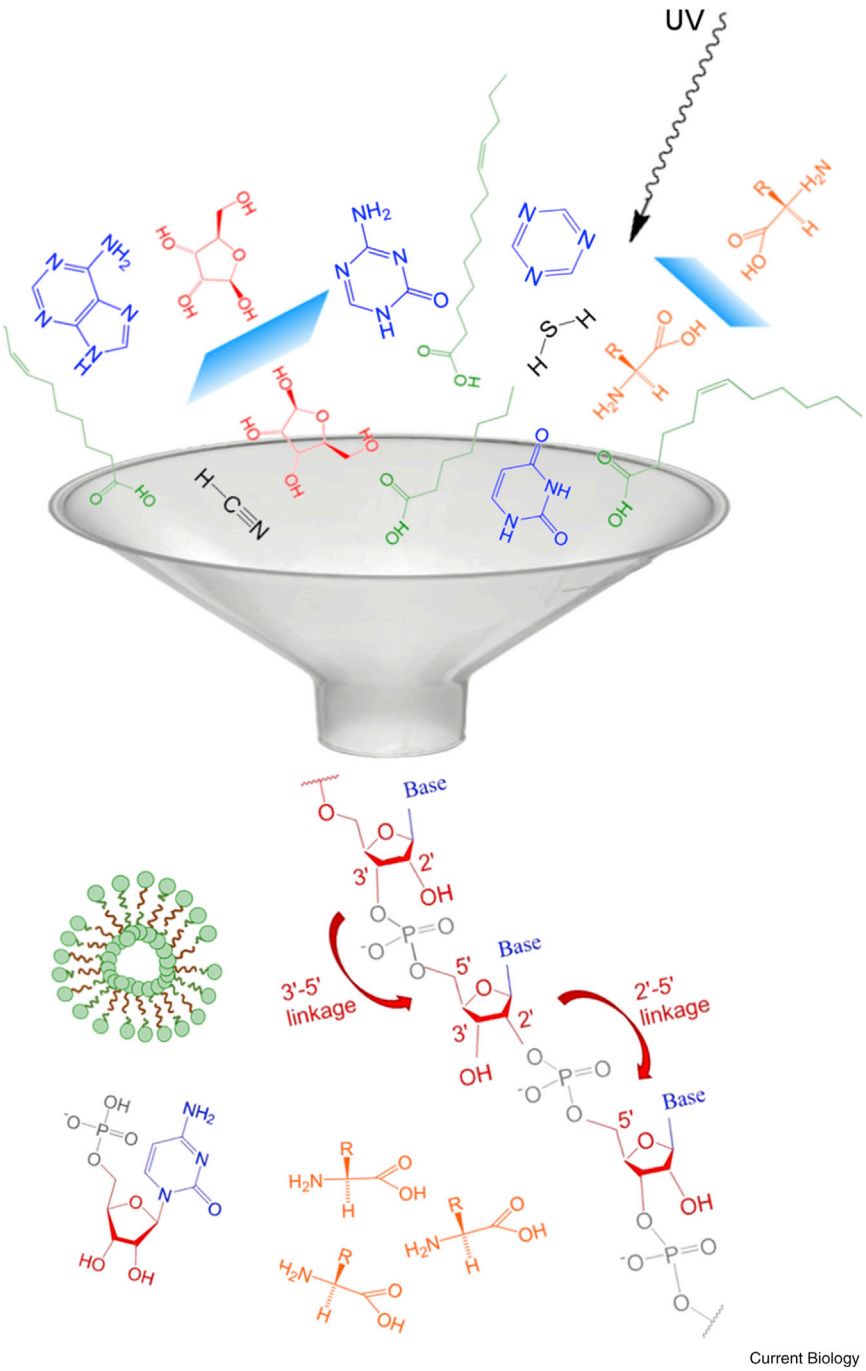

Figure 1. Overcoming the problem of a chemically heterogeneous prebiotic soup. Mechanisms to funnel this complexity include reduced diversity of synthetic products, amplification of small initial differences in abundance, and tolerance of biopolymers and self-assembled structures to heterogeneous components and linkages. Note the production of RNA nucleotides (red: sugar; blue: nucleobase: gray: phosphate).

RNA is not prebiotically plausible. However, in 2009, Powner *et al.* demonstrated that pyrimidine nucleotides could be prepared from prebiotically plausible substrates [16], using a systems chemistry synthetic analysis to avoid the problematic formation of the C–N bond that links sugar and base. A mixture of hydrogen cyanide (HCN) and hydrogen sulfide (H_2S), activated by ultraviolet light, creates the required precursors, and remarkably, the same experimental conditions also create precursors for some amino acids and lipids [17]. This suggests that many of the essential building blocks for life could form in closely related geological settings. A caveat of this work is that the conditions of the syntheses undergo specific changes (e.g., in pH), as opposed to a one-pot synthesis. However, the chemical principles illustrated may be generalizable, such that further work may uncover mechanisms to reduce the level of experimental control. At the same time, other researchers continue to study the separate formation of sugars and bases. The repertoire of conditions under which nucleobases are synthesized continues to expand (e.g., [18,19]).

While RNA itself may be prebiotically plausible [16,20,21], another avenue toward genetic molecules hypothesizes that the contemporary components of RNA (nucleobases, ribose, and phosphate) are not the first or only possible elements of the informational polymer(s) of life [22]. Although it is not obvious that function can be transferred when copied into an alternative backbone (but see discussion on backbone heterogeneity below), the rapid decomposition of nucleobases and sugars in water [23–25] suggest that alternatives should be investigated [26–30]. For example, triazines have been considered as possible alternative nucleobases [31], and they have been found in prebiotic synthesis experiments [18,19] and detected in carbonaceous chondrites [32]. Unnatural base pairs, which differ from those present in today's biology, have been investigated for prebiotic understanding as well as for synthetic biology [33–36]. A recent systematic study attempted to identify the best possible alternative nucleobase candidates out of all pyrimidines and purines with $-NH_2$, =O, or −H as exocyclic groups. From more than 80 possible candidates, 2,4,6-triaminopyrimidine (TAP) was suggested as having the highest reactivity with ribose, and indeed it forms supramolecular assemblies with complementary heterocycles [37,38]. Other nucleobases may have even participated in early molecular evolution, with the four canonical RNA bases proposed as a more stable replacement for an earlier more irregular set [39]. Backbone alternatives to RNA have also been extensively investigated [40,41]. The question of why RNA backbone sugars in nature are pentose and not hexose has been studied [42,43], and

the base-pairing properties of alternative nucleic acids constructed from hexo- and pento-pyranoses and tetrofuranose have been reviewed in [44]. In fact, several alternative backbones have been subjected to *in vitro* evolution, resulting in functional nucleic acids [41,45]. One caveat is that an alternative nucleic acid should be capable of transferring catalytic function to RNA and DNA, in order to be a plausible precursor stage of biology on Earth; how this might happen is not yet clear. Nevertheless, in the synthetic view, such studies are important for understanding the possible chemical pathways toward life that is not necessarily our own.

Not So Nightmarish

A major issue that prebiotic chemistry must address is the vast heterogeneity of possibly synthesized chemicals. In particular, most prebiotic syntheses result in a large number of products, each at low yield, a trend also observed in the contents of carbonaceous chondrites. Joyce and Orgel famously called this problem 'the prebiotic chemist's nightmare' [46], because it seems nearly impossible to produce the highly regular biopolymers of life in the presence of such a diverse mixture of chemical precursors. Recent progress points toward at least three ways to solve this problem of heterogeneity (Figure 1). First, in the systems chemistry approach to RNA synthesis described above, reactants for one reaction step can also control other steps, such that a moderate increase in the complexity of chemical inputs can actually lead to a decrease in the diversity of reaction products [47]. This approach anticipates a major function of enzymes in biology, namely control over the flux and channeling of substrates through a metabolic network by modulation of the relative rates of reaction.

Second, heterogeneity can be reduced through processes that amplify initial differences in abundance. The fundamental property of chirality has been heavily investigated for such processes. Chirality arises when two different molecules have identical atoms, bonding and electronic structure, but are mirror images of each other; such molecules are known as enantiomers. Although simple, prebiotically available compounds are generally synthesized as racemic mixtures (equal amounts of each enantiomer), or are achiral, modern biomolecules are nearly completely homogeneous with respect to handedness. How might the initial symmetry be broken so completely? As pointed out by Mislow, a perfectly racemic mixture of enantiomers is chemically impossible to achieve [48]. Therefore, the origin of homochirality could theoretically be explained by amplification of a tiny stochastic imbalance of enantiomers (i.e., Spontaneous Mirror Symmetry Breaking (SMSB)). Autocatalytic reactions (in which the product catalyzes its own production and inhibits the other enantiomer) are excellent candidates for SMSB processes [49,50]. The Soai reaction [51–54] demonstrated absolute asymmetric synthesis from an autocatalytic reaction, leading to nearly enantiomerically pure product even when starting from a racemic mixture of product/catalyst. Alternatively, physical processes can achieve a similar effect. In Viedma ripening, a racemic mixture precipitates to form crystals in which each enantiomer preferentially interacts with enantiomers of the same handedness, forming small domains of enantiomerically pure compound. If the enantiomers can interconvert in solution, a 50/50 mixture of enantiomers will spontaneously deracemize given some energetic input (e.g., grinding [55–59] or temperature gradients [60–62]). Viedma ripening has received significant attention in experimental and theoretical studies in the last decade [63–73]. Another experimental model is the autocatalytic crystallization of a system containing glycine and α-amino acids at the air–water interface, which generates enantiomerically enriched amino acids in prebiotic conditions [74,75]. This system may be especially relevant to the origin of homochirality, as it involves amino acids that are the major components found in modern prebiotic synthesis experiments and in meteorite and comet analysis [76–79]. Finally, although one might expect polymerization reactions of racemic mixtures of monomers to yield mostly mixed polymers, polymerization of racemic α-amino acids might actually yield homochiral oligopeptides [80–85]. Once an early biopolymer achieved homochirality, others could follow as a consequence. For example, the specificity of ribozymes for amino acids of a certain handedness could enforce homochirality of the peptide products [86], propagating the early enantiomeric excess to new molecules.

Third, it is possible that the difficulty that chemical heterogeneity presents to early life has been exaggerated. Recent work from Szostak and others suggests that some chemical heterogeneity is actually tolerable in RNA. For example, heterogeneity in the sugar (ribose vs. deoxyribose) can be tolerated in functional RNA. Mixed backbone aptamers (sequences that bind to specific targets) against ATP and GTP were evolved from libraries of polynucleotides containing a random mix of ribo- and deoxyribo-nucleotides. Despite this seemingly chaotic mixture, the aptamers exhibited highly specific molecular recognition, demonstrating that nonheritable backbone heterogeneity does not preclude the evolution of functional RNAs [87]. Another source of heterogeneity in RNA is the backbone linkage, which might occur as 2'–5' or 3'–5' in RNA during non-enzymatic synthesis [88]. While both 2'–5'-linked RNA and mixed 2'–5'/3'–5'-linked RNA can template primer extension reactions [89], this heterogeneity had been assumed to prevent the non-enzymatic emergence of functional RNAs such as ribozymes [47]. However, recent experiments show that not only is this heterogeneity compatible with RNA folding and function, but it also would lower the melting temperature of RNA duplexes that would otherwise be too stable for thermal strand separation [21]. Backbone heterogeneity might still have a negative effect on the folding stability and replication of early nucleic acids, but the extent to which this would have precluded evolution and function of such molecules remains to be seen, and may be far less than previously thought. Outside of nucleic acids, system-wide heterogeneity could have also provided advantages in the composition of early lipid vesicles. Model prebiotic lipid syntheses yield primarily short, single-chain amphiphiles that can form bilayer vesicles only at very high concentrations in isolation (critical aggregation concentration, i.e., *cac*). However, a mixture of fatty acids of different chain lengths exhibits more robust vesicle formation: small amounts of long-chain lipids lower the *cac* and allow assembly of vesicles in dilute environments with primarily short-chain components [90]. Although much remains to be understood, the recent surge in new approaches to address the prebiotic chemist's 'nightmare' indicates that the hurdles are not insurmountable.

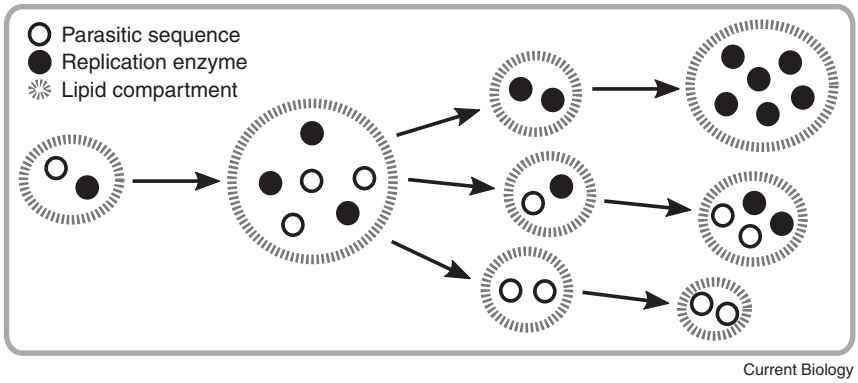

Figure 2. The stochastic corrector model.
Compartmentalization counters the emergence of sequences (white) that parasitize replication enzymes or ribozymes (black). Even with random segregation, cells with a higher proportion of replicases would copy their contents more quickly, leading to greater fitness of the encapsulated replicases.

The RNA World Emerges

While the earliest replicating sequences may not have been strictly ribonucleic acid, RNA is a plausible molecule for early life. More practically, it is also an effective model system for research on early biocatalysts. The first polynucleotides would have been small oligomers formed randomly or by non-enzymatic template copying. Under conditions promoting feedback between molecular activity and fitness, sequences that aided in their own replication or survival could gain a competitive advantage. Ligase and polymerase ribozymes are two particularly interesting candidates for the first replicating ribozymes. New ribozyme activities are discovered through *in vitro* evolution, in which a pool of random RNA sequences undergoes a biochemical selection (e.g., reacting with a substrate) and successful molecules are purified, amplified by RT–PCR, and re-selected over multiple rounds, thus resulting in the isolation of active sequences [91–93]. Polymerase ribozymes have been selected *in vitro* over decades, showing improvement over a series of selections, and now have sufficiently high fidelity to accurately copy sequences 80–200 nucleotides long [94–100]. Polymerase ribozymes generally require annealing of a short RNA primer, a role perhaps filled by random oligomers formed either through nonenzymatic polymerization or as the product of other ribozymes catalyzing non-templated synthesis. Such small oligomers could also act as substrates for ligase ribozymes in the early RNA World. Interestingly, RNA ligation can be accomplished by shorter ribozyme sequences compared to the polymerase (and the first RNA polymerase was evolved from the Class I ligase [95,101]). Ligase ribozymes can self-replicate from shorter oligo precursors, with high fidelity over many cycles [102–105], forming a self-sustaining chemical system capable of Darwinian evolution. This synthetic system thus fulfills NASA's working definition of life, although the definition may be argued (see [106] for an updated analysis of this definition, and [107] and associated commentary for a recent discussion of the issue of definitions). Although the known RNA polymerase ribozymes are not yet able to copy an arbitrary sequence due to obligatory base-pairing between enzyme and substrate, a recent approach neatly sidesteps this problem using a 'cross-chiral' ligase; in this system, a ligase copies RNAs of the opposite handedness, and vice versa for its enantiomer [108]. This system represents an unconventional pathway to RNA replication that takes advantage of molecules of both handedness, illustrating how the synthetic view can spur new approaches.

Work on the RNA World model has highlighted fascinating subtleties in the evolution of self-replication. For example, ribozymes selected *in vitro* are usually a few dozen to a couple hundred nucleotides in length, but short sequences are copied more quickly and thus have an intrinsic advantage during natural selection. At best, this effect would inhibit the development of complex genomes, and at worst, the replication machinery might grind to a halt, overwhelmed by short parasitic sequences. However, recent work has demonstrated how a thermal gradient across an open pore in a submerged rock could lead to size-based differences in transport, with short sequences washing away while longer sequences remain and replicate [109]. In another scenario, thermal or evaporation cycles in early Earth conditions, resulting from a variety of proposed sources [110–113], may have promoted alternating phases for templated replication and de-annealing in a replication cycle. If strand melting is infrequent relative to the copying process, polymerization phases could be long enough for long sequences to fully copy alongside shorter ones [111,114]. Simulations have also demonstrated that ligation-based replication, with sequences copied through the joining of short complementary oligomers, would help favor longer sequences compared to replication by polymerization [115].

A related challenge for early replication is the maintenance of genetic information over many rounds of imperfect replication. Indeed, a naive calculation suggests that even the shortest ribozymes are too long to be replicated by non-enzymatic mechanisms. Given this, how would the first ribozymes emerge from a chemical system? This classic problem is known as Eigen's paradox [116]. Several mechanisms have been described that could alleviate this paradox. First, nonenzymatic RNA replication has been shown to stall substantially following introduction of a mismatch, leading to slower than expected rates of incorrect copy formation and thus an effectively greater copying accuracy [117–119]. Second, homologous recombination of RNA sequences, originally observed in viruses [120–122], can operate *in vitro* in communities of short RNA molecules [122,123], leading to the speculation that quasispecies of related ribozymes could recover their original site variants [124,125]. Ribozymes consisting of multiple cooperative oligomers could also 'recombine' fragments for similar effect [126,127]. Third, the 'stochastic corrector' model suggests that compartments (e.g., vesicles) with more active ribozymes would enrich their contents while compartments with deleterious mutants or parasitic sequences would find their contents selected against (Figure 2) [128–131]. Compartmentalization essentially creates group selection among compartments, resulting in an analogous error threshold

at the higher level of selection [132]. Finally, the problem of accuracy might be tackled directly. The greatest contributor to inaccuracy during replication is the stability of G:U wobble pairing in RNA [133]; this might be circumvented by a different base pairing system that improves this discrimination [134].

Another interesting problem for replicators is the tradeoff between favorable folding stability and ease of unfolding for templating during replication. More effective ribozymes, with likely higher folding stability, are expected to be less prone to unfold for use as a replication template, and would therefore have lower fitness. At least two mechanisms have been proposed to counter this tendency. The presence of non-homogeneous linkages in RNA backbones could create a broad distribution of folding stability without changing RNA sequence, with some copies serving as better templates and others as better ribozymes [21,87,135,136]. Another way to 'divide the labor' of templating and activity is based on the ability of RNA to form wobble pairs. This feature can cause a ribozyme and its reverse complement to have quite different folding energies, a pattern which can be observed in RNA viroids [137].

In addition to its importance for the origin of life, the RNA World is also an ideal model system for studying fundamental issues about evolution. One such issue is the balance between natural selection, which is largely deterministic, and chance events. To address this question, one would ideally like to analyze all possible pathways for evolution through sequence space to determine the extent to which alternative pathways are present. Although this is clearly impossible for biological genomes due to their size (i.e., even considering a small viral genome of a few hundred base pairs, the number of possible sequences is astronomical), ribozymes and other functional RNAs are short enough that their entire sequence space could be analyzed in principle. *In vitro* evolution of ribozymes typically begins with a randomized library of 10^{15}–10^{16} sequences, which is a relatively sparse sample of sequence space. Nevertheless, the success of these selections suggests that there must be numerous functional families in the totality of sequence space [138,139]. More recently, extensive studies of the fitness landscapes of various functional RNAs have shown mostly isolated, sharp peaks with distinct structural motifs linked by generally unfavorable paths of mutation [140–142]. One recently proposed hypothesis is that molecular crowding and confinement may have played a role in increasing pathways of evolution [143], since crowding effects contribute to the folding stability of ribozymes [144–148] and reduce the effect of otherwise deleterious mutations [149]. Despite the apparent lack of pathways to evolve among sequence families performing the same function, a few pathways for evolving new functions have been found [150–152]. The frequency of such pathways and their evolutionary importance are still unknown.

Given an effective system of self-replication and evolution, how might greater complexity arise? Eigen and Schuster proposed several decades ago that a system of cooperating enzymes (a hypercycle) could allow high complexity despite high mutation rates [153] (see [154] for a brief review), but no experimental implementation of a hypercycle has been put forth. However, recent work has demonstrated how an experimental system of cooperating recombinase ribozymes can spontaneously create a network of sequences that aid in each other's formation [126]. Much attention has also been paid to the origins of the genetic code as perhaps the pinnacle of emergent complexity during the RNA World.

Encapsulation of the RNA World

Most proposed models of early RNA-based life suggest that enzymatic functions would evolve more readily within an encapsulated, cell-like system. Lipid vesicle compartments would have kept similar sequences in close proximity, since daughter copies would be initially contained in the same compartment. As vesicle division split ribozyme populations, compartments with a greater number of inactive sequences could be selected against, enforcing selection of higher activity sequences and preventing takeover by parasitic sequences (the stochastic corrector, discussed above) [129]. While long RNAs cannot permeate through membranes, RNA monomers and other small metabolites could diffuse through, allowing a heterotrophic lifestyle [155,156]. Experiments with a biologically derived self-encoding replicase have shown much higher rates of replication and sequence preservation within a compartmentalized system than in a simple aqueous one [157,158], and mathematical analysis suggests that membrane-bound 'protocells' spontaneously dividing while holding random replicase populations is sufficient to select for replicases [132]. Other mechanisms of physical separation, such as attachment to surfaces, may achieve a similar effect [159], and may be particularly relevant if the surface itself catalyzes RNA polymerization [160].

Simple lipid vesicles would have other positive effects on an RNA World as well. The confined volume of a membrane-bound reaction creates crowded conditions [143] and could preserve a high concentration of RNA polymers. The presence of a membrane allows energy storage in the form of transmembrane gradients, such as the pH gradients that form during growth of fatty acid vesicles [161]. An intriguing finding is that the environment of dehydrated lipid membranes could promote non-enzymatic RNA polymerization [162]. Furthermore, simple lipids, such as fatty acids, are found in prebiotic syntheses as well as in meteorite samples [163–168]. Thus, membrane compartments appear to be available, desirable and perhaps essential for the RNA World.

The model system of encapsulated RNA, the protocell, has proven to be surprisingly rich in biophysical phenomena. Self-replicating vesicles that spontaneously grow and divide can be produced robustly in the laboratory [169–173]. Small molecules potentially available in the prebiotic inventory can further promote both membrane and RNA stability, increasing the plausibility of the protocell model [90,155,174]. Since membranes are generally impermeable to macromolecules, internal RNA polymerization would raise osmotic pressures and cause protocell growth at the expense of inactive vesicles [175]. Furthermore, genetic replication can itself cause vesicles to divide [176]. Thus, the best replicators would have the fastest growing and dividing membranes, creating a cellular unit of high fitness. The synthetic view toward the origin of life has been foundational in guiding the study of such phenomena.

Inventing the Genetic Code

At some point, life on Earth transitioned to protein-based catalysis, with the ribosome and tRNAs as the last known remnants

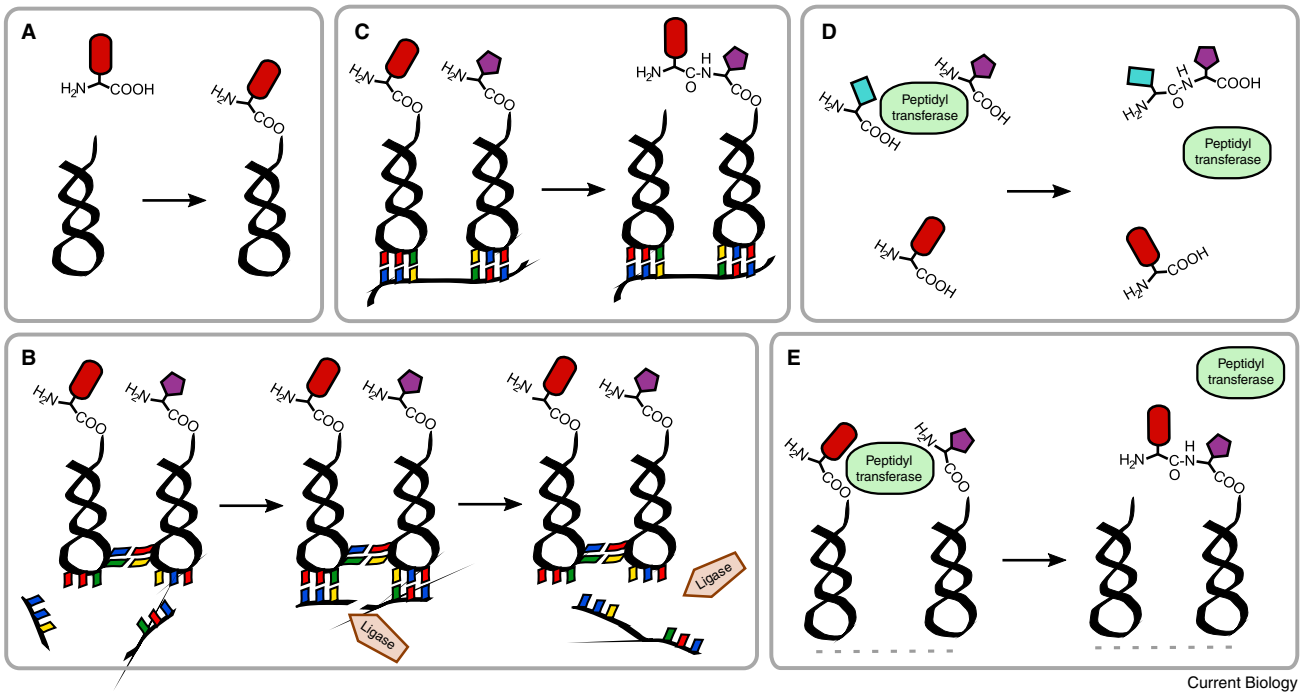

Figure 3. Possible steps toward the genetic code.
(A) Aminoacylation ribozymes covalently attach to substrate amino acids. Such modifications may have conferred a functional benefit. (B) The first guide sequences may have formed from interactions among aminoacyl-RNAs that promoted ligation of small complementary oligonucleotides. (C) Primitive mRNAs may have been oligonucleotides that annealed to specific aminoacyl-RNAs in a configuration that would promote condensation of functionally useful small peptides. Alignment of aminoacyl-RNAs through base-pairing interactions is known to cause the non-enzymatic polymerization of amino acids [197]. (D) The first peptidyl transferases may have been ribozymes catalyzing nonspecific peptide bond formation between amino acids. As small peptides may have contributed useful functions to early life, peptidyl transferases might have evolved before, after, or independently from the genetic code itself. (E) Eventually, the combination of aminoacyl-RNAs as early tRNAs, a peptidyl transferase as an early ribosome, and oligonucleotides aligning aminoacyl-RNAs as early mRNAs may have constituted a primitive translation machinery.

of the RNA World conserved across all living organisms [177]. Amino acids were likely present in prebiotic environments, and ribozymes have been discovered to catalyze formation of peptide bonds as well as other amide bonds [178,179]. Even without a genetic code, randomly formed dipeptides may have been useful catalysts or cofactors in early metabolic processes or for stabilization of protocellular components.

The genetic code has two fundamental components: the specific association of amino acids with adapter RNAs (now performed by aminoacyl-tRNA synthetases) and the base pairing of the adapter RNAs to the linear genetic molecule (mRNA) to promote ordered protein synthesis (now by the ribosome). With regard to the first element, an RNA world would need to evolve a way to selectively capture amino acids. Artificial selections have identified ribozymes capable of aminoacylating their own sequence or other sequences (and tRNAs) in *trans* at specific sites, using a variety of activated amino acids or modified precursors (Figure 3A) [180–185]. Site-specific aminoacylation of ribozymes may have added useful diversity and new functional chemistry to ribozymes, possibly favoring the evolution of aminoacyl-RNAs before peptide synthetase activities [186–188]. Recognition of specific amino acids would have been important to such processes, but known aminoacylation ribozymes vary widely in their substrate specificity [189,190]. It remains an open question whether ribozymes specific for different amino acids would evolve independently versus from a single promiscuous ancestor. The observed similarity among modern tRNAs has been suggested to be the result of diversification from a single ancestral ribozyme evolving specificity to a number of different amino acids [191–193].

Secondly, the RNA would need a system for creating ordered peptides. It has been proposed that the triplet codon sequences now in the genetic code may have originally functioned in amino acid binding [194]. Alternatively, the first codon triplets may have been small oligomers that bound to and stabilized early tRNAs, which were eventually ligated into small mRNAs that stabilized a series of tRNAs in turn (Figure 3B) [195]. Such early mRNAs may have evolved from random sequences that happened to coordinate the synthesis of favorable small peptides using primitive tRNAs [186,196–198] (Figure 3C). At the same time, a simple peptide synthetase ribozyme might evolve into an increasingly large and complex system of molecular alignment, leading to the modern ribosome. Indeed, the ribosome can be thought of as essentially an entropy ‘trap’ for carefully aligned substrates [199], and the inferred oldest core of the ribosome consists of a few surprisingly simple sequences [200]. Perhaps ribozymes catalyzed nonspecific peptide formation initially [201] (Figure 3D,E), with ordering of amino acids emerging later. Alternatively, early peptides may have been aligned by aminoacyl-RNAs, but ribozyme catalysis was not initially part of the

mechanism for peptide bond formation [197,202]. Simple oligopeptides might help to stabilize folded RNAs, or even catalyze RNA replication or ligation, and therefore improved coding functions (e.g., higher fidelity, longer peptides) may have been selected. Recent work has also highlighted a dipeptide capable of stimulating vesicle growth, suggesting that some of the earliest peptides may have aided in vesicle growth and competition as well [203]. Eventually proto-mRNAs with their protein products presumably out-competed ribozymes of similar function, erasing many, but not all, traces of the RNA World [204]. The details of such a process are still not clear, however. A more radical hypothesis is that the earliest mRNAs may have served as templates for RNA synthesis by ligation, with the peptidyl transferase center of the ribosome having its origin as an RNA replicase ribozyme [200]. The mystery of this major evolutionary transition remains to be solved.

Conclusion

The question of how life can arise from chemicals is a fascinating topic that appeals to the child in almost every scientist. Recent discoveries in other disciplines, from the detection of hundreds of exoplanets to the evidence for ancient liquid water on Mars, have contributed to a sense of excitement in this field. At the molecular and protocellular levels, much progress can be made, and some debate avoided, by taking a synthetic view of the problem. This view has driven the field into exciting new territory over the past decade. Studying this fundamental problem also serves as a gateway toward new biophysical and evolutionary insights.

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