CHAPTER

5

From building blocks to cells

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1 Introduction

Within the scope of astrobiology we are faced with a truly expansive picture of life. A picture that encompasses not only life as it appears before us now, and on our own planet, but also as it would have appeared in Earth's ancient past, and how it might present itself elsewhere in the universe. In this context, we are forced to think deeply and carefully about life at every level of organization: from building blocks to biospheres. In this chapter, we will examine multiple levels of biological organization as we bridge the gap between the abiotic chemical processes discussed in the previous chapters and the emergence of early cellular life. We will begin by discussing the terms that appear in the title of this chapter, "building blocks" and "cells."

What are the building blocks of life? At the molecular scale, all known forms of life rely on the transformation and recombination of a shared set of molecular subunits to support their basic functions. The identity of these molecular subunits is known, and they most certainly should be considered "building blocks" of life, but they only represent the building blocks used by modern cells to sustain life (and for that matter only to sustain Earth life). From an astrobiological perspective, a definition of building blocks that only includes these molecular subunits is incomplete. In astrobiology, we must concern ourselves with both the origin of life and alternative forms of life that may have arisen on other planets. We must consider the molecular building blocks used in the construction of life from non-living systems, and the range of building blocks that may subsequently arise to sustain life in a range of planetary environments.

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What about cells? At the ecological scale, we see that cells, like molecules, serve as subunits of life that are transformed and recombined to sustain living communities. Over the course of evolution, cells have been transformed and recombined to sustain various organisms and ecosystems. And, although we observe a wide range of cell types that have evolved diverse mechanisms of survival, growth, and reproduction, these cells still only represent the units of a mature form of life on a single planet. Once again, from an astrobiological perspective, we are left with an incomplete picture. In the following sections, we will discuss both building blocks and cells in terms of what they do in modern biological systems on Earth, their role in the transition from abiotic chemistry to life, and their potential roles in life elsewhere in the universe.

2 Coming together: From building blocks to protocells

It seems likely that the emergence of life from abiotic precursors required the recombination of modular molecular building blocks, but the identity of these building blocks and the manner in which they were combined could have been quite different from those used by life on Earth today. Understanding the emergence of life requires us to identify which materials were available, how they could have come together, and what functions they needed to support in order to initiate and sustain life.

When considering which building blocks are available in prebiotic environments, there are two primary sources of information that we can examine, astronomical sources of organic molecules (e.g., meteorites, interplanetary dust particles, comets, asteroids) and laboratory experiments that attempt to simulate either astronomical processes or processes at play on the early Earth. The organic molecules present in extraterrestrial materials and laboratory simulations include many small molecules used by modern biology along with a broad suite of additional molecules that may have served as building blocks in earlier forms of life on Earth or in life elsewhere in the universe. And laboratory simulations of early Earth conditions have identified potential routes to the formation of macromolecular building blocks of life. Still, many of the important questions about the connection between abiotic chemistry and cellular life are far from being answered. Which building blocks did emerging life use and from which prebiotic reservoirs were they drawn? Which of these molecules were delivered to the early Earth, which were the results of geological processes, and which were only introduced to biology through pre-existing biological systems? (Fig. 1).

2.1 Building compartments

Cellularity is a fundamental characteristic of contemporary life, and the formation of cell-like compartments was likely a key aspect in the transition from non-living matter to life. For both established and emerging life forms, compartmentalization provides many advantages that can support survival, reproduction, and the ability to evolve. Important advantages provided by compartments include the ability to establish controlled internal conditions shielded from environmental fluctuations, to selectively retain specific molecules that are acquired from the environment or synthesized internally, and to use disequilibria between the interior and exterior to extract energy from the environment. We know that a wide variety of compartments can provide these advantages, as evidenced by the range of observed cell types

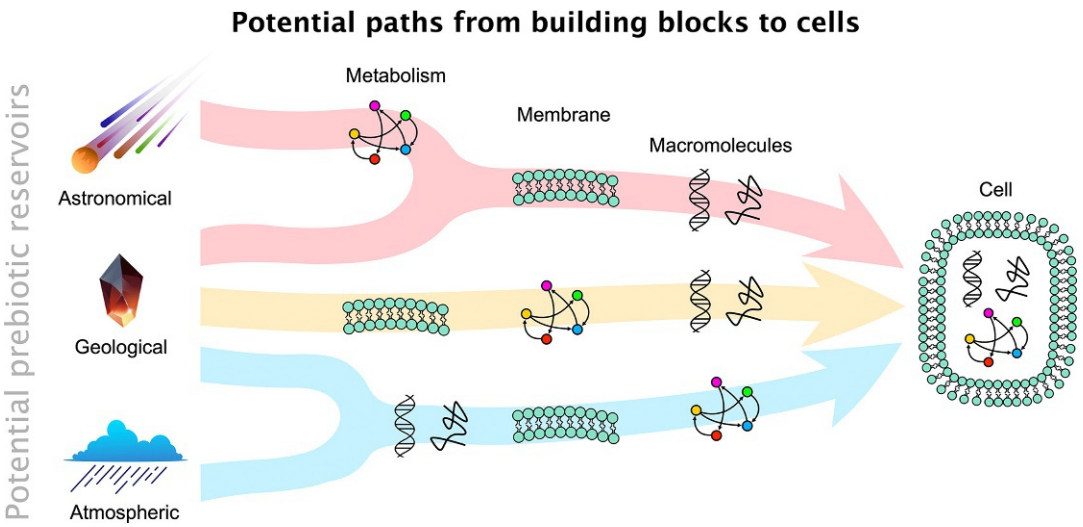
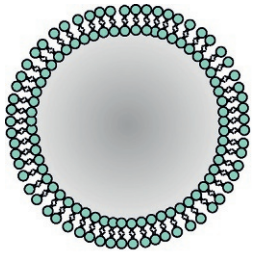


FIG. 1 Potential paths from building blocks to cells. There are several possible paths life may have taken in transitioning from abiotic building blocks to cells. There are many competing theories about how this occurred on Earth or how it may occur elsewhere in the universe. Three paths are illustrated here to serve as representatives of the many different proposed paths from building blocks to cells.

on Earth, and we can imagine several additional types of compartments that may have played a role in the origin of life or may support life on other planets; these include the following: amphiphilic assemblies (Cornell et al., 2019; Jordan et al., 2019; Maurer, Deamer, Boncella, & Monnard, 2009; Maurer et al., 2018; Namani & Deamer, 2008), heterogeneous phases in aqueous solutions (Jia et al., 2019; Koga, Williams, Perriman, & Mann, 2011; Mann, 2012; Pir Cakmak & Keating, 2017; Tena-Solsona, Wanzke, Riess, Bausch, & Boekhoven, 2018), aerosols (Dobson, Ellison, Tuck, & Vaida, 2000; Griffith, Tuck, & Vaida, 2012), and mineral surfaces or pores (Lane & Martin, 2012; Mielke et al., 2010, 2011; Mizuuchi et al., 2019; Tena-Solsona et al., 2018) (Fig. 2).

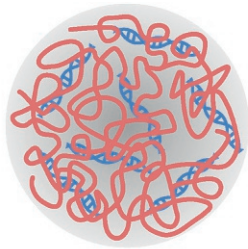
In modern cells, boundaries are formed by the assembly of various complex amphiphilic molecules, which are molecules that are composed of both segments that "love" water and segments that "love" nonpolar organic molecules. The amphiphilic nature of these molecules drives them to assemble into bilayers with hydrophobic interiors that form a barrier to the diffusion of charged molecules in and out of cells (Fig. 2). One of the most important classes of amphiphiles in biology is the phospholipids, which constitute a major component of cell membranes. Phospholipids are made differently in different domains of life. Bacterial and eukaryotic phospholipids consist of fatty acids linked to glycerol-phosphate through ester bonds, while in the membranes of Archaea, phospholipids consist of isoprene chains linked to glycerol-phosphate through ether bonds (Jain, Caforio, & Driessen, 2014). Although they differ in the specifics of their structures, phospholipids in all forms of life share the same general architecture of a hydrophilic "head" group and hydrophobic "tails" which drives their assembly into bilayers. Several other amphiphiles coassemble with phospholipids (e.g., sterols in eukaryotes (Dufourc, 2008), hopanoids in bacteria (Sáenz et al., 2015), and amphiphilic proteins in all three domains of life), and this complex collection of amphiphiles determines the fluidity and permeability of cell membranes.

Liposomes



Amphiphilic molecules can spontaneously assemble to form bilayers that enclose an aqueous solution. The bilayer is a barrier to the exchange of material, particularly charged molecules, and may thereby establish a distinct internal environment that favors processes necessary for survival and reproduction of the enclosed system.

Coacervates

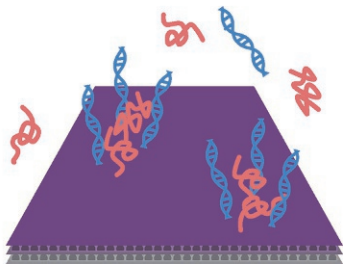


Through liquid-liquid phase separation, macromolecules can spontaneously form dense phases in aqueous mixtures and provide stable compartmentalization without the need for a membrane. Coacervates are permeable to most small molecules and selectively permeable for some macromolecules. This process forms an internal environment much like in liposomes, but without membranes.

Aerosols

Atmospheric aerosols are droplets formed at water-air interfaces that can be of cell-like sizes and persist in air for extended periods of time. Aerosols rich in organic molecules, relying in part on the unique properties of the water-air interface, could have served as small chemical reactors capable of supporting early biological functions.

Mineral surfaces



Surfaces of common minerals can concentrate monomers and macromolecules. Mineral surfaces may have contributed to the organization of molecules by concentrating prebiotic molecules from a dilute aqueous environment into ordered and spatially separated domains of biomolecules.

FIG. 2 A gallery of potential protocells. Several different types of compartments could have served as protocells, the earliest cell-like entities. Here we illustrate four different types of compartmentalization that may have played a role in the origin of life.

Biological amphiphiles are complex and the result of long evolutionary processes. It should therefore come as no surprise that the amphiphiles present in modern cell membranes are not found in astronomical materials nor are they readily produced in simulated prebiotic syntheses. But, if we break up these complex amphiphiles into smaller units, we find that the component parts appear in the inventory of molecules likely available to emerging life. For example, glycerol is available in great abundance in astronomical materials (Cooper et al., 2001) and fatty acids of up to 12 carbon chain length have been detected in meteoritic samples (Lai, Pearce, Pudritz, & Lee, 2018). A variety of other amphiphiles have also been extracted from meteorites (Cooper et al., 2001) and produced in simulations of astronomical environments (Dworkin, Deamer, Sandford, & Allamandola, 2001). And laboratory experiments based on predicted conditions of the prebiotic Earth have generated heterogeneous mixtures of amphiphiles of up to 34 carbon chain length, including fatty acids of up to 26 carbon chain length (McCollom, Ritter, & Simoneit, 1999; Rushdi & Simoneit, 2001).

We certainly do not expect the earliest biological compartments to display the complexity of modern cells. As mentioned before, the complex amphiphiles that dominate modern membranes were likely not available to the earliest forms of life. But many of the simpler amphiphiles likely available to emerging life can readily form vesicles with semi-permeable membranes in aqueous solutions (Deamer & Pashley, 1989; Deamer, 1985; Dworkin et al., 2001), analogous to modern cells. These simpler vesicles would have differed from the phospholipid membranes of modern cells in important ways. For example, vesicles formed by fatty acids are more sensitive to the changes in environmental conditions and more permeable to nutrient molecules including ions, sugars, and nucleotides (Mansy et al., 2008; Sacerdote & Szostak, 2005; Schrum, Zhu, & Szostak, 2010). Nevertheless, simple vesicles can support some of the basic functions of a cell. By maintaining a unique internal environment and a barrier to the loss of useful internally generated products, simple vesicles may have served as protocells that preceded the emergence of more complex cells.

Several forms of compartmentalization could have preceded compartments formed by organic bilayers. These other potential protocellular compartments include coacervates, aerosols, and mineral surfaces (Fig. 2). Membraneless compartments such as coacervates can be formed by phase separation in aqueous systems (Jia et al., 2019; Koga et al., 2011; Mann, 2012; Pir Cakmak & Keating, 2017; Tena-Solsona et al., 2018), and aqueous phase-separated subcellular compartments are common in contemporary biology where they localize RNA and proteins (Hyman & Brangwynne, 2011). Coacervates could have served as protocells composed of high concentrations of polymers which generated localized internal solvent environments that were distinct from their surroundings (Drobot et al., 2018; Strulson, Molden, Keating, & Bevilacqua, 2012). Aqueous aerosols containing high concentrations of organic molecules also could have easily formed on the early Earth and acted as protocells. Within aerosol protocells, condensation reactions at the water-air interface could have led to the accumulation of functional macromolecules, and a proposed surfactant monolayer could have facilitated protocell division (Dobson et al., 2000; Griffith et al., 2012). Compartments based on inorganic components have also been proposed as participants in the earliest biological processes. This includes micro-scale mineral pores (Lane & Martin, 2012; Mielke et al., 2010, 2011) and simple mineral surfaces (Baum, 2018; Mizuuchi et al., 2019), which could have served as protocells, selectively adsorbing chemical species and catalyzing useful reactions.

But what about cells on other planets? For planetary environments that resemble, even remotely, those found on Earth, membrane-bound organic compartments may also be present. The presence of amphiphiles in meteorites indicates that we should expect to find them on other planetary bodies. For planetary systems containing water-rock interfaces, such as alkaline hydrothermal vents, mineral compartments like those proposed for early life on Earth are also a possibility (Mielke et al., 2010, 2011). In more exotic planetary environments, more exotic forms of cells are a possibility. For example, in cold planetary environments rich in organic solvents such as Saturn's moon Titan, computer simulations indicate that amines and nitriles with short hydrocarbon chains, which are known to be present on Titan, can form vesicles in liquid methane (Stevenson, Lunine, & Clancy, 2015). Vesicles like the ones predicted by these simulations could therefore conceivably provide the basis for cellular life in such environments.

As we consider the different types of compartments potentially used by early life, we must ask: how could have these compartments evolved into more complex cells, similar to those we observe today? In order for any population to evolve, there must be diversity for selection to act upon and there must be some form of reproduction that results in offspring that resemble their parents, i.e., some form of heritability. Several of the compartments we have discussed are known to exhibit diversity in their ability to grow and divide. For example, fatty acid vesicles vary significantly in their stability depending on which other simple molecules coassemble with the fatty acids (Cornell et al., 2019; Jordan et al., 2019; Maurer et al., 2009, 2018; Namani & Deamer, 2008) and simple vesicles can vary in their ability to grow and potentially divide depending on membrane composition and the materials encapsulated within them (Adamala & Szostak, 2013; Chen, Roberts, & Szostak, 2004; Wei & Pohorille, 2021). As for heritability, it could have been initially supported by encapsulated nucleic acids as it is in modern cells, but this is not the only possibility. Heritability could have also been supported by the fact that when a parent compartment divides the resulting compartments will inherit their composition from the parent (Gutierrez, Hinkley, Taylor, Yanev, & Cronin, 2014; Segré, Ben-Eli, & Lancet, 2000; Segré, Shenhav, Kafri, & Lancet, 2001; Wu & Higgs, 2008). This so-called compositional inheritance could have supported a primitive form of evolution at the beginning of life that only later gave way to the nucleic acid-based mechanism of inheritance observed in life today.

2.2 Building a metabolism

For life to emerge and thrive in any environment it must have the ability to harness available energy and consistently assemble complex structures from simple and abundant materials, i.e., it must have a metabolism. In modern cells, complex metabolic processes supply the lipids, sugars, and amino acids needed to construct membranes and cell walls, which define the boundary of the cell; and they supply the nucleotide and amino acid monomers needed for construction of functional macromolecules. And in all known life, the transformation of small organic acids lies at the center of these metabolic processes, most notably as part of the tricarboxylic acid (TCA) cycle. Many different proteins must work in concert to support these complex and highly regulated processes that support the ever-changing synthetic needs of the cell, and it is no small task to explain how such a system could have arisen from abiotic processes.

When we look to astronomical sources for evidence of what molecules were likely available to emerging life, we find a wide variety of small molecule metabolites, including many different amino acids (Burton et al., 2014; Glavin et al., 2021; Shock & Schulte, 1990) and several hydroxy- and keto acids, including those present in central metabolic cycles (Cooper et al., 2001; Cooper, Reed, Nguyen, Carter, & Wang, 2011; Pizzarello, Cooper, & Flynn, 2006). The presence of the labile keto acids that are in the TCA cycle is, in some respects, a surprising observation, given the chemical instability of these molecules (Cooper, Ginos, & Meister, 1983). It seems likely that many of these organic acids are continuously produced in complex abiotic reaction networks (Cooper et al., 2011). Understanding these and similar abiotic reaction networks is an important frontier in astrobiology because it could potentially establish continuity between the abiotic synthesis of simple organic molecules and the emergence of complex chemical processes that characterize biological metabolism.

The nature of the earliest metabolic processes has been the subject of much debate. Attempts to understand ancient metabolism are rooted either in the reaction networks observed in modern biological systems or in identifying self-sustaining chemical reactions that could arise from abiotically generated small molecules. Inspired by the centrality of the TCA cycle in modern metabolism, many proposals for the earliest forms of metabolism are closely tied to the TCA cycle and its organic acid intermediates. For example, the reductive tricarboxylic acid (rTCA) cycle, or something very similar, may have been one of the earliest forms of metabolism (Morowitz, Kostelnik, Yang, & Cody, 2000; Muchowska, Varma, & Moran, 2019; Smith & Morowitz, 2004; Stubbs, Yadav, Krishnamurthy, & Springsteen, 2020). The proposed early rTCA cycle is envisioned as an autocatalytic reaction network (a network in which one or more products catalyze reactions in that network) that provides a simple route for converting CO_2 into complex biomolecules. These characteristics make the rTCA cycle appealing as an early metabolic network. Recent work in this area has shown that large portions of the rTCA cycle can occur in the absence of the enzymes that promote these reactions in modern biology (Muchowska et al., 2017, 2019), but a convincing prebiotic route to an autocatalytic rTCA cycle has not been fully demonstrated and the potential for unproductive side reactions may prevent the formation of this and other proposed autocatalytic networks (Orgel, 2008). Several reactions and networks that are part of other modern metabolic processes also may have been precursors to modern metabolism (Goldford, Hartman, Smith, & Segrè, 2017; Keller, Turchyn, & Ralser, 2014). Experiments that explore these metabolic cycles in the context of other co-emerging protobiological components such as vesicles and functional macromolecules are likely to drive important new discoveries in this area.

It is, of course, entirely possible that the earliest forms of life relied on the production of biomolecules through mechanisms much less directly related to modern metabolism than those described in the preceding paragraph (Tran, Adam, & Fahrenbach, 2020). One mechanism of biomolecule synthesis that has long been proposed as important in the emergence of life, and is distinct from modern metabolic cycles, is the formose reaction. The formose reaction is a multistep process in which formaldehyde, the simplest possible aldehyde, serves as a feedstock for the production of complex sugars (Breslow, 1959). In the initial steps of the reaction, one molecule of formaldehyde and one molecule of glycolaldehyde (which can itself be formed from formaldehyde, though very slowly) are consumed to give rise to one molecule of dihydroxyacetone. In the next few steps, the dihydroxyacetone molecule and another formaldehyde molecule are consumed to produce the sugar aldotetrose.

Aldotetrose can then undergo a reaction to produce two molecules of glycolaldehyde. Critically, the net result of these reactions is that two molecules of glycolaldehyde can be produced for every one that is consumed, which means that the formose reaction is autocatalytic and can therefore drive the production of sugars from formaldehyde indefinitely. In addition to generating sugars, the formose reaction can support the production of other important small molecules used by modern life, e.g., amino acids, which may have allowed the formose reaction to support the emergence of life (Omran, Menor-Salvan, Springsteen, & Pasek, 2020; Weber, 1998). If, in fact, early biosynthetic processes did not share features with modern metabolism, then it will be necessary to identify specific mechanisms by which life could have transitioned away from these early processes and toward the metabolic pathways observed in modern cells.

2.3 Building functional macromolecules

Macromolecules support a wide range of functions essential to living cells. These functions include storing and transmitting information, controlling the nature and timing of essentially chemical reactions, and regulating the movement of materials in and out of the cell, just to name a few. The two most important types of macromolecules in life as we know it are proteins and nucleic acids. Understanding how and when these macromolecules became part of the transition from prebiotic chemistry to modern cellular life is essential to our understanding of this process (Fig. 1).

Proteins are built by linking amino acids together in the ribosome. For each protein, the ribosome uses information encoded in a corresponding messenger RNA to determine the order in which the amino acids are connected, a process referred to as translation. The order in which the amino acids are strung together determines the three-dimensional structure of the protein, and that structure, in turn, determines the protein's function. Within modern cells, proteins catalyze chemical reactions, replicate DNA, respond to external stimuli, provide structure, transport materials in and out of the cell and within the cell, and control cell movement. The other key class of macromolecules, the nucleic acids, is built by linking nucleotides together. Their function is also determined by the order in which their monomers are assembled. The templated polymerization of nucleotides to form DNA is how modern cells store and transmit information from one generation to the next. And the templated polymerization of nucleotides to form RNA serves two primary purposes: (i) it can generate messenger RNAs whose sequences are used to determine which proteins are made by the ribosome, and (ii) it can generate RNAs whose function is determined by their three-dimensional structure, a prime example of this being the ribosomal RNAs (ribosomal RNAs make up the core of the ribosome where they catalyze peptide bond formation) (Nissen, Hansen, Ban, Moore, & Steitz, 2000).

Amino acids are a broad class of molecules and only a limited subset appear in proteins. Specifically, α-amino acids (amino group is attached to the α-carbon) are used to make proteins, and among the α-amino acids only a select few are present in proteins. Amino acids that are not used by the ribosome to make proteins also play roles in biology; for example, β-alanine is a β-amino acid that is used to make coenzyme-A, which plays a major role in the TCA cycle and in fatty acid synthesis. Both meteoritic analyses and laboratory simulations

of the early Earth indicate that amino acid synthesis is a common abiotic process. The collection of amino acids identified in meteorites includes a wide diversity of amino acids, more diverse than those with prominent roles in biology today, including several different α-, β-, γ-, and δ-amino acids (Burton et al., 2014; Glavin et al., 2021; Pizzarello et al., 2006; Shock & Schulte, 1990). A wide range of amino acids are also detected in laboratory experiments that simulate both abiotic planetary and interplanetary processes (Cleaves, Chalmers, Lazcano, Miller, & Bada, 2008; Muñoz Caro et al., 2002; Parker et al., 2011). These observations provide strong evidence that amino acids were readily available on the early Earth and are broadly distributed throughout the universe. In either the emergence of life on Earth or life on other planets, abiotically synthesized amino acids were likely available to participate, though the identity of the amino acids that were used may very well differ from those primarily used by the life forms found on Earth today.

In biology, proteins are generated through a complex series of reactions carried out by a specific set of proteins and nucleic acids, which would not have been available during the very earliest stages of life. Although amino acids can be linked together through a simple condensation reaction, this reaction is thermodynamically unfavorable in water. Much of the work in understanding how polypeptides may have formed abiotically has focused on overcoming this barrier to synthesis. It has been shown that heating and other mechanisms for eliminating water drive this reaction forward (Campbell et al., 2019; Fox & Harada, 1958; Rodriguez-Garcia et al., 2015; Surman et al., 2019). However, even when condensation is favored, the formation of condensation products that arrest polymerization can act as a barrier to the abiotic synthesis of polypeptides. One interesting solution to this problem is to include simple hydroxy acids in the reaction, which results in the production of intermediate oligomers made of a mixture of peptide and ester bonds and prevents the formation of dead-end products (Forsythe et al., 2015; Frenkel-Pinter et al., 2019).

Experiments simulating abiotic processes indicate that any polypeptides present in the earliest forms of life were likely short and simple relative to modern proteins. Without the sophisticated coding mechanism of modern translation, early peptides would be limited in length and complexity. Nevertheless, several studies have shown that short and simple peptides can form functional structures. In fact, even a simple dipeptide can potentially drive protocellular growth in membrane-bound vesicles (Adamala & Szostak, 2013; Wei & Pohorille, 2021). It has also been shown that short oligopeptides can assemble into simple membrane channels (Ma et al., 2009; Pohorille, Schweighofer, & Wilson, 2005; Wilson, Wei, Bjelkmar, Wallace, & Pohorille, 2011) and that amyloid fibers are capable of forming functional structures of intermediate complexity (Rout, Friedmann, Riek, & Greenwald, 2018; Rufo et al., 2014). It is also possible for the oligomers of non-α-amino acids to form functional structures (Seebach, Beck, & Bierbaum, 2004), opening the possibility that earlier forms of life or extraterrestrial life could rely on these other types of amino acids to build functional macromolecules. If simple polypeptides did serve as functional macromolecules prior to translation, then we are left to answer the question of: what connection, if any, exists between non-coded polypeptides and the first coded protein products of translation as carried out by the ribosome?

Nucleotides are the monomers that form the information molecules of DNA and RNA. In contrast to amino acids, these building blocks have never been observed in astronomical materials and demonstrating their synthesis through likely prebiotic mechanisms has been

challenging. Nucleotides are often described as being composed of three subunits that include a five-carbon sugar (ribose or deoxyribose), one of two types of nucleobase classes (purines and pyrimidines), and a simple inorganic phosphate. Unlike intact nucleotides, these subunits have been observed in astronomical materials (Callahan et al., 2011; Furukawa et al., 2019; Martins et al., 2008). However, these components play only minor roles as isolated subunits in living systems. They arise only as nucleotide degradation products, and therefore if these isolated subunits played a role in the formation of nucleotides used by early life, then early nucleotide synthesis looked nothing like the process used by biology today. Similar to amino acids, the nucleobases and sugars found in meteorites (Callahan et al., 2011; Martins et al., 2008) and laboratory experiments (Materese, Nuevo, McDowell, Buffo, & Sandford, 2018; Miyakawa, Cleaves, & Miller, 2002; Nuevo, Materese, & Sandford, 2014; Oba, Takano, Naraoka, Watanabe, & Kouchi, 2019) are diverse and only partially overlap with what is found in the biological world. If these molecules are/were used by emerging biological systems it could result in the evolution of nucleic acids with different nucleobases or backbones than those observed in biology on Earth today.

The role of nucleic acids in early life is key to a suite of theories about the emergence of life that fall under the description of "RNA World." These theories posit that RNA played a much larger role in early life than it does now by acting as both the dominant biocatalyst and the repository of genetic information (Benner, Ellington, & Tauer, 1989; Cochrane & Strobel, 2008; Crick, 1968; Orgel, 1968; Robertson & Joyce, 2012; White, 1976). Several characteristics of RNA and modern biology provide support for this view. RNA, like DNA, is capable of storing and transmitting heritable information, and, like proteins, RNA is capable of catalyzing chemical reactions (Fedor & Williamson, 2005; Guerrier-Takada, Gardiner, Marsh, Pace, & Altman, 1983; Kruger et al., 1982; Nissen et al., 2000). RNA's ability to support both catalysis and heritability opens the possibility that a single polymer can support two of life's essential functions. Thus RNA World theories eliminate the need for emerging life to invent two kinds of macromolecules to carry out these two distinct types of function. Additional support for RNA World theories comes from specific features of modern biology, which include the pervasive involvement of nucleotides in enzymatic cofactors (even when the nucleotide moiety is not essential to the chemistry involved) (Benner et al., 1989; White, 1976), a biosynthetic pathway in which deoxynucleotides are produced from ribonucleotides (Freeland, Knight, & Landweber, 1999; Lazcano, Guerrero, Margulis, & Oró, 1988), and the central role of ribosomal RNA in protein synthesis (Nissen et al., 2000).

Broadly speaking, RNA World theories fall into two categories, an RNA-first view and an RNA-later view (Robertson & Joyce, 2012). In the RNA-first view, RNA was the first functional macromolecule and RNAs were formed under abiotic conditions on the early Earth. In the RNA-later view, RNA was either preceded by other macromolecules or if it was the first macromolecule its synthesis was supported by a pre-existing form of biology that did not depend on macromolecules. Several potential routes to the prebiotic synthesis of nucleotides have been identified (Benner, Kim, & Carrigan, 2012; Patel, Percivalle, Ritson, Duffy, & Sutherland, 2015; Powner, Gerland, & Sutherland, 2009), and the primary frontier in this area is in improving our understanding of the likelihood of abiotic synthesis of nucleotides, and more specifically, determining if a prebiotic synthesis is more likely than emergence from a pre-existing biological system. Alternatively, RNA-later theories are faced with the challenge of determining how RNA might emerge within a pre-existing biological system. One possibility for the emergence of RNA from a pre-existing biological system involves so-called pre-

RNAs, i.e., macromolecules that served a similar role to RNA, but were more compatible with prebiotic synthesis. Experiments using alternative nucleobases and sugar moieties have proven that several other polymers, besides DNA and RNA, can support information storage and transmission through base pairing (Hoshika et al., 2019; Yu, Zhang, & Chaput, 2012). It is therefore reasonable to imagine that alternative, RNA-like macromolecules were used by early life on Earth or are currently being used by life on other planets.

2.4 Integration and continuity on the path to protocells

From interstellar ice grains to prebiotic deep-sea vents, it is clear that many different abiotic systems can generate molecular building blocks that are either used by known forms of life, may have been used by past life, or may be used by life on other planets. However, there is far more to understanding the emergence of life than identifying its potential building blocks. After all, life, perhaps more than anything else, is more than the sum of its parts. And because this is so, truly understanding how life first emerged requires an approach that examines life's various building blocks, not only as individual components, but also as parts in a larger integrated system. Encouragingly, there appears to be a growing appreciation among astrobiologists for the need to apply a holistic approach to questions surrounding the emergence of life. Evidence for this trend can be found in recent work that highlights how multiple building blocks can be synthesized simultaneously (Omran et al., 2020; Patel et al., 2015), in studies that couple vesicle growth with either internal RNA replication (Chen et al., 2004) or internal peptide synthesis (Adamala & Szostak, 2013; Wei & Pohorille, 2021), and in emerging computational tools aimed at modeling massive networks of prebiotic chemical reactions (Wołos et al., 2020). And it is by continuing in this direction of increasingly integrated experimental systems that we find our best hope for establishing continuity between our understanding of the living and non-living universe.

3 The path to LUCA: From protocells to cells

All known forms of life can be traced back through billions of years of evolution to a shared ancestral community, a community generally referred to as the last universal common ancestor (LUCA). It may seem an impossible task to uncover the nature of this ancestral community and the communities that preceded it, but the sequences and structures of modern macromolecules are a surprisingly rich source of information that can be used to deduce some of the basic features of LUCA and the microbial communities that preceded it.

There is considerable disagreement about what life might have been like up to and including the time of LUCA, especially with respect to the metabolic processes used by these communities (Berkemer & McGlynn, 2020; Gogarten & Deamer, 2016; Weiss, Neukirchen, et al., 2016; Weiss, Sousa, et al., 2016), but several characteristics are broadly agreed upon. In general, features universally conserved in modern organisms were likely present in LUCA. On this basis it can be safely inferred that the LUCA community had a sophisticated system of coded protein synthesis (Bernier, Petrov, Kovacs, Penev, & Williams, 2018; Fournier, Andam, Alm, & Gogarten, 2011; Harris, Kelley, Spiegelman, & Pace, 2003); had some form of lipid

membrane, and exploited electrochemical gradients across those membranes to synthesize adenosine triphosphate (ATP) using complex membrane proteins (Gogarten & Taiz, 1992; Mulkidjanian, Makarova, Galperin, & Koonin, 2007); used DNA for the storage of genetic information (Freeland et al., 1999); and utilized a wide range of coenzymes to support metabolism (Benner et al., 1989; White, 1976). But what about life before LUCA, can modern biology tell us anything specific about these even older communities? It appears that the answer is yes. Several modern macromolecules retain substructures within their conserved structural cores that likely supported early cellular life prior to lineage-specific divergence among these macromolecules (Gogarten & Taiz, 1992; Li, Francklyn, & Carter, 2013; Petrov et al., 2014, 2015). This allows us to use information embedded within modern biological systems to explore the nature of pre-LUCA life.

3.1 The progenote era and the emergence of translation and the genetic code

The translation system that was present by the time of LUCA was clearly already too complex to have emerged spontaneously from prebiotic components. There must have been a period of biological evolution during which both the genetic code and translation machinery matured to its current form. The early form of life in which the genetic code and the machinery of translation were still emerging is known as the progenote (Woese, 1998; Woese & Fox, 1977). As envisioned by Woese, the progenote era was dominated by lateral gene transfer, so much so that evolutionary histories of organisms did not yet exist. In contrast to the modern world, in which evolutionary innovation tends to become established through selection acting on organisms, in the progenote era innovations would have come in "waves" of genes readily passing between cellular lineages. As a consequence, life's component parts would have initially had independent ancestries separate from other associated parts. Over time, compatibility with the emerging translation system would have provided advantages to individual components, ultimately leading to a single universally adopted system of gene-directed protein synthesis. Once a mature system of gene-directed protein synthesis emerged, cells would have been able to reliably produce a wide range of specialized proteins. The evolution of more complex proteins would become possible, and given the complexity of several universally conserved proteins (e.g., ATP synthase), significant advances in protein evolution must have occurred after the evolution of the core components of translation but before the divergence of the three domains of life.

Ribosomes are complex (>50 macromolecules) and massive (~2 million Daltons) assemblies of RNA and protein molecules (Fig. 3), which are responsible for the coded synthesis of proteins in all known cellular life. Within the ribosomes, there is a core structure that is common to all ribosomes from across the tree of life. This universal core structure closely resembles the structure of modern bacterial ribosomes, for which the common core encompasses ~90% of the RNA component and ~65% of the protein component (Bernier et al., 2018) (Fig. 3). And it is within this universal core structure that many of the clues needed to understand pre-LUCA evolution can be found. Although there are many different theories on the early evolution of the ribosome (Agmon, Bashan, Zarivach, & Yonath, 2005; Belousoff et al., 2010; Bokov & Steinberg, 2009; Calkins et al., 2019; Fox, 2010; Petrov et al., 2014, 2015), each with its own unique set of inputs and assumptions, one thing they hold in common is a

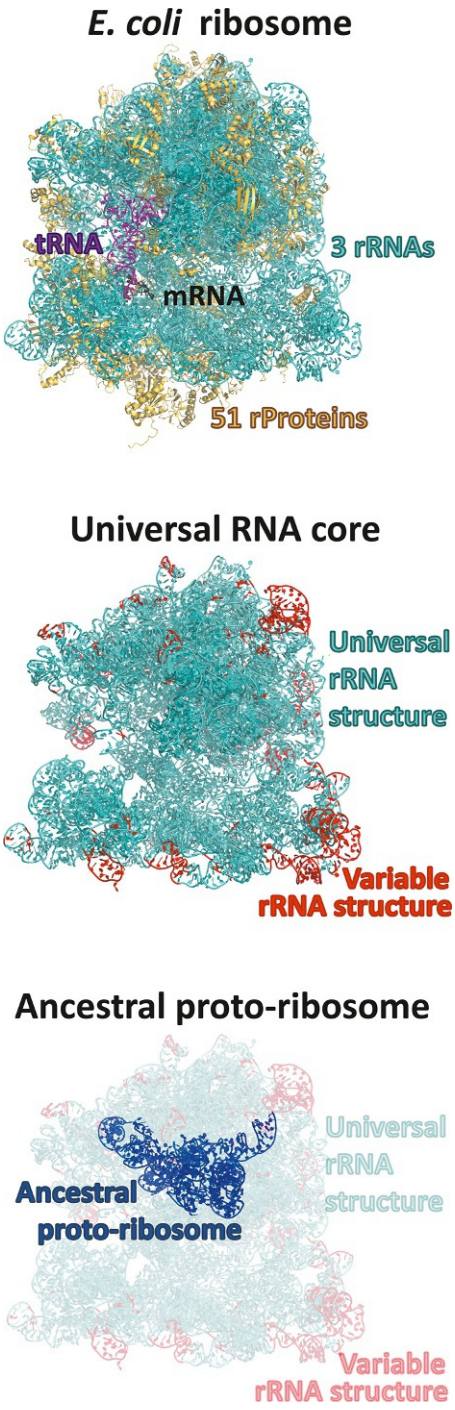


FIG. 3 Using macromolecular structures to work our way back to the pre-LUCA era. At the top of the figure, the structure of the ribosome from the bacterial species *Escherichia coli* is shown. The structure is composed of 3 ribosomal RNAs *(cyan)* and 51 ribosomal proteins *(yellow)*. The ribosome is bound to a transfer RNA *(magenta)* and a messenger RNA *(black)*. The structure was determined using X-ray crystallography (PDB ID: 4V9D). In the middle of the figure, just the ribosomal RNAs are shown. The portions of the ribosomal RNAs that are conserved across the entire tree of life are shown in *cyan*. Those parts of the RNA that are not universally conserved are shown in *red*. At the bottom of the figure, the substructure within the modern ribosome that corresponds to one of the earliest forms of the ribosome as predicted by the "accretion model" of ribosome evolution (Petrov et al., 2014) is shown in solid blue and the rest of the RNA is transparent.

reliance on the preservation of ancestral structures within the core of modern ribosomes. In other words, these models assume that the addition of new components to the ribosome generally did not disrupt the structures that were already present. Across the tree of life, patterns of structural conservation among ribosomes support this assumption. Lineage-specific changes to the ribosome, which have been particularly extensive among the Eukaryotes, have not disrupted the core ancestral structures that are shared across the tree of life (Bernier et al., 2018). We can therefore think of the core of the ribosome as a "living fossil" (Lupas & Alva, 2017), preserving many ancestral features in its molecular structure.

Despite their differences, multiple models of ribosome evolution make similar predictions about the earliest forms of the ribosome (Agmon et al., 2005; Belousoff et al., 2010; Bokov & Steinberg, 2009; Fox, 2010; Petrov et al., 2014, 2015). They generally predict that the earliest forms of the ribosome looked very similar to the site at which peptide bonds are formed between consecutive amino acids during protein synthesis in modern ribosomes, the so-called peptidyl transferase center. And, like the peptidyl transferase center in modern ribosomes, the earliest ribosomes are predicted to have been primarily, if not exclusively, made of RNA (Fig. 3). Evolutionary models disagree on the exact details of the earliest ribosomes and on the order in which the various components came together on the path to modern ribosomes. Fortunately, both the predictions and underlying assumptions associated with these models can and are being put to the test in the laboratory. Predicted ancestral states of the ribosome have been generated and subjected to structural analyses (Hsiao et al., 2013; Lanier, Roy, Schneider, & Williams, 2017), and experimental studies have been used to explore the mechanisms by which RNAs could have grown in size and complexity during the early evolution of life (Mutschler et al., 2018; Plebanek et al., 2019; Popović et al., 2021; Smail, Clifton, Mizuuchi, & Lehman, 2019). Continued efforts along these lines should help to resolve differences among models and will hopefully lead to a clear and accurate consensus view of ribosome evolution.

During translation the ribosome reads information encoded in a messenger RNA's sequence to produce the corresponding protein. To read this code, the ribosome relies on the delivery of amino acids to the peptidyl transferase center by transfer RNAs, and on interactions between the transfer RNAs and the messenger RNA inside the ribosome that ensure the correct tRNA, carrying the correct amino acid, is used to add an additional amino acid to the protein being synthesized. For the genetic code to operate properly, a collection of proteins called the aminoacyl-tRNA synthetases (aaRS) ensure that the correct amino acid is attached to the correct tRNA for delivery to the ribosome. Understanding the evolution of aaRSs is therefore key to understanding the evolution of the genetic code.

As with the ribosome, ancestral forms of the aaRSs can be inferred based on their modern sequences, structures, and functions. Encouragingly, primordial aaRS enzymes, inferred from modern sequences and structures, have been synthesized in the laboratory and shown to be catalytically active (Carter et al., 2014; Li et al., 2013; Li, Weinreb, Francklyn, & Carter, 2011; Martinez-Rodriguez et al., 2015; Pham et al., 2010). The modern aaRSs recognize the correct transfer RNA by interacting with multiple features of the transfer RNA, which in most cases includes specific contacts with the transfer RNA's anticodon (this is the part of the transfer RNA that interacts with messenger RNAs in the ribosome to ensure that amino acids are incorporated into proteins in the order that is encoded in the messenger RNA). Interestingly, predicted ancestral forms of the enzyme, which are truncated to remove their anticodon

recognition domains, retain catalytic activity (Li et al., 2011, 2013; Martinez-Rodriguez et al., 2015; Pham et al., 2010) and some level of specificity (Carter et al., 2014). This observation is consistent with the proposal that ancestral aaRSs relied on a simpler "operational code" embedded within the acceptor stem of the transfer RNAs rather than the anticodon (Carter & Wills, 2018; Schimmel, Giegé, Moras, & Yokoyama, 1993), and it is consistent with the more general proposal that ancestral forms of transfer RNAs lacked the modern anticodon containing stem (Sardesai, Green, & Schimmel, 1999; Schimmel & Ribas de Pouplana, 1995; Widmann, Di Giulio, Yarus, & Knight, 2005). Continued efforts to reconstruct ancestral forms of these aaRS proteins, which define the genetic code, will likely shed important light on how the code emerged. And future research focused on integrating the evolution of the aaRSs with the ribosome and other components of translation has the potential to establish a complete picture of one of life's earliest and most significant innovations.

3.2 The emergence of complex metabolic processes

Many of the details of the metabolic processes at play during the time of LUCA remain hidden, but some features of metabolism at that time can be clearly established by examining modern cells. Notably, we can safely infer that LUCA employed a complex, electrochemically driven membrane protein (ATP synthase) to synthesize ATP as an energy source for the cell. The ATP synthases are key players in extracting energy from the environment; are present in all cellular life; and are highly conserved in structure, function, and sequence, and therefore were undoubtedly present at the time of LUCA (Gogarten & Taiz, 1992; Mulkidjanian et al., 2007; Nirody, Budin, & Rangamani, 2020). The complexity of their conserved structure and function clearly indicates that the ATP synthases present at the time of LUCA were the result of a prior, multistep evolutionary process (Mulkidjanian et al., 2007), with at least one clear instance of gene duplication in which both copies were incorporated into the ATP synthase (Gogarten & Taiz, 1992). In addition to ATP synthesis, this protein assembly can operate in the reverse direction by consuming ATP to drive ion movement in the thermodynamically unfavorable direction, and it is possible that ATP-driven ion transport was its primary function in the pre-LUCA era (Gogarten & Taiz, 1992; Mulkidjanian et al., 2007). Regardless of how the ATP synthases evolved, their presence in LUCA has important implications for the nature of early cells. Specifically, because the ATP synthases rely on a thermodynamically favorable flow of ions across a membrane to support ATP synthesis, we can safely infer that organic cellular membranes that were relatively impermeable to ions existed prior to LUCA (Gogarten & Taiz, 1992; Mulkidjanian, Galperin, & Koonin, 2009).

Although we can learn much about LUCA and the ancestral communities that preceded it by identifying characteristics that are universally conserved across the tree of life, this is not the only way we can use information from modern biology to learn about the nature of early life. Early cells undoubtedly had characteristics that are not universally conserved in modern biology but are still present in some lineages. To identify these characteristics, we can look for genes present in multiple lineages that are far apart on the tree of life and that show up in lineages within both of the two primary branches, i.e., genes present in both the bacteria and the Archaea/Eukarya lineages. The presence of a gene in multiple distant branches of the tree of life could indicate that it was inherited from LUCA, but it could also indicate that

it is a useful gene that has been transferred between lineages after LUCA through horizontal gene transfer. Distinguishing between these two possibilities is an important technical challenge and is often the source of disagreement between predictions about early metabolic processes (Berkemer & McGlynn, 2020; Weiss, Sousa, et al., 2016). Outside of the features that are universally conserved in modern cells, there is little consensus on the features of LUCA and its predecessors. Nevertheless, it seems likely that our picture of early metabolism will become clearer in the near future as genetic data expand to include more genes from more organisms, data curation continues to improve, and novel methods of analysis are applied to these data.

3.3 Integration and continuity on the path to LUCA

The molecular record of life's history on Earth, as preserved in modern biological systems, provides opportunities to understand major evolutionary events, from the emergence of biopolymers, to early cellular processes, to the evolution of multicellularity and beyond. There have been many attempts to infer ancestral biological processes through the analyses of both small molecule metabolites and functional macromolecules. Much of the work in this area has been separately focused on individual molecules, assemblies, or processes, which provide limited insight into the coordinated evolution needed to sustain the complex interconnected molecular systems that drove the proliferation of life on Earth. One of the important new frontiers in this area of research is in the integration of multiple ancestral components to better understand how they were able to work together to support ancestral cells and ecosystems.

4 Conclusion

In the last century, we have learned much about the composition of living systems and the production of life's component small molecules by abiotic processes. The new frontier for this century lies in establishing continuity between abiotic chemical processes and the emergence of biological systems capable of undergoing open-ended Darwinian evolution. As we set out to explore this frontier, we will need to apply highly integrated approaches that consider (i) the interplay of different sources of abiotic building blocks in setting the stage for life to emerge, and (ii) the interconnection of diverse protobiological processes and materials in transforming life into a robust planet-wide phenomenon.

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