

Citric Acid and the RNA World**

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How did the first life form originate? This fundamental question, which has fascinated philosophers and scientists alike, will almost certainly remain unanswered. Most scientists assume that today's terrestrial life forms originated on earth; but how? To identify the chemistry of the first life forms, one could, in principle, analyze sediments that might contain the first signs of life from more than 3.4 billion years ago.^[1] However, only a small proportion of the surface of the earth has escaped severe metamorphosis since then, and most of the early biomolecules have degraded. Researchers have therefore attempted to identify universal principles that likely governed the emergence of life as we know it, and subjected them to constraints that are assumed to have existed on the early earth. The anticipated results would address several questions, such as: Were there only few or many different chemical possibilities for life to arise? Under the right conditions, would the origin of life be an unlikely phenomenon or would life inevitably arise?

What are the universal principles that guided the origin of life, and can they be identified by research today? Life forms can be defined as self-replicating molecular systems that are amenable to open-ended evolution. This requires a genetic polymer that is able to store information and accumulate beneficial mutations during evolution (corresponding to the DNA of today), genome-encoded catalysts that facilitate self-replication (today mediated by proteins), and a separation between self and non-self to define individuals and enable evolution (corresponding to today's cell membranes). The challenge is to identify chemical systems that display all of these characteristics, and that could have arisen in the early earth environment. Relatively little is known, however, about the early earth environment. Its atmosphere was dominated by nitrogen, lacked oxygen, and included numerous other gases (e.g., methane, ammonia). This environment facilitated very different reactions compared to today's oxygen-rich atmosphere, including the synthesis of amino acids.^[2] Furthermore, we do not know where on the earth the earliest life

forms developed. Chemists therefore have to consider only a few strict constraints when trying to mimic early-life-forming conditions in the laboratory.

Which molecules could have given rise to our earliest, ancestral life forms? In other words, how could today's organisms have arisen from a prebiotic soup, when today's DNA depends on proteinaceous enzymes for replication, and today's proteins depend on DNA for their genomic information? This chicken-and-egg problem was famously solved by the postulation of an RNA world,^[3] which relies on RNA molecules that not only store genetic information (because of their polymeric arrangement of nucleotides), but also catalyze reactions. Although it is possible that RNA was predated by a different polymer fulfilling its roles,^[4] the RNA world hypothesis itself is now supported by several independent observations in today's organisms (e.g., RNA catalysis and RNA-catalyzed protein synthesis, nucleotide-based protein cofactors, the biosynthesis of deoxynucleotides from ribonucleotides)^[5] and by *in vitro* selected catalytic RNAs that are capable of catalyzing a wide range of chemical reactions that could sustain a metabolism.^[6]

To recreate an RNA world organism in a test tube, the aim is to find a self-replicating and evolving set of catalytic RNAs (ribozymes) that are encapsulated by lipid vesicles (Figure 1a). Encapsulation is required for two reasons: 1) It generates a boundary between self and non-self, which is necessary to keep out parasitic molecules and to facilitate Darwinian evolution, and 2) It allows the RNA world organism to contain small molecules and harvest the fruits of their metabolic processes. Fatty acids, which can assemble into lipid vesicles with double-lipid membranes, could have been generated in a prebiotic environment by Fischer–Tropsch synthesis in hydrothermal vents and were also likely delivered to the prebiotic earth by meteorites.^[7] Fatty acid vesicles have been shown to grow and divide in the laboratory under prebiotically plausible conditions,^[8] which solved an important piece of the puzzle. In contrast, the prebiotic syntheses of ribonucleotides and oligoribonucleotides from a prebiotically plausible mixture of compounds are much more problematic and suffer from limited regio- and stereo-selectivity and detrimental side reactions.^[9] In recent years, progress has been made and includes, for example, advances towards a prebiotically plausible synthesis of nucleotides, a ribozyme-mediated conversion of the 5'-hydroxy groups of RNA into 5'-triphosphates, and the ribozyme-mediated polymerization of nucleoside triphosphates into RNA.^[10] We note, however, that aside from identifying appropriate

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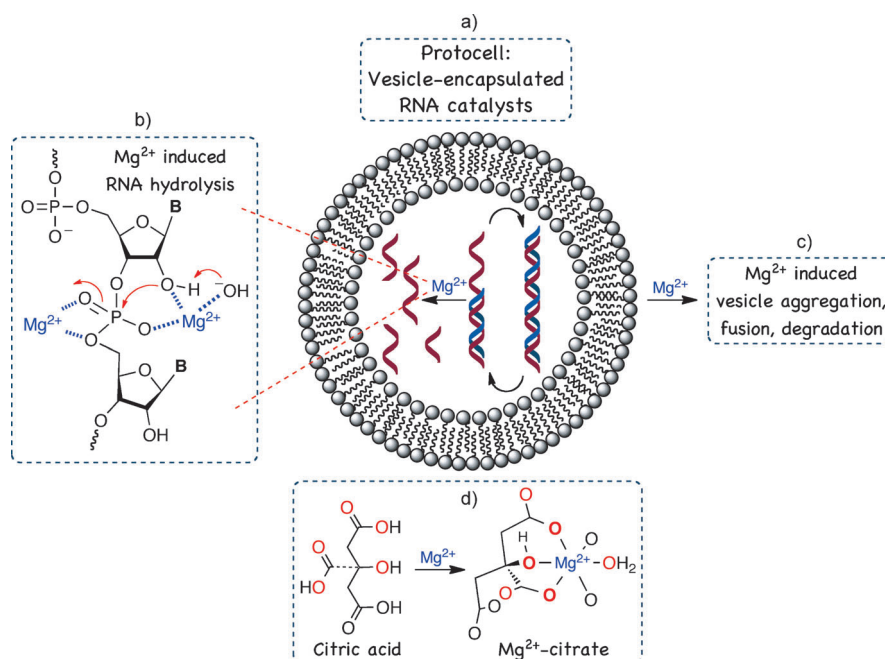


Figure 1. a) A protocell is schematically represented by a set of replicating RNAs that are encapsulated by a lipid vesicle. b) Mg^{2+} ions can catalyze RNA degradation by various mechanisms, including the activation of the phosphate group as well as of the 2'-hydroxy group; the ultimate products of this reaction are a 2',3'-cyclic phosphate and a liberated 5'-hydroxy group, leading to strand cleavage. c) Mg^{2+} ions can also degrade vesicles through their interactions with the carboxylates. d) The structure of citric acid and the core structure of the Mg^{2+} coordination sphere as seen in the crystal structure of magnesium citrate; in this highly networked structure, all carboxylates are interacting with the Mg^{2+} ion, leaving one potentially labile coordinating water molecule (Ref. [11]).

lipid vesicles and sets of self-replicating RNA, the identification of conditions under which both systems coexist and function in concert remains challenging.

A major predicament and an obstacle on the pathway to the recreation of an RNA world organism is presented by divalent metal ions (e.g., Mg^{2+}). On one hand, high concentrations are required for most ribozymes. On the other hand, divalent metal ions can lead to the fragmentation of RNA and the aggregation of lipids, thereby incapacitating both components of such RNA world organisms (Figure 1 b,c). A recent study by Adamala and Szostak^[11] showed that both problems could be remedied by citric acid: Citrate chelates Mg^{2+} ions with an affinity that is high enough to substantially reduce RNA degradation and lipid aggregation while leaving open coordination sites, which are necessary for productive interactions with RNA (Figure 1d). In particular, the study investigated the template-directed nonenzymatic polymerization of nucleotides that are activated as 5'-(2-methylimidazole)s in lipid-based vesicles. Concentrations of 50 mM of Mg^{2+} ions, which are typically detrimental to such assemblies, were tolerated by lipid vesicles if the citrate was present in a concentration of 200 mM. The same conditions also facilitated nonenzymatic RNA polymerization in the interior of the vesicles. Intriguingly, isocitrate was less potent than citrate in protecting the vesicles. Other chelating agents, such as ethylenediaminetetraacetic acid (EDTA) or nitrilotriacetic acid (NTA), while protecting the vesicles, did not facilitate RNA polymerization. Furthermore, citrate reduced the rate of magnesium-induced RNA fragmentation by a factor of ten per nucleotide. Citrate therefore appears to display a favor-

able combination of stability constants and coordination features among the chelators, endowing it with unique abilities with regard to RNA world organisms.

What is so special about citrate compared to isocitrate, its constitutional isomer, in terms of the ability to coordinate Mg^{2+} ions (Figure 2 a)? Although the three pK_a values that have been reported for the two tricarboxylic acids are essentially identical, stability-constant measurements suggest citrate to have a much higher affinity for Mg^{2+} ions than isocitrate (ca. ten-fold).^[12] This likely reflects the rather optimal coordination geometry that is accessible for the *meso*

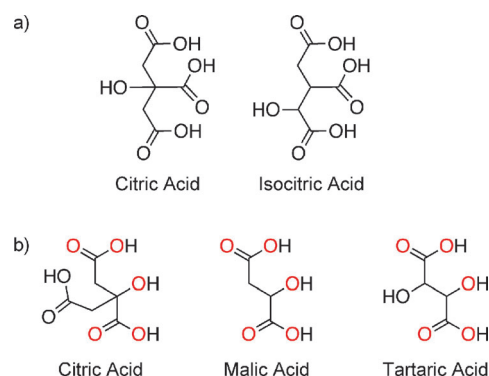


Figure 2. a) The structures of citric acid and isocitric acid. b) The structure of citric acid is shown next to the structures of malic acid and tartaric acid, highlighting their similarity and the presence of functional groups that are capable of coordinating Mg^{2+} ions. Note the different representations in (a) and (b) and in Figure 1.

citrate, but inaccessible for the asymmetric isocitrate (Figure 1 d, 2 a). As a result of its lower chelating ability, isocitrate likely only partially coordinates Mg^{2+} ions, leaving enough unchelated ions to degrade lipid assemblies and catalyze RNA degradation.

What sources of citrate would have been available to an early RNA world organism? Citrate, its precursors, and related compounds could have been delivered by meteorites.^[13] The complete citric acid or related cycles could have formed before the first self-replicating genetic systems existed, but this hypothesis remains contentious.^[14] Instead, related compounds with potential Mg^{2+} chelating properties, such as tartrate (Figure 2 b), could have been generated by simpler prebiotic processes.^[15] Inefficient prebiotic syntheses of such compounds would have created a selection pressure for RNA world organisms to evolve catalytic RNAs that catalyze limiting steps in the synthesis of such protective agents. In this way, sub-sets of the citric acid cycle could have operated in RNA world organisms to later evolve into today's citric-acid-centered metabolisms.

What remains to be done to recapitulate the first putative steps in the origin of life? The influence of citrate on the structure and function of ribozymes is unclear. It is important to determine this influence, as most catalytic RNAs depend on Mg^{2+} ions, and the catalytic function of RNAs is fundamental for RNA world organisms. Furthermore, complete, prebiotically plausible syntheses of nucleosides are yet to be demonstrated. The ribozyme-catalyzed polymerization of activated nucleotides is far from being efficient enough for self-replication, and it is not yet clear how the products of RNA polymerization, highly stable RNA double strands, can dissociate and re-fold to form catalytically active RNAs. The observations made by Adamala and Szostak suggest that the synthesis of citrate or related compounds could have carried a fitness benefit for RNA world organisms. This synthesis may have formed the core of today's citrate-centered metabolism. Unexpected findings like those of Adamala and Szostak continue to propel the field forward, and we may soon be able to greet our distant ancestors during a visit to the laboratory.

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