On the origin of life: an RNA-focused synthesis and narrative

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ABSTRACT

Darwin's assertion that "it is mere rubbish thinking, at present, of origin of life" is no longer valid. By synthesizing origin of life (OoL) research from its inception to recent findings, with a focus on (i) proof-of-principle prebiotically plausible syntheses and (ii) molecular relics of the ancient RNA World, we present a comprehensive up-to-date description of science's understanding of the OoL and the RNA World hypothesis. Based on these observations, we solidify the consensus that RNA evolved before coded proteins and DNA genomes, such that the biosphere began with an RNA core where much of the translation apparatus and related RNA architecture arose before RNA transcription and DNA replication. This supports the conclusion that the OoL was a gradual process of chemical evolution involving a series of transitional forms between prebiotic chemistry and the last universal common ancestor (LUCA) during which RNA played a central role, and that many of the events and their relative order of occurrence along this pathway are known. The integrative nature of this synthesis also extends previous descriptions and concepts and should help inform future questions and experiments about the ancient RNA World and the OoL.

Keywords: RNA World; origin of life; evolution; ribosome; translation; polymerase

THE ORIGINS OF THE RNA WORLD

The RNA World hypothesis: historical and theoretical background

The canonical DNA ⇒ RNA ⇒ protein mode of information flow is universal in modern organisms. That information polymers are a sine qui non for life implies that the origin of life (OoL) on earth must have involved the emergence of molecularly encoded biological information. Though both nucleic acid-based and amino acid-based information polymers dominate the contemporary biosphere, it is far less plausible that the first self-replicating systems contained both functional nucleic acid polymers and functional proteins that simultaneously arose by stochastic chemical reactions (Cech 2012). A more parsimonious view is that the ternary mode of information flow of contemporary life was preceded by a unary system involving a single class of information polymers. In such a unary system, one dominant class of information polymers with sufficient structural and functional versatility would have given rise to different lineages of information polymers with different structures and functions that ultimately led to the origins of the last universal common ancestor (LUCA). In this view, a single type of primordial information polymer with catalytic activity carried out its own self-replicative catalysis. This idea is not new, as the hypothesis that catalytic information polymers played a role in the OoL is consistent with the view that life began with primordial "genetic enzymes" as suggested by Troland in the 1910s (noted in Lazcano 2010).

The view of RNA as a primordial information polymer was postulated in the 1960s (Woese 1967; Crick 1968; Orgel 1968). The fundamental roles played by tRNA and rRNA in protein synthesis led to the hypothesis that protein synthesis emerged in a milieu of functional RNAs, for instance, a protein-less ribosome and an RNA replicase (Crick 1968). Without knowledge of RNA's potential catalytic function, however, the primordial nature of RNA remained largely unsupported until the discovery of RNA catalysts or ribozymes by the Cech (Kruger et al. 1982) and Altman (Guerrier-Takada et al. 1983) laboratories. Spiegelman's work on a small 218 nt RNA molecule that underwent in vitro replication using RNA-dependent RNA polymerase (Kacian et al. 1972), later named Spiegelman's Monster, can also be viewed as early evidence in favor of primordial

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RNA. Though the known natural ribozymes were initially limited to group I self-splicing introns (Kruger et al. 1982) and the catalytic component of RNase P (Guerrier-Takada et al. 1983), the catalytic repertoire of RNA was further increased by the discovery of multiple other naturally occurring RNA catalysts. The reader is directed to Deng et al. (2023) for a list of the known natural ribozyme families and classes which include group II self-splicing introns, hepatitis delta virus, the Varkud satellite, hairpin, hammerhead, GIR1, *glmS*, and notably ribosomal RNA. The list of artificially generated ribozymes is also extensive and now includes RNA molecules capable of aminoacylation (Turk et al. 2010; Ishida et al. 2020), phosphorylation (Biondi et al. 2012; Dolan et al. 2015), ligation (Zhou et al. 2020b), alkylation (Wilson and Szostak 1995; Deng et al. 2022), coenzyme attachment (Breaker and Joyce 1995), nucleotide synthesis (Unrau and Bartel 1998), and importantly, RNA-dependent RNA polymerase activity (Johnston et al. 2001; Zaher and Unrau 2007; Bentin 2011; Wochner et al. 2011; Horning and Joyce 2016; Tagami et al. 2017; Tjhung et al. 2020; Cojocaru and Unrau 2021).

The large and ever-increasing repertoire of RNA enzymatic function provided a priori support for the idea of RNA-based protein-less life, for what became known as the RNA World (Gilbert 1986). Catalytic RNA now appeared to resolve the chicken–egg paradox of the origins of DNA replication, that is, that DNA requires proteins to replicate, yet proteins require DNA to store their sequence information. Given the observation that different authors have had various interpretations of the RNA World (Joyce and Orgel 1993), this synthesis does not claim to offer a universal definition of the hypothesis. A working definition is, however, suggested: the RNA World hypothesis denotes the view that life incorporated RNA before the emergence of coded proteins and DNA genomes. We argue that sufficient evidence exists to support this view beyond reasonable doubt.

Did RNA descend from an earlier information polymer?

That RNA evolved before coded proteins and DNA genomes, as the RNA World hypothesis asserts, does not necessarily mean that RNA was the first information polymer to emerge in earth's history. Some have suggested that in part due to the difficulties associated with prebiotic RNA syntheses, RNA descends from an earlier RNA-like polymer (Cafferty et al. 2018). Other potential information polymers are indicated (Orgel 2004), and include 2′–3′-dideoxy-D-glucose (homo-DNA), pyranosyl-RNA (p-RNA), threose nucleic acid (TNA), and peptide nucleic acid (PNA). The possibility that the first RNA enzymes were established in a "PNA/RNA World" was presented by Nielsen (1993) who speculates that RNA peptidyl transferases might have helped form the polyamide backbone of PNA, which may have served as a template for RNA polymerization (Nielsen 1993). Despite these considerations, the RNA World molecular fossils of contemporary life imply that proto-life must have incorporated RNA at some stage, even if it was initially based on a non-RNA polymer. Xu et al. (2022) show that isoxazole nucleosides, proposed to be prebiotically plausible (Becker et al. 2019), can form base pairs with guanine in a selective and stable manner, and can rearrange into cytidine, all within RNA strands. It is therefore possible that pre-RNA gradually evolved into RNA through a series of hybrid polymers composed of both monomers.

The origins of prebiotic chemistry

The view that the OoL is an evolutionary process implies that a continuum of increasingly life-like entities must have existed between inanimate matter and the first cells. Events along this continuum included the transition from inorganic to organic chemistry, which likely involved the origins of the first nucleotides, fatty acids, and other biomolecules, as well as the subsequent emergence of encapsulated self-replicating information polymers that ultimately led to cellular life. Several factors support this view: (i) gradualism (Aguirre et al. 2018), the observation that many small changes over a long enough time period can produce large changes; (ii) the mechanist notion that organisms are ultimately material systems with properties that derive from the laws of physics and chemistry (Allen 2005); and (iii) the principle of hybridism defined to mean that hybrid forms can, and often do, bridge the gap between two or more seemingly distinct biological categories.

Demonstrating prebiotically plausible syntheses of complex monomers has been a key objective of OoL experimentation. An early example showing that starting materials consisting of methane, ammonia, water, and hydrogen gas could produce a variety of amino acids after electric discharge was described by Miller (1953). Though some assumptions of the Miller experiment such as the degree to which the early earth's atmosphere was reducing have been challenged (Orgel 2004), what is significant is that Miller showed the ease with which complex biomolecules could form from simpler starting materials. Given the elasticity of the term prebiotic (Orgel 2004), we do not attempt to establish what makes something prebiotically plausible, nor do we review specific chemical challenges associated with particular prebiotic syntheses, but we note that numerous syntheses for biologically relevant monomers via potentially prebiotically plausible routes have been proposed, including ribonucleotides (Powner et al. 2009; Kim and Benner 2017; Stairs et al. 2017; Xu et al. 2017; Pérez-Villa et al. 2018; Becker et al. 2019), carbohydrates (Kim et al. 2011; Haas et al. 2020), lipids (Bonfio et al. 2019; Liu et al. 2020), and amino acids

(Jiang et al. 2014; Magrino et al. 2021; Pulletikurti et al. 2022). Endogenous mechanisms from hydrothermal vents, electrical spark discharges, geothermal hot springs, ultraviolet radiation, and volcanic eruption, as well as objects, such as meteoroids, asteroids, and comets have been described as potential sources of energy for the accumulation of biologically relevant monomers on the prebiotic earth (Oberbeck et al. 1991; Chyba and Sagan 1992; Deamer 1999; Bernstein 2006; Brunk and Marshall 2021).

Compartmentalized chemistry and polymerization

With the gradual increase in the complexity of molecules on the early earth, there must have been a point at which ancestral molecules evolved a spatiotemporal link with their molecular offspring. Compartmentalization of prebiotic chemistry into distinct loci which may have included lipid membranes, hollow condensates, organic hydrogels, clay, and lipid lamellar phases (Caliari et al. 2021) is necessary to satisfy the assertion that "molecules that stay together evolve together" (Orgel 2004). That spatiotemporally linked molecules tend to coevolve is evident, for instance, in the observation that proteins with similar coexpression levels often coevolve with their interactors to preserve protein–protein interactions (Fraser et al. 2004). Another early potential compartmentalization mechanism described by Poudyal et al. (2018) is that coacervates resulting from liquid–liquid phase separation (LLPS) facilitated the accumulation of nucleotides and their subsequent evolution into RNA polymers. Though a variety of pathways for compartmentalization exist, the dependence of contemporary life on lipid-based membranes ultimately implicates this mechanism in the OoL. Due to their greater permeability, lower polarity, and overall simplicity, it has been suggested that the first membranes were based on fatty acids (Jin et al. 2018).

Because lipids submerged in water spontaneously form spherical vesicles at the appropriate pH (Cohen et al. 2022), it is conceivable that fatty acid vesicles encapsulated the first biomolecules prior to the origins of information polymers (Apel et al. 2002). Eventually, nucleotides within the vesicles evolved into self-replicating RNA polymers coupled to vesicular growth and division. Contemporary phospholipid membranes likely evolved when fatty acid membranes gradually incorporated phospholipids through a series of hybrid membranes (Jin et al. 2018). Montmorillonite, a clay catalyst for RNA polymerization, also has catalytic properties that enhance membrane formation (Hanczyc et al. 2003), and montmorillonite-enhanced proto-cellular membrane formation can bring RNA polymers into the newly formed membranes via the clay particles that adsorbed RNA (Hanczyc et al. 2003). Since volcanic ash weathering that may have occurred on the early earth is known to produce montmorillonite (Ferris 2006), these findings provide a potential link between prebiotic RNA polymerization and prebiotic membrane evolution. Though montmorillonite has been widely used to model prebiotic polymerization, generating RNA polymers nonenzymatically has also been achieved via eutectic ice (Kanavarioti et al. 2001; Monnard and Szostak 2008; Attwater et al. 2010; Zhang et al. 2022). How RNA or RNA-like polymers generated nonenzymatically via the aforementioned mechanisms and remained intact over multiple generations to eventually become encapsulated by lipid membranes remains unclear.

Events that led to the formation of the first proto-cells might have resembled the growth and division cycle proposed by Zhu and Szostak (2009). Their experiments showed that larger multilamellar fatty acid vesicles could be fed by smaller fatty acid vesicles (micelles) such that the larger fed vesicles produced thread-like protrusions after which modest shear forces caused the protrusions to break off and divide into new fatty acid vesicles (Zhu and Szostak 2009). These new vesicles could reaggregate into the larger multilamellar vesicles to restart the cycle. Throughout these cycles, RNA molecules encapsulated within the vesicles remained encapsulated after division and could be redistributed into the resultant vesicles (Zhu and Szostak 2009). More recently, Zhou et al. (2020a) showed that within model proto-cells, oligonucleotides modified with 3′-amino groups could incorporate tetranucleotides through step-wise ligation reactions in a template-dependent manner which produced RNA polymers up to 200 nt in length. Though this demonstration represents a key advance in proto-cell research, the 3′-amino oligonucleotides along with the artificially controlled ligation reactions might not be prebiotically plausible (Zhou et al. 2020a). While both models provide some support for the view that a physicochemical process could have produced self-replicating vesicles with encapsulated information polymers, there has yet to be a complete demonstration of template-directed nonenzymatic RNA self-replication directly coupled to a self-replicating proto-cell. Such a demonstration would shed light on how the prebiotic chemistry we refer to as the pre-RNA World transitioned into the RNA World.

Another point of discussion is the extent to which the polymerization of RNA, without template-directed replication, could produce RNA polymers with adaptive biological functions. Wu et al. (2022) proposed loop-closing ligation as a mechanism by which RNA oligomers made in a template-free manner are converted into functional RNA hairpin stem–loop structures, for instance, tRNA mini-helices and hammerhead ribozymes. The former structure might have played a key role during the very early stages of the origins of translation (Petrov et al. 2015). It is therefore conceivable that prior to the origins of template-directed RNA synthesis, the sporadically formed RNA polymers on the early earth were functional.

The structural and functional versatility of RNA in the ancient RNA World: ribozymes, riboswitches, and self-splicing introns

There is a large and growing body of proof-of-principle research demonstrating the structural and functional versatility of RNA. These findings provide support for the view that RNA is sufficiently versatile to have served as the primary catalytic and information polymer before coded proteins and DNA genomes. A notable example of RNA's wide functional repertoire is its ability to undergo self-regulation as a function of ligand binding. Since their discovery in the early 2000s, riboswitches, now known to exist in all three domains of life (Pavlova et al. 2019), were immediately thought to have an early origin (Winkler et al. 2002). At present, there are over 50 classes of riboswitches (Kavita and Breaker 2022) and there is evidence that some of the contemporary riboswitches possibly evolved from ancient sensors and switches based on RNA that arose during the preprotein stages of evolution (Kavita and Breaker 2022). Though riboswitches are located within mRNA and are involved in the regulation of transcription, translation, and RNA processing (Breaker 2012), the general principle of RNA undergoing conformational changes in response to ligand binding can in theory apply to RNA molecules other than mRNA. The observation that many cofactors for riboswitches, such as FMN and coenzyme B_{12}, contain nucleotides within their biochemical structure is indicative of potential stages within the RNA World which may have involved the ancestors of contemporary riboswitches.

As a potential bridge between the origins of genetics and metabolism during the ancient RNA World, it is also conceivable that some ribozymes gradually developed the ability to undergo allosteric regulation in response to specific metabolites. Such riboswitch–ribozymes could have functioned as the first allosterically regulated RNA catalysts, allowing for a Jacob–Monod-like form of gene regulation prior to the origins of contemporary ligand-dependant proteins. Consistent with this idea is the existence of a group I self-splicing ribozyme with the ability to undergo allosteric regulation in which cyclic-di-GMP binding determines the particular spliced product obtained (Chen et al. 2011). Prior to their discovery, various nonnatural ligand-dependent ribozymes were obtained using in vitro selection (Tang and Breaker 1997; Soukup and Breaker 1999), thereby demonstrating that artificial functional RNA can provide clues about natural functional RNA. The *glmS* self-cleaving ribozyme is also of interest, partly because its self-cleaving activity is promoted by the selective binding of glucosamine-6-phosphate (McCown et al. 2012).

Speculation about possible scenarios for the origins of the RNA architecture found within the spliceosome have included the RNA World (Doolittle 2013; Fica et al. 2013). It was postulated (Sharp 1991) that during the origins of the spliceosome, group II self-splicing introns broke into five pieces that gave rise to the five snRNAs (U1, U2, U4, U5, and U6). Consistent with Sharp's hypothesis, Zimmerly and Semper (2015) review the structural and functional similarities between spliceosomal introns, snRNAs and group II introns, and conclude that "important insights over the past 2 yr have largely erased doubts about the longstanding hypothesis that the spliceosome descended from group II introns." Though the evolution of snRNA from group II introns likely occurred after eukaryogenesis and therefore long after the ancient RNA World, the hypothesis that self-splicing introns predate spliceosomal introns is consistent with the view that RNA, not protein, was the initial primary molecule of intron processing. It also contributes to the expansion of RNA's known structural and functional versatility. Indeed, the existence of self-splicing introns as evidence for the ancient RNA World was one argument presented by Gilbert (1986) when the term was first proposed.

An additional observation relevant to the RNA World is that group II introns encode a reverse transcriptase known as intron encoded protein (IEP) (Lambowitz and Zimmerly 2011). Since reverse transcriptase may have provided the path for RNA genomes to generate complementary DNA, which then led to the replacement of RNA genomes by DNA genomes, this finding could implicate group II introns and their reverse transcriptase in the evolutionary transition from RNA to DNA. This scenario would agree with the view that protein enzymes were necessary for the transition from RNA to DNA genomes, that is, that the initial reverse transcriptase was not a ribozyme but a protein enzyme, perhaps related to the IEP of group II introns. Alternatively, the initial reverse transcriptase for the RNA-to-DNA genome transition might have been a ribozyme, in which case the IEP of group II introns evolved long after the emergence of DNA genomes and coded proteins. Though a posteriori evidence for either scenario is lacking, the hypothesis that the first reverse transcriptase was a ribozyme is likely to be evolutionarily simpler, and is consistent with the existence of an artificial ribozyme capable of reverse transcription (Samanta and Joyce 2017).

The list of known natural and artificial ribozymes previously mentioned increases the known versatility of RNA, while providing clues about the nature of the ancient RNA World. These findings indicate that functional RNAs in the ancient RNA World might have been capable of phosphorylation, nucleotide synthesis, ligation, alkylation, coenzyme attachment, RNA-dependent RNA polymerization, and more. Perhaps the eventual coupling of amino acid chemistry with functional RNA dynamics facilitated the coevolution of ribozymes and amino acids, leading to the emergence of aminoacylation and peptidyl transferase ribozymes during the birth of primitive polypeptide synthesis.

THE EVOLUTION OF THE RNA WORLD

The RNA World and the origins of coded proteins

The working definition of the RNA World hypothesis—that life incorporated RNA before the emergence of coded proteins and DNA genomes—requires a distinction between coded proteins and noncoded polypeptides as the former require a genetic decoding apparatus for information flow from nucleic acids to amino acids, and the latter do not. From this working definition, it follows that a key difference between the ancient RNA World and subsequent evolutionary stages is the presence of coded proteins. Despite this difference, noncoded amino acid chains, that is, oligopeptides and/or polypeptides, synthesized in a random and nonspecific manner might still have been present and could have provided adaptive value before, during and after the RNA World (Cech 2009). For instance, it has been shown that lysine decapeptides can reduce the extent to which ribozyme RNA polymerases depend on Mg^{2+} (Tagami et al. 2017) which could decrease the overall need for Mg^{2+} in RNA proto-cell(s). This feature is especially useful given that Mg^{2+} disrupts fatty acid vesicles (Adamala and Szostak 2013). The accumulation of short noncoded polypeptides in the first RNA-based cells could therefore have facilitated RNA-dependent RNA polymerase activity. Though the pervasiveness of noncoded polypeptides in the RNA World remains unknown, the wide structural and functional versatility of RNA contributes to the view that the dependence of RNA-based proto-life on amino acid chains of any sort could have been little to none. That the first bona fide coded proteins could not have arisen before the production of the first noncoded polypeptides, and the view that noncoded polypeptides antedate coded proteins is an assumption of many models for the origins of translation (Smith et al. 2008; Petrov et al. 2015). Proto-life must therefore have gained the ability to produce amino acid chains before such chains became encoded by a complex system of information flow from nucleic acids to proteins, that is, translation.

The translation-centric nature of the RNA World

Given the central role of translation and the genetic code in sustaining life, it is not unexpected that the RNA World and the OoL are both thought to involve the ribosome's origins (Wolf and Koonin 2007; Fox 2010; Root-Bernstein and Root-Bernstein 2015). Consistent with this notion is the observation that translation-related genes dominate the set of <100 genes common to all living organisms, termed the Universal Gene Set of Life (Bernier et al. 2018). By comparison, genes involved in DNA replication and transcription are found less often in this gene set; that is, they are less conserved than translation-related genes across evolution (Harris et al. 2003).

The observation that the catalytic core of the ribosome is an RNA enzyme (Nissen et al. 2000) is consistent with the RNA-centric and translation-centric nature of the OoL, and was later used to support the view that RNA preceded coded proteins in the early evolution of life (Strobel and Cochrane 2007; Cech 2012; Son et al. 2021). That proteins occupy the periphery of the ribosome (Noller 2012), while RNA forms the ribosome's core-portions and exit tunnel (Nissen et al. 2000) and also performs its aminoacyl-tRNA selection function (Noller 2012) suggests that the ribosome's evolution began with RNA, not protein. These considerations allow one to conceive of a primitive RNA-based ribosome without r-proteins, in accordance with the view postulated by Crick (1968), who wrote that "it is tempting to wonder if the primitive ribosome could have been made entirely of RNA." These suggestions are consistent with the postulate that the peptidyl transfer center (PTC), the RNA catalyst at the heart of the ribosome, is thought to be one of the structure's earliest features (Smith et al. 2008; Bernhardt and Tate 2010; Fox 2010; Petrov et al. 2014, 2015). It is postulated that if the ribosome is needed to make coded proteins, then coded proteins did not exist before the first ribosomes or proto-ribosomes. If the ribosome began with RNA, it follows that RNA evolved before coded proteins. This supports the RNA World hypothesis beyond reasonable doubt, while making questions such as "how did the RNA World arise and transition into contemporary life" and "is RNA stable and simple enough to have emerged under prebiotic conditions" less important than the potential historical fact that RNA emerged before coded proteins. By analogy, based on molecular fossil data one can infer that the mitochondrion evolved from a free-living bacterium without the precise knowledge of how the bacterium itself originated and transitioned into the mitochondrion.

Consistent with the translation-centric nature of the OoL, as well as the necessity of compartmentalization, Wolf and Koonin (2007) describe the origins of translation in the context of "a compartmentalized ensemble of replicating, coselected RNA segments." Such a scenario might reflect that a late stage of the RNA World consisted of a membrane-bound minimal translation apparatus. The origins of the ribosome and translation should not be conflated with the origins of the RNA World itself, since the emergence of the latter, that is, a sufficiently versatile pool of functional RNA, is a necessary condition for the emergence of bona fide ribosomes and the translation apparatus. In this view, fully formed ribosomes and the translation apparatus were inventions of the late RNA World, and their RNA-centric nature supports the hypothesis that their evolution required an antecedent pool of diverse functional RNAs. Despite the lateness of translation and the ribosome in the RNA World, it cannot, however, be ruled out that smaller RNA fragments with functional properties resembling aspects of the ribosome, that is, small functional RNAs with the ability to catalyze peptide bond formation for the production of small noncoded

oligomers, could have formed at relatively earlier stages of the RNA World.

Based on the principles of hybridism and gradualism previously described, it is likely that the contemporary translation system descended from increasingly translation-like processes beginning with the formation of a sufficiently functional minimal translation apparatus. Progress toward artificially synthesizing aspects of this minimal translation machinery is underway. For example, it has been shown that amino acids joined to single nucleic acids can form template-based dipeptides, without ribosomes (Jash et al. 2021). Additionally, a recent mass spectrometry-based study (Bose et al. 2022) found that various minimal ribosome constructs derived from the PTC can form peptide bonds, leading to the conclusion that the proto-ribosome might bridge the gap between the RNA World and DNA–RNA–protein dominated life (Bose et al. 2022). Though the findings from Bose et al. (2022) are possibly ground-breaking, the study would benefit from the inclusion of experimental conditions with defective or random minimal ribosome constructs, given that the current negative experimental controls consist only of conditions without the ribosome or RNA. Successful attempts to artificially synthesize or reconstruct translation-related elements of the current biosphere, that is, the synthesis of 5 nt long aminoacylation ribozymes (Turk et al. 2010), the artificial in vitro synthesis and assembly of the prokaryotic 16S rRNA (Michael et al. 2020), and the protein-less RNA-based synthesis of 15-aa long polypeptides (Müller et al. 2022) can also help elucidate potential events during the OoL. Given that some of the most conserved nucleotide sequences in life are found within rRNAs (Noller et al. 2022) and the translation-centric nature of the Universal Gene Set of Life (Bernier et al. 2018), it follows that contemporary rRNAs should contain at least some fossilized sequences from LUCA and possibly pre-LUCA stages of life's origins. The relative order of emergence of different segments of rRNA can also be used to infer evolutionary events at the molecular level that took place during the OoL. This reasoning has been applied by some (Mears et al. 2002; Harish and Caetano-Anollés 2012; Petrov et al. 2014) to elucidate potential facts about the origins of the ribosome and translation.

Though most theories predict that ribosomal evolution began with the PTC, Harish and Caetano-Anollés (2012) conclude that some components of the small ribosomal subunit (SSU) are older than the PTC and by extension, that the ribosome's decoding function arose before its ability to catalyze peptide synthesis. As part of the model, they propose that r-proteins and their precursors played important roles in the early stages of ribosomal evolution prior to the origins of the PTC, such that coded proteins were produced before translation emerged, potentially by autocatalysis or nonribosomal protein synthesis (Harish and Caetano-Anollés 2012). Nonribosomal peptide synthesis, however, requires complex multifunctional enzymes (Finking and Marahiel 2004) and it seems unlikely that coded proteins, composed of a specific sequence of amino acids, evolved before the genetic code itself. An important limitation of the model proposed by Harish and Caetano-Anollés (2012) is therefore its prediction of a system that produced and replicated functional proteins prior to the origins of ribosomal protein synthesis (Bernhardt 2012; Petrov and Williams 2015).

Another model of the ribosome's origins (Petrov et al. 2014) argued that the oldest segments of the large subunit are located within the PTC. This publication was followed by a comprehensive summary of ribosomal history proposed by Petrov et al. (2015), involving the proposition that the PTC evolved before ribosomal decoding. It is worth noting that the authors of these two publications (Petrov et al. 2014, 2015) have published a response (Petrov and Williams 2015) to the model proposed by Harish and Caetano-Anollés (2012), which is part of a larger exchange that the reader is directed to (Caetano-Anollés 2015; Caetano-Anollés and Caetano-Anollés 2015, 2016; Petrov and Williams 2015). The ribosomal history presented by Petrov et al. (2015) also described the origins of other translation-related elements such as tRNA and mRNA segments. In terms of tRNA, they postulate that a collection of RNA stem–loops and mini-helices interacted during which the proto-CCA arose at a hypothetical early stage of ribosomal origins when the ancestral versions of the large ribosomal subunit (LSU) and SSU evolved independently. Afterwards, their gradual evolution became coupled as primitive tRNA molecules emerged, leading to the origins of ribosomal translocation (Petrov and Williams 2015). Consistent with this, it was proposed earlier that the architectural similarity between the LSU and SSU rRNA suggests a common evolutionary origin of the proto-LSU and proto-SSU (Petrov et al. 2013).

Considering the importance of aminoacylation in translation, several studies have explored potential mechanisms for the prebiotic aminoacylation of RNA (Lee et al. 2000; Turk et al. 2010; Roberts et al. 2022). As the RNA World eventually gave rise to peptides, it could have generated an RNA–peptide World prior to the localization of proto-life's catalytic function to coded protein (Müller et al. 2022). Relevant to this, Müller et al. (2022) show how RNA–peptide chimeras can be generated by the synthesis of peptides directly on RNA molecules. The critical roles of ribonucleoprotein (RNP) enzymes in modern organisms also demonstrate that proteins did not completely override RNA as the molecule of catalysis, even though most catalysis is now achieved by protein (Cech 2009).

Given the centrality of rRNA and tRNA in translation, it is necessary that the origins of translation involved the ancestors of both molecules. A notable and widely cited postulate is that the joining together of two hairpin-like RNA molecules is one central event during the origin of tRNA

(Di Giulio 2012). In this view, each hairpin contained one segment of nucleotides specific to a given amino acid. When the two hairpins joined, one of these sequences developed into the acceptor stem, while the other evolved into the contemporary anticodon. This led to the formation of a cruciform structure which then evolved into the cloverleaf appearance of current tRNAs. It is also conceivable that during the establishment of the genetic code, primitive tRNA genes underwent duplication and mutation in a manner that incrementally expanded the genetic code by matching new amino acids with new codon–anticodon interactions. This indicates that the near-universal genetic code of extant life emerged by descent with modification from smaller and simpler codes containing fewer codons, potentially where glycine, alanine, aspartic acid, and valine were among the first amino acids to join the genetic code (Trifonov 2004; Macé and Gillet 2016). As the genetic code increased in complexity, coded proteins would have gradually replaced many of the functions once carried out by RNA. The observation that several translation factors have tRNA-like 3D shapes classified as molecular mimicry (Nissen et al. 1995; Nakamura et al. 2000), might also suggest that at least some RNA structures were gradually imitated by proteins until proteins replaced them altogether (Nakamura 2001).

Though much of the evidence for the RNA World hypothesis involves the ribosome, it is important to consider RNA World relics found within the riboswitches, self-splicing introns, and other RNA-based molecular architecture in contemporary life. This RNA architecture might, for instance, involve M1 RNA, given its catalytic role in tRNA maturation and the suggestion that contemporary ribonuclease P holoenzymes in all three domains descend from an ancestral protein-free RNase P (Lai et al. 2010).

The RNA World origins of polymerases

The existence of two classes of nucleic acid information polymers entails four types of possible polymerase activity: (i) RNA-dependent RNA polymerase (RdRp), (ii) RNA-dependent DNA polymerase (RdDp), (iii) DNA-dependent DNA polymerase (DdDp), and (iv) DNA-dependent RNA polymerase (DdRp). In principle, each of these types of polymerase activity can be catalyzed either by ribozymes or protein enzymes. The ancient world of RNA genomes undergoing self-replication in the absence of proteins, as predicted by the RNA World hypothesis, indicates that the first RdRp was a ribozyme that arose before all other classes of polymerase. That the transition from RNA genomes to DNA genomes likely required reverse transcriptase (Samanta and Joyce 2017) indicates that the first RdDp emerged after RdRp. It is unclear whether this polymerase was made of RNA, protein, or perhaps both. If the RNA-to-DNA transition could only have occurred after the origins of a sufficiently extensive repertoire of coded proteins, then it is more likely that this RdDp was protein-based. As noted earlier, however, the RNA-based scenario might be evolutionarily simpler.

The transition from RNA genomes to DNA genomes was likely a gradual process involving hybrid RNA–DNA molecules (Gavette et al. 2016) as well as ribonucleotide reductase and thymidylate synthase (Forterre 2005). It is conceivable that short information-rich DNA fragments were produced from RNA genomes, until these polymers grew in size to replace RNA genomes as the dominant molecule of information storage. Only once there were sufficient amounts of information-rich DNA polymers was there a need to evolve DdDp and DdRp. Thus, the first reverse transcriptase likely predates the first DdDp and DdRp. It however remains unclear which of DNA-dependent DNA replication or DNA-dependent RNA transcription is older, and whether the first DdDp or DdRp catalysts were made of RNA, proteins, or a mixture of the two. Although we do not provide definitive answers to these questions, we argue that both contemporary protein-based polymerases have a common origin, given the common polymerase active site found in DdDp, DdRp, RdDp, and RdRp (Steitz 1993) and the model proposed by Koonin et al. (2020), which posits that DdDp and DdRp descend from an ancestral RdRp. These considerations indicate that the likely order of replicase evolution is: RdRp ⇒ RdDp ⇒ DdDp and/or DdRp.

That DNA synthesis always begins with an RNA primer (Kuchta and Stengel 2010) is also consistent with a common origin of DNA replication and RNA transcription. Indeed, both replication and transcription involve the de novo synthesis of RNA ribonucleotides from a DNA template. For replication, the ribonucleotides are removed and replaced by deoxyribonucleotides, whereas in transcription, the ribonucleotides form a polymer of RNA which detaches from the DNA to perform a function. A functional similarity therefore exists between primase, the enzyme responsible for synthesizing RNA primers during DNA replication, and RNA polymerase, the enzyme that synthesizes mRNA from a DNA template during transcription. In mitochondria, these two functions are carried out by the same enzyme, mitochondrial RNA polymerase, also known as POLRMT (Wanrooij et al. 2008; Kühl et al. 2016). A plausible interpretation of this is that an ancestral primase-like enzyme with a conditional DNA or RNA polymerase activity analogous to POLRMT could have existed prior to the division of biological labor into DNA replication and RNA transcription. Thus, contemporary RNA polymerase and DNA polymerase activity could have descended from an all-in-one ancestral primase with a dual function. At this stage of life's origins, the function of translating nucleic acids to polypeptides would have not yet been coupled to the production of a nucleic acid message from another nucleic acid polymer. The flow of information of the form DNA ⇔ RNA ⇒ protein was therefore not yet continuous but rather involved separate information flow

between DNA and RNA (DNA ⇔ RNA) as well as unidirectional information flow from RNA to protein (RNA ⇒ protein). At some point, these two information flow pathways, initially evolved in parallel, would have been coupled into the conventional scheme DNA ⇔ RNA ⇒ protein.

A third question about the origins of DNA genomes is the extent to which DNA monomers, oligomers, and polymers were present before the origins of homogenous RNA genomes. Part of the RNA World hypothesis as defined here asserts that DNA genomes began to dominate life only after the pervasiveness of RNA-based organisms with RNA genomes. This fact is still compatible with the claim that deoxynucleotides could have been made prebiotically (Teichert et al. 2019; Xu et al. 2020) and that several information polymers during life's origins might have contained both RNA and DNA (Trevino et al. 2011; Gavette et al. 2016; Bhowmik and Krishnamurthy 2019). Consistent with the principle of hybridism, homogenous RNA genomes arose from a pool of heterogeneous information polymers containing both RNA and non-RNA monomers. That proto-life likely evolved a unary system of catalytic information polymers rather than a binary or ternary system, implies that catalytic RNA information polymers and catalytic DNA information polymers are mutually exclusive options to dominate this stage. While many catalytic DNA molecules or DNAzymes have been artificially generated (Breaker and Joyce 1994; Achenbach et al. 2004; Aranda et al. 2019), there are no known natural DNAzymes (Zhou et al. 2017; Zhang 2022), and the 2′-hydroxyl group in ribonucleotides expands RNA's functional repertoire by contributing to its increased ability to undergo folding, hydrogen bonding, metal ion coordination, and catalysis (Gordon et al. 2004). These facts are consistent with the view that the unary system of self-replicative catalytic information polymers was based on RNA genomes before life incorporated DNA genomes.

While it is claimed here that sufficient evidence exists for the propositions that (i) coded proteins evolved after RNA genomes and that (ii) DNA genomes evolved after RNA genomes, we have not made any firm claims about the relative order of the emergence of coded proteins with respect to DNA genomes. Though it is possible that the first DdDp and/or DdRp catalysts were made of RNA or perhaps both RNA and protein, it does seem more likely that they began with protein. If DdDp and DdRp catalysis were initially RNA-based, then why is it that unlike the ribosome and the spliceosome, both of which almost certainly began with catalytic RNA, do contemporary DdDp and DdRp enzymes not contain any clear RNA-centric features indicative of their ribozymal beginnings? It therefore seems more plausible that coded proteins were present before the emergence of the protein-centric processes of DNA replication and RNA transcription. Another consideration that accords with this view is that the initial unary mode of information flow involving RNA-centric proto-life did not directly evolve into a ternary system with DNA, RNA, and protein, but rather transitioned through a binary intermediate, that is, unary ⇒ binary ⇒ ternary, given that DNA genomes were probably unnecessary for the origins of a minimal translation apparatus from the RNA World. RNA and protein, rather than RNA and DNA, were likely the two main information polymers during this binary stage. Indeed, the order of emergence RNA ⇒ protein ⇒ DNA has wide acceptance (Cech 2012).

The RNA core of life

While the postulate that life began with RNA rather than protein or DNA suggests a fundamental RNA character of the primordial world, it also argues for a more RNA-focused view of the contemporary biosphere. The two RNA Worlds (Cech 2012), that is, the primordial and the contemporary, implies that the ancient RNA World never truly disappeared. Indeed, much of the biology established at different stages of the RNA World played an essential role in life's 4-billion-year evolution and is still critical for the architecture of contemporary life. Though the proportion of the contemporary RNA World that derives from the ancient RNA World remains uncertain, it is clear that much of life's nonuniversal RNA architecture represents newer evolutionary additions to different lineages. Clearly, however, the contemporary biosphere does contain some universal RNA architecture, that is, the PTC of the ribosome and much of the translation apparatus. This RNA core of life is intricately linked with the translation apparatus, which we define as the universally conserved RNA-based architecture that arose either late or early during the ancient RNA World and is still present in contemporary organisms. Other components of the RNA core could include preprotein riboswitches, self-splicing introns, the RNA monomers present in some cofactors (Goldman and Kacar 2021), the RNA primers from DNA replication, and the universal process in which DNA monomers are synthesized from RNA monomers (Lazcano et al. 1988). All of these observations challenge the statement that "modern cells present no signs of a putative prebiotic RNA World" (Kurland 2010). Evolutionary approaches aimed at identifying universally conserved RNA architecture in the contemporary biosphere would advance the characterization of the RNA core. This identification of specific RNA World fossils could then be followed by their synthetic reconstruction under prebiotically plausible conditions, to shed light on life's RNA past.

THE TIMELINE: A SYNTHESIS OF INDEPENDENT MODELS AND OBSERVATIONS

Timeline aims and objectives

We propose a plausible timeline for the OoL that integrates and contextualizes independent models and

TABLE 1. Proposed timeline of events during the OoL

Stage	Order	Suborder	Potential event	Type
Pre-RNA World	1	A	Pre-RNA monomer formation	N
		B	Ribonucleotide formation	N
		C	Deoxyribonucleotide formation	N
		D	Lipid formation	V
		E	Amino acid formation	P
	2	A	Montmorillonite polymerization	N
		B	Eutectic ice polymerization	N
		C	Fatty acid vesicle formation	V
	3	A	Fatty acid vesicle growth and division	V
		B	Pre-RNA hybrid polymers	N
		C	RNA/pre-RNA hybrid polymers	N
		D	RNA/DNA hybrid polymers	N
		E	Nontemplated DNA oligomers	N
		F	Nontemplated oligopeptides	P
	4	-	Homogenous RNA polymers	N
Early RNA World	5	-	Pretemplated functional RNAs	N
	6	A	Phosphorylation ribozymes	N
		B	Hammerhead ribozyme	N
	7	A	Fatty acid vesicle phospholipid incorporation	N
		B	Template-based RNA self-replication	V
	8	A	Phospholipid vesicle formation	V
		B	RdRp ribozyme	N
		C	Self-splicing RNA	N
		D	RNA proto-cell(s) coreplication mechanism	V
	9	-	Riboswitches and riboswitch–ribozymes	N
Late RNA World	10	-	Aminoacylation ribozymes	T
	11	-	Aminoacylated RNAs	T
	12	-	Peptidyl transferase ribozyme	T
	13	-	Noncoded polypeptides	T
	14	-	Proto-PTC	T
	15	-	Proto-tRNA fragments	T
	16	-	Primitive anticodon and acceptor stem	T
	17	-	Primitive decoding	T
	18	-	Proto-M1 RNA	T
	19	-	LSU and SSU coevolution	T
	20	-	Primitive translocation	T
Near-LUCA	21	-	tRNA duplications and amino acid coexpansion	P
	22	-	Coded proteins mimic functional RNAs	P
	23	A	Proto-ribosome proteinification	T
		B	Proto-M1 RNA proteinification	T
	24	-	Universal canonical protein active sites	P
	25	A	Ribonucleotide reductase	P
		B	Thymidylate synthase	P
	26	-	RdDp protein/ribozyme	P
	27	-	Coded DNA polymer expansion	N
	28	-	Replication–transcription hybrid process	N
	29	A	DdDp protein	P
		B	DdRp protein	P
	30	-	Replication–transcription split	N

Major stages during the OoL (column 1). Chronological ordering (column 2) and achronological subordering (column 3) of potential events (column 4) and respective event types (column 5).
Event types: (N) Nucleotide-related, (V) vesicle-related, (T) translation-related, and (P) protein-related.

observations into four general stages: (i) the pre-RNA World, (ii) the early RNA World, (iii) the late RNA World, and (iv) the near-LUCA stage. The key difference between the pre-RNA World and the early RNA World is that the transition into the latter stage involved the establishment of RNA as the central molecule of function. While the late RNA World is defined by the origins and evolution of a minimal translation apparatus from pre-established RNA chemistry, the near-LUCA stage involves the division of biological labor into coded proteins and DNA genomes

as the main molecules of catalysis and information storage, respectively. In this view, the completion of the OoL is marked by the establishment of the canonical DNA ⇒ RNA ⇒ protein mode of information flow.

Rather than providing approximate dates and geophysicothermal conditions of such events, the events presented are organized into one plausible timeline (Table 1) based on their relative chronology. The four stages are depicted (Table 1, column 1) along with the approximate order of such events or groups of events (Table 1, column 2) with respect to other events or event groups. When the exact chronological order of events within a set of events is uncertain but the general notion that such events occurred in a similar interval is supported, we have added suborders without indicating a precise chronology to distinguish between such events for references to the table (Table 1, column 3). Potential events are indicated (Table 1, column 4) and grouped into nucleotide-related (N), vesicle-related (V), translation-related (T), and protein-related (P) event types (Table 1, column 5) to focus on overarching evolutionary pathways; for instance, items that pertain specifically to the origins of translation or the formation of proto-cellular vesicles. Metabolism, however, is not a focus of this meta-narrative. The complete OoL timeline, that is, the actual chain of all atomic and molecular events between prebiotic chemistry and the first cells extends to atomic resolution and must account for every structure and function of LUCA. Such an analysis is beyond the scope of this presentation and the timeline we propose is therefore useful but not exhaustive.

Conclusions

The analysis and timeline that we present contributes to science's understanding of the OoL as an evolutionary process. Though the timeline proposed here lacks metabolism-related events, that is, the origins of ATP, glycolysis, the Krebs cycle, the electron transport chain, etc., it does identify many potential and definite steps between inanimate matter and the first cells. Future OoL timelines would benefit from the inclusion of evolutionary events related to RNA viruses and viroids, given their potential role in the ancient RNA World. Most of the steps we propose relate to the flow of biological information, that is, the central dogma of molecular biology. The key tenets of the central dogma, the ribosome, the genetic code, polymerase, etc., are fundamentally linked to the OoL.

The OoL is the sum of all suborigins, extending from prebiotic chemistry to the formation of LUCA. Computational approaches that characterize universally conserved structures and functions, with a focus on contemporary life's RNA core, as well as attempts to synthetically reconstruct life's minimal RNA architecture, could help identify many of these events. Investigating the OoL cannot be described as "mere rubbish thinking" (Darwin as quoted in Peretó et al. 2009). Life is the result of particular arrangements of matter and at no single point was inanimate matter transformed into a vital substance. As presented here, decades of OoL research have shown that the OoL was a gradual evolutionary process involving a continuum of intermediates between inanimate matter and the first cells. Many events along this continuum are now known, such as the fact that the biosphere must have incorporated RNA before the first coded proteins which supports the RNA World hypothesis beyond reasonable doubt. As more continues to be learned about the structural and functional versatility of RNA, the RNA character of both contemporary life and ancient proto-life will continue to be elucidated, providing additional support for the details of the timeline that we present.

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