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RNA World and The Development of RNA Protocells

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Abstract

Origins of life research, also known as pre-biotic chemistry or astrobiology, aims to unravel the mystery of the first cell's origin on Earth. This interdisciplinary field encompasses biology, chemistry, and physics, with the primary goal of understanding the conditions necessary for life to emerge from abiotic environments. The RNA world hypothesis suggests that early life initially used RNA instead of DNA to store genomic information and for enzymatic functions. Protocells, membrane-bound entities with metabolic processes and self-replication capabilities, likely preceded the emergence of true cells. The challenges associated with RNA world is currently an active field of research. Advancements in our understanding of artificial protocells could shed light on the conditions necessary for natural protocell formation as well as the feasibility of an RNA world.

Introduction

Origins of life research (also known as pre-biotic chemistry or astrobiology) is a broad field that is at the intersection of many disciplines including biology, chemistry, and physics. The primary goal of the field is to determine the origin of the first cell on Earth. With this knowledge we would have a better understanding of the conditions necessary for life to form on exoplanets, as well as a fundamentally stronger grasp of what it means for something to be alive.

Chemical evolution is a core concept within probiotic chemistry; it is the process by which simple chemicals can abiotically reproduce themselves and become more complex over time, given the right conditions. For example, lipids left in aqueous solution will clump together to form empty lipid-bilayer membranes that given enough lipids and time can abiotically split themselves and create two 'daughter' membranes (Kauffman et. al., 2013). This is similar in appearance to how cells split themselves during cytokinesis (McIntosh et. al., 2016). Many of the chemical building blocks required for life, such as RNA and amino acids, can be formed abiotically through chemical evolution (Cronin et. al., 1983). These building blocks can be thought of as also being acted on by a force like natural selection; only the most stable isomers will persist long enough to form more complex molecules. The primordial soup model for origin of life takes the idea of chemical evolution, and postulates that if a solution in the early Earth had enough of these building blocks for life; by chance, they could mesh with each other creating the first cell (Perry et. al., 2004). This cell could then go on to replicate itself and through Darwinian evolution begin to diverge into different species.

This first cell lineage however likely went through many changes before it began to undergo differentiation. Pre-biotic chemists often use the term last common universal ancestor, or LUCA to describe the end of this first linage, as all life on Earth should be able to trace its heritage back to this LUCA cell (Penny et. al., 1999). Hud (2013),

uses "my grandfather's axe" as an analogy to describe this process. An axe is passed down as an heirloom in a family and as it is used parts of the axe wear out and are replaced. Eventually, the axe is completely different from its original version. The first cell to be formed from a so-called primordial soup likely went through many such changes before speciation was possible.

RNA world is a hypothesis within origin of life research that postulates that life originally adopted RNA instead of DNA to store genomic information (Bartel et. al., 1999). It also suggests that the first cells used RNA in place of proteins for enzymes. RNA alone can catalyze reactions that produce ester, peptide, and glycosidic bonds which are all very significant for life (Bartel et. al., 1999). RNA as opposed to DNA is much less complex, so if life were to form because of chemical evolution it would make sense that it would utilize chemicals with simpler structures. RNA is only single stranded, while DNA is double stranded. Ribose sugar due to having an extra oxygen is less stable than deoxyribose meaning that it would be easier for it to form in an abiotic system, and lastly the nitrogenous base uracil is structurally simpler than its thymine counterpart, as it lacks a methyl group (Ferenczy et. al., 1986; Perry et. al., 2004).

All life today, with limited exceptions, uses DNA as its primary means of information storage (Hiyoshi et. al., 2011). This suggests that by the time LUCA evolved, DNA had already been adopted by the first cell lineage. Forterre (2002), suggests that the first DNA organism may have been a virus, as it would be impervious to the RNAses of a host cell, giving it a significant selective advantage. Once established in viruses, it would be relatively trivial for DNA to be laterally transferred into the first cell lineage.

One of the largest pieces of evidence in support of the RNA world is the existence of ribosomes. Ribosomes are necessary for cells to undergo translation and produce proteins, a function that is essential for life (Kaeberlein et. al., 2007). Ribosomes are just large ribozymes with protein acting as supports. A ribozyme is a specialized RNA strand that can catalase reactions, like a protein enzyme (Scott et. al., 2007). The universality of ribosomes in modern day life also suggests that ribosomes may have been present in LUCA. Following the concept of chemical evolution and moving backwards (removing complexity) from the LUCA cell it would make sense that one would eventually find a cell within the lineage that exclusively used RNA and ribozymes to carry out cellular processes, or in other words a cell from the RNA world. Ribosomes have been dubbed as being 'molecular fossils' of the RNA world (Bowman et. al., 2015).

More evidence for the RNA world comes from the discovery of non-coding regions in the genomes of modern life that code for RNA products, referred to as non-coding RNA or ncRNA (Eddy et. al., 2001). Detecting such genes is difficult due to their products not being proteins, however it is still possible to screen for these genes (Eddy et. al., 2001). Work by various other researchers to find ncRNA genes suggests that their abundance is much greater than previously thought (Eddy et. al., 2001). Like ribozymes it is possible that these ncRNA genes are another genetic fossil from the RNA world (Eddy et. al., 2012).

There are two camps within prebiotic chemistry on how RNA could have been incorporated into the first cells. One states that it was the direct result of chemical evolution, while the other states that simpler precursors to RNA served as the steppingstones for early life to achieve RNA genomes (Hud et. al., 2013).

A protocell defined broadly is a membrane bound sac that can localize some chemical processes, has a metabolism, and the ability to self-replicate (Bedau et. al., 2009). In chemical

evolution, the formation of protocells would be one of the last steps before the formation of the first 'true' cell. Biologist Joyce (2018), remarks about the difficulty of creating a protocell simple enough to form from chemical evolution, as well as intricate enough to allow for future complexity.

Currently the most advanced artificial protocells lack the ability to undergo self-repair and organize themselves into communities (Green, 2021). Most artificial protocells are developed with the interest of facilitating drug delivery or for other medical purposes (Green et. al., 2021). However, a better understanding of artificial protocells could lead us to a better understanding of the conditions that would be necessary for protocells to form naturally, ultimately giving us a better understanding of the condition's necessary for life to develop.

The purpose of the present study was to explore the viability of RNA based protocells as precursors to modern cells. Through this research, a better understanding of the mechanisms underlying the origins of life may be accomplished.

RNA Protocells

Following the idea of RNA world, before the first cells began to emerge there must have been RNA [or simple RNA precursors] based protocells. RNA was likely incorporated into protocells directly from chemical evolution (Joyce et. al., 2018). Because the cellular membranes of these cells would have been much simpler than modern day cells, they would not contain sophisticated ion transport channels. As a result, it is unlikely that early protocells would have been able to support internal membranes that could specialize in certain functions, as the protocell would have difficulty removing products, and reintroducing reactants into these internal membranes (Joyce et. al., 2018). These protocells could then 'compete' with each other to obtain more amphipathic molecules to further grow their membranes and split into daughter protocells (Joyce et. al., 2018). This competition could give a way for selection to act on these early protocells; where the fittest, and in this case most complex protocells will be able to acquire the most amphipathic molecules and thus create the greatest number of daughter protocells.

Even with a mechanism for selection, RNA protocells still face the challenge of being able to replicate their genomes with only abiotic substrates. Historically mechanisms to accomplish this have been found, but they have high error rates and are incredibly slow; with the extension of just three nucleotides potentially taking up to 24 hours (Bartel et. al., 1999; Joyce et. al., 2018). Another factor that should be noted is that although RNA is single-stranded, to replicate it using RNA as a template you must create a double-stranded RNA. These double RNA strands can reanneal together much faster than template copying can occur, making slow RNA replication even more impractical in an abiotic world (Joyce et. al., 2018).

A novel solution to this dilemma proposes that cells of the RNA world could have used circular RNA chromosomes (Ma et. al., 2013). With the use of a computer simulation a circular RNA chromosome was found to be viable, given the presence of specialized ribozymes (Ma et. al., 2013). The RNA analog to the DNA replication sense strand would need to be easily broken down by ribozymes, with cut sites being between the chromosomes respective 'genes'. Interestingly, replication is the only process that would be necessary for an RNA based protocell to express its gene products; as the now cut RNA sense strand can proceed directly to folding, ultimately creating the desired encoded ribozyme product.

Another promising development in RNA replication speed found that it was possible to use RNA oligomers to catalyze RNA replication (O'Flaherty et. al., 2018). 5'- phosphoro-2-methylimidazoyl-activated trinucleotide

downstream binders acted as great catalysts for RNA template replication, greatly decreasing the total RNA extension time using some test RNA template sequences (O'Flaherty et. al., 2018). The presence of magnesium inside the protocell would be significant for the feasibility of this mechanism, as lipid membranes have relatively low permeability towards oligomers, the presence of magnesium inside the cell significantly increased this permeability (O'Flaherty, 2018).

Conclusion

Great strides have been made in the field of prebiotic chemistry towards gaining a better understanding of LUCA and what life looked like before its inception. We have found many pathways for how macromolecules could have formed via chemical evolution, as well as other life-building blocks (i.e., lipid membranes). However, there are still holes in our understanding on how chemical evolution could go about 'molding' these building blocks into complex protocells, or even the exact conditions on the early Earth needed to permit protocell formation.

The RNA world and how cells could adequately function with RNA genomes also still needs more time to be flushed out. The main hurdle facing RNA cells and protocells alike is the process of RNA only replication. As discussed, many hypotheses have been proposed on how an RNA protocell could be made more viable through different catalysts or RNA arrangements, but none of these have been applied into real RNA based protocells. Further research needs to be conducted to determine the feasibility of these different hypotheses, or perhaps if a combination of these hypotheses can produce a viable cell.

Armed with the knowledge of how to create an artificial protocell, prebiotic chemists could begin to piece together the exact conditions that would have been necessary for a natural environment to form such a cell. A great insight would be gained not only into the history of life on our planet but also into the range of exoplanets that could possibly harbor life.

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