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Environmental Adaptation from the Origin of Life to the Last Universal Common Ancestor

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Abstract: Extensive fundamental molecular and biological evolution took place between the prebiotic origins of life and the state of the Last Universal Common Ancestor (LUCA). Considering the evolutionary innovations between these two endpoints from the perspective of environmental adaptation, we explore the hypothesis that LUCA was temporally, spatially, and environmentally distinct from life's earliest origins in an RNA world. Using this lens, we interpret several molecular biological features as indicating an environmental transition between a cold, radiation-shielded origin of life and a mesophilic, surface-dwelling LUCA. Cellularity provides motility and permits Darwinian evolution by connecting genetic material and its products, and thus establishing heredity and lineage. Considering the importance of compartmentalization and motility, we propose that the early emergence of cellularity is required for environmental dispersal and diversification during these transitions. Early diversification and the emergence of ecology before LUCA could be an important pre-adaptation for life's persistence on a changing planet.

Keywords: origin of life, last universal common ancestor (LUCA), environmental adaptation

I. Introduction

Extensive molecular and biological evolution took place alongside significant environmental and planetary shifts during Earth's early history (Figure 1).Life emerged and transitioned from a proposed RNA-based world to the modern DNA/RNA/Protein world very early in planetary history, possibly during a proposed late heavy bombardment, the formation of continental crust, and the emergence of liquid water on its surface, among other geologically significant events (Feulner 2012; Mojzsis et al. 2001; Wilde et al. 2001; Koeberl 2006; Myers 2001; Bowring and Williams 1999; Stern 2005; Tang et al 2016; Brown 2006; Lyons et al. 2014; Allwood et al. 2006; Wacey 2010; Sugitani et al. 2010; McLoughlin et al. 2012; Ohtomo et al. 2014; Schopf 1993; Barboni et al. 2017; Ueno et al. 2006; Tarduno et al. 2006; Badro et al. 2016). This biological innovation against a geologically active backdrop invites consideration of how early life may have interacted with its dynamic planetary setting: in what environment or environments did life originate and make the transition to the modern RNA-DNA-Protein world?

While current evidence about the origin of life and the nature of LUCA allows identification of key evolutionary events between these two points, many of these studies cannot constrain the ecological or environmental history of early life. These studies have focused on the diversification of ancient protein families that became distinct pre-LUCA, including families such as aminoacyl-tRNA synthetase protein classes, elongation factor proteins, ATP synthase proteins, and the elaboration of the ribosomal machinery (e.g., Fournier et al. 2011; Stephenson and Freeland 2013; Fournier et al. 2015a; Fournier et al. 2015b; Iwabe et al. 1989; Gogarten et al. 1989; Hilario and Gogarten 1993; Lanier et al. 2017; Lanier et al. 2016; Petrov et al. 2015; Nagel and Doolittle 1995; Lazcano 1995). Because these very ancient protein families are highly

functionally conserved, and relate to fundamental processes that operate largely independently of the specific environmental context of organismal systems, inferences from their functional history cannot constrain the ecological history of early life. A broader range of investigations is required.

By considering evolutionary innovations relevant to environmental adaptation, we explore the possibility that LUCA was temporally, spatially, and environmentally removed from life's earliest origins in a hypothesized RNA world. Finally, we discuss the potential impact of considering environmental diversification and adaptation as a crucial part of life's earliest evolution on Earth and elsewhere.

II. UV radiation and the evolution of nucleic acid systems

Implications of the RNA world hypothesis. Early life is frequently proposed to have been composed of self-replicating polymers in an "RNA world" that emerged from cycles of prebiotic biochemical synthesis. In the RNA world, RNA formed both the molecule of inheritance and the primary catalyst for biological reactions (Bernhardt 2012; Moulton et al. 2000). Much work has been done on the prebiotic synthesis of the components required to build the RNA world (for a detailed review, see McCollom 2013).

RNA, like all biomolecules, is optimally functional within a specific range of conditions (temperatures, pH, radiation fluxes, etc.). If RNA were a component of life from its origin, limits on the stability and function of RNA are also limits on the environments in which early life could have originated, especially since RNA-based repair mechanisms or stabilizing RNA modifications could only have developed after establishment of RNA as the biomolecule of inheritance and expression. If abiotic or biotic mechanisms *were* available to enhance early RNA

properties beyond what is observed for native RNA molecules today, then the observed properties of modern synthetic or naturally observed RNA systems do not necessarily inform the environments in which life could have emerged. Alternatively, if RNA usurped an earlier, simpler genetic system, limits on RNA stability would still constrain the environments in which this switch could take place, and this additional transition would also then require explanation. In either case, without evidence for such a pre-RNA system or such additional transitions, the simper, uniformitarian hypothesis of a primordial RNA-based earliest life remains the most compelling (Bernhardt 2012). Therefore, the observed biofunctional range of RNA molecules today likely constrains the environments in which early life could have emerged. Since life in an RNA world is inferred to rely on RNA for so many additional functions, such as replication, catalysis, and perhaps even structural roles (Bernhardt 2012), physiological constraints may have been even more stringent than what is observed for individual RNA systems today.

Nucleotide stability under UV constrains life's first use of RNA. Both UV and ionizing radiation damage DNA and RNA. This damage can result in mutations or transcription errors that have significant, deleterious consequences for organisms at the cellular level. Organisms have evolved a variety of mechanisms to repair radiation damage to their genomes, and these mechanisms make it possible for organisms to thrive on Earth's surface, exposed to ultraviolet light from the Sun.

Because radiation damage has strongly negative consequences for the stability and fidelity of genetic material (Ganesan and Hanawalt 2016; Kladwang et al. 2012), radiation exposure would present a significant challenge in the development of an early nucleotide-based system. Organisms have developed a variety of complex mechanisms to repair radiation damage. For example, excision repair of DNA in modern *Escherichia coli* makes use of six proteins; these

proteins must be highly specific for DNA damage in order to avoid mutagenesis (Petit and Sancar 1999). This complexity makes their emergence in the early RNA world implausible. Without a known abiotic mechanism or catalyst to repair radiation damage, any scenario wherein RNA emerged in an environment with significant radiation flux seems unlikely.

UV radiation decreases during the lifetime of a star, and the young Sun would have emitted more of its energy in the ultraviolet spectrum than today (Feulner 2012). The poorly constrained composition of Earth's early atmosphere--missing a protective ozone layer, but possibly containing other UV-limiting agents, such as fractal organic hazes or volcanic sulfur dioxide (Cockell 1998, Wolf and Toon 2010, Westall et al. 2006)-- make it difficult to estimate the total UV flux on the surface of the early Earth. However, it has long been expected that UV flux would represent a challenge for early life on Earth's surface (Sagan 1957; Cnossen et al. 2007). Even current solar UV radiation presents a significant challenge to modern microbes, whether in experiments on Earth or in space (Horneck 1993). Similar damage can occur from sources of ionizing radiation, so an origin of life within a radioactive, mineral-rich environment (e.g., Garzón and Garzón 2001, Draganić et al. 1977) faces similar, if not even greater, challenges as an origin with direct exposure to UV.

Despite UV's potentially deleterious effects on life, UV is thought to have played a significant role in prebiotic synthesis (eg., Ranjan and Sasselov 2016, Beckstead et al. 2016, Sutherland 2017), and plays a key role in the prebiotic synthesis of uracil and cytosine achieved by Powner et al. (2009), an important achievement in understanding the prebiotic emergence of RNA. It is difficult to define a threshold before which UV levels are critical for achieving prebiotic synthesis, but after which UV levels are too damaging to nucleic acids. The unknown atmospheric composition of the early Earth makes determining the exact flux of UV to the

surface difficult. However, it has been observed that wavelengths below 290 nm are most damaging to DNA, and that the Archean Earth would have experienced a higher flux of shorter-wavelength UV radiation though water and atmospheric carbon dioxide may have efficiently blocked wavelengths much below 200 nm (Cockell 1998; Ranjan and Sasselov 2016). Modern experiments exploring the role of UV-mediated prebiotic chemistry often use ultraviolet light of wavelength 254 nm due to the commercial availability of mercury lamps emitting light at this wavelength (e.g., Ritson and Sutherland 2012; Powner et al. 2009; see Ranjan and Sasselov 2016 for an analysis of how the emission spectra of these lamps may compare to plausible conditions on the early Earth). While ultraviolet light between 200-280 nm (UVC) is today blocked by the modern atmosphere and ozone layer, it was likely a significant force on the early Earth (Ranjan and Sasselov 2016, Cockell 1998). The apparent paradox of UV as both a threat to life and aid for prebiotic chemistry should motivate both 1) a search for shielded environments for life's origin and the organization of molecules, and 2) a process by which these shielded environments might be connected to unshielded environments, where prebiotically significant molecules might be assembled with UV, and be delivered to regions with lower fluxes.

Prebiotic synthesis can be considered in terms of processes and environments that serve as either sources or sinks of precursors. Sinks, or areas where precursors accumulate, should be favored as locations for the origin of life, but the precursors in these sinks may be allochthonous, originating at a separate location under different conditions. Prebiotic synthesis with UV was likely important on the early earth, but the persistence, fidelity, and longevity of an RNA polymer in the presence of such a flux is a different question.

We assume that a UV flux sufficient to drive significant prebiotic synthesis would also damage RNA. Therefore, a protected environment that would permit the RNA world to emerge

in the presence of substantial environmental flux at the Earth's surface should be preferred as a potential habitat for life's origin. This has long been one of the arguments against life's origin on a comet or asteroid (Rothschild and Mancinelli 2001), but such a line of reasoning can also be applied to terrestrial habitats. Radiation shielding can be effectively provided by a water column, ice cover, sediment, organic material or other barriers (Cockell 1998), but the importance of UV in prebiotic chemistry suggests that shielded environments which could plausibly be in fairly close contact with unshielded environments should be preferred. For this reason and others (e.g., Jackson 2016; Sutherland 2017), we prefer a scenario where life originates near the surface. Mechanisms to provide shielding at the surface bear further investigation. Clay minerals, in particular, possess interesting properties: the clay mineral montmorillonite has been shown to protect the RNA molecule ADHR1 from UV-induced damage (Biondi et al. 2007) and clay minerals have long been proposed as potential sites for the accumulation and assembly of prebiotically significant chemicals (e.g., Bernal 1951).

As previously mentioned, non-nucleotide-based polymers have been proposed as precursors to a nucleotide-based replicative information molecule. One could speculate that such precursors could be more UV-resistant, and not experience the same environmental restrictions. However, moving from a UV-resilient pre-RNA system to a UV-sensitive RNA system would appear to be selectively disadvantageous. It requires that later stages of early life became more, not less environmentally constrained, which we consider highly unlikely. That being stated, subsequent events in life's early history may also themselves be responses to a changing UV flux; for example, the replacement of RNA by DNA may have been driven by DNA's greater resilience to UV damage (Lazanco et al. 1988).

Inheritance and distribution of UV protection mechanisms. While bottom-up arguments, like the one just presented, consider environmental and other conditions in terms of their plausibility as a starting point for life, top-down arguments consider genetic and other evidence from modern organisms in an effort to reconstruct information about earlier life forms. Analysis of modern organisms has shed light on the probable habitat and lifestyle of LUCA. One type of radiation damage, pyrimidine dimerization, is exclusively caused by UV radiation (Ravanat and Douki 2016). Therefore, the presence of cellular repair mechanisms specific for pyrimidine dimer repair, also known as photoreactivation (PHR), is an indication that an organismal lineage experiences exposure to ultraviolet radiation.

Proteins in the photolyase family use near-UV light as an energy source for photoreactivation (Heijde et al. 2010). Several categories of photolyases exist, each specific to a particular type of UV lesion (Heijde et al. 2010). Photolyases occur widely across the tree of life, in all three domains, and emerged early in the history of life (Rambler and Margulis 1980). Phylogenetic analyses indicate that photolyase was present in the lineage of the Last Universal Common Ancestor (LUCA) and that the observed paraphyletic distribution across the three domains is due to widespread gene loss (Heijde et al. 2010, Kanai et al. 1997). Not only is the damage repaired by PHR caused by ultraviolet light, but the photolyases responsible for PHR rely on near-UV light to drive the repair reaction. The presence of photolyase in LUCA is a strong indication that LUCA and/or its ancestors contended with both UV and near-UV light, suggesting a surface environment. Because the recombination of DNA and the repair of ultraviolet-induced damage to DNA use some of the same enzymes, it has been suggested that the evolution of these repair mechanisms preadapted organisms for DNA recombination (Marguilis 1981; Witkin 1969; Bernstein 1977).

An environmental adaptation to UV flux. These observations suggest that between the origin of life and LUCA, life diversified into environments unlike the one in which it originated. Evolutionary changes across this interval should be considered in light of this environmental adaptation, which may hold explanatory power for additional physiological and genetic changes during the early evolution of life.

More subtly, this example of environmental adaptation hints at an important role that cellularity played in early evolution. In order to diversify across environments and move away from their environment of origin, organisms would need to be able to contain their genetic information and metabolisms within a portable envelope. This is consistent with a pre-LUCA emergence of cellularity, with the cell as the fundamental organizing unit of life.

III. Evidence for a cold-start origin of life

Early psychrophily. Both a "hot-start" and a "cold-start" origin of life have been proposed (Russell and Hall 1997 Forterre 1996; Bada et al. 1994). The chemistry of minerals and of the serpentinization process near deep sea vents, the traditional understanding of a hot early Earth, and the observation that most of the most deeply branching lineages in the bacterial and archaeal phylogenies are thermophiles or hyperthermophiles have made the "hot start" model attractive (Russell and Hall 1997, Forterre 1996, Martin et al. 2008).

However, with a faint young sun, the surface of the early Earth may have been cold enough for significant ocean ice to form (Kienert et al. 2012; Feulner 2012), and the environment of the early Earth may have been more temperate than the traditional model suggests (Hren et al. 2009, Vincent et al. 2004). Other differences between the modern Earth and the early Earth, such as a faster rotation and less continental area, have been suggested to heighten the polar-equatorial

temperature gradient between Earth's faster rotation during the Hadean and early Archean (Feulner 2012). These observations suggest that low-temperature scenarios for the origin and early evolution of life are environmentally plausible, while the hot-start model presents challenges for the stability and accumulation of prebiotically significant compounds (Islas et al. 2003).

The cold-start model is not necessarily incompatible with models that suggest life emerged near hydrothermal vents. Hydrothermal fluids can be vented through lower temperature off-axis systems; for example, the Lost City Hydrothermal Field produces water with temperatures as low as 10 degrees C, which then mix with even cooler ocean water (DeChaine et al. 2006, Martin et al. 2008). Additionally, surface hydrothermal systems can directly interface with or be in close proximity to ice (Bargar and Fournier 1988; Lipenkov et al. 2016). Plausible scenarios for life's origins in cold, subsurface environments can inform the search for life on icy worlds such as Europa, Enceladus, and possibly Ganymede (Chyba and Phillips 2001; McKay et al. 2014; Chyba and Hand 2001).

Prebiotic amino acid synthesis and use of amino acids. Experiments by Miller and colleagues (Bada et al. 1994, Levy et al. 2000) showed that prebiotic synthesis of key amino acids occur most readily under cold conditions. Both eutectic freezing and ice surfaces may concentrate prebiotic materials (Miyakawa et al. 2002, Price 2007; Trinks et al. 2005). Work on the addition of amino acids to the canonical repertoire suggest that the first amino acids used by life are also consistent with a temperate, rather than hot, origin of life (Brooks et al. 2002, Zeldovich et al. 2007, Fournier and Gogarten 2010).

Thermostability of RNA, nucleotides and ribozymes. As with UV radiation, limits on the stability of RNA and nucleotides constrain the environmental boundaries for the origin of life.

RNA folding, which is required for RNA to function, is strongly limited at higher temperatures (Moulton et al. 2000). The rapid rates of hydrolysis of nucleotides at temperatures above 0 degrees C would make accumulation of these nucleotides difficult in a hot-start scenario (Kua and Bada 2011; Levy and Miller 1998, Kawamura et al. 2005). In the laboratory, cold conditions in icy environments promote the activity of synthetic RNA polymerase ribozymes, while also protecting the macromolecule from hydrolysis. While colder temperatures would slow down RNA polymerization rates, the enhanced stabilization and reduced degradation of the ribozyme counteract this effect, permitting synthesis of remarkably long molecules (Attwater et al. 2013; Attwater et al. 2010). Also, the first ribozymes were likely short; an experimental short ribozyme evolved from a longer parent ribozyme was observed to have a lower temperature optimum (Akoopie and Muller 2016). This may be a general feature of these systems. While chemical reactions occur more quickly at higher temperatures, early life was likely under less selection from competition, and not under rate-related pressure to the same degree as modern life. While the temperature conditions at the origin of life remain controversial, the evidence for a colder start is compelling.

IV. An early origin for membranes

Temperature, membranes, and motility. One of the most important properties of extant life is the cell membrane, the boundary between an organism and its environment. Within this boundary, the outside world is present in a representational form, abstracted to chemical signals and gradients, and complex sets of molecular and inter-molecular states. Consistent with this, the catalogue of the minimal characteristics of a primitive "organismal" biological system are typically identified as a molecule or molecules encoding genetic information; catalysts

responsible for the synthesis and replication of the system; and a membrane containing these components and interfacing with the outside world (Szostak et al. 2001). Under the RNA world hypothesis, RNA serves as both a molecule of inheritance and forms the catalysts needed for its replication (Bernhardt 2012); however, a membrane, as a barrier between organism and environment, is still required for homeostasis, metabolism, and motility. Additionally, compartmentation is required for Darwinian evolution; as membranes keep closely related molecules in proximity to each other, preferential selection can occur (Szostak et al. 2001).

Timing of membrane emergence. The relative timing of cellular membranes' appearance remains controversial. Work focusing on early metabolism has suggested that life developed within mineral structures that sheltered self-replicating genetic molecules until membranes developed at a later point as a biological invention of this system (Martin and Russell 2003). Alternatively, "membrane-first" models propose that the first lipid membranes contributing to cellular biological systems arose abiotically and independently of heredity and metabolism. Membrane-like vesicles form in solutions containing fatty acids, which were abundant from both endogenous and exogenous sources to the early Earth (Deamer et al. 2002). These bilayer membranes are capable of growth and division through abiotic processes, including shear forces and concentration changes; in the course of growth they transition from small and spherical to long and filamentous (Zhu et al. 2012, Budin et al. 2012). It has been suggested that these membranes pre-date the origin of life, and that this compartmentation permitted not only the Darwinian evolution of early protocells (Szostak et al. 2001; Chen et al. 2004; Bianconi et al. 2013), but also possibly the concentration necessary for prebiotic chemical reactions to occur without dilution and dissipation (Deamer et al. 2002).

Working backward from extant membrane-dependent biological systems is also informative. ATP synthase protein complexes utilize a proton or sodium gradient across a membrane, requiring an enclosed lipid bilayer—i.e., cellularity—to function. Therefore, enclosed cellular membranes must have existed at the time of the origin of ATP synthase complexes. Work on the early evolution of ATP synthases has demonstrated that the catalytic and non-catalytic rotor subunits of these systems evolved via gene duplications in a pre-LUCA lineage. By extension, this evidence, together with analysis indicating that signal recognition particle RNA was present in LUCA, strongly supports cellularity and cell membranes emerging prior to LUCA (Gogarten and Taiz 1992; Kawamura et al. 2005; Hoeppner et al. 2012; Harris et al. 2003; Jékely 2006).

Membranes and temperature. Vesicles composed of fatty acids, fatty alcohols or fatty-acid glycerol esters are stable from 0 to 100 degrees C (Mansy and Szostak 2008). Stability across these ranges could permit temperature fluctuations to drive cellular processes, with template-copying chemistry enhanced at lower temperatures and strand-separation and nutrient uptake enhanced at higher temperatures (Mansy and Szostak 2008). This broad range of temperature resilience would also mean that early organisms could inhabit and/or disperse into environments with a wide range of temperatures before membrane stability became a limiting factor.

The behavior of vesicles under different temperature and concentration regimes also illustrates how replication, metabolism, and heredity could have become membrane-associated. Within an enclosed area subject to temperature variation, the concentration of vesicles, the permeability of membranes, and the stability of genetic material would change as a function of temperature. Mansy and Szostak (2008) proposed that, during higher temperature periods,

increased membrane permeability would permit an influx of nucleotides while strands of genetic material would separate. With a shift to lower temperatures, replication of the genetic material and growth of the vesicle would be favored. Through these temperature-driven processes, early cells could complete a primitive "cell cycle" of growth, strand separation, template replication, and division without requiring dedicated cellular mechanisms. Experimental fatty-acid vesicles like those used by Mansy and Szostak (2008) are highly thermostable and retain internal RNA and DNA olgionucleotides at temperatures from 0° to 100°C. Because of this broad thermostability range, the properties of nucleic acids, rather than of membranes, may be more informative about the temperature of the early RNA world. However, membranes' stability range may provide other environmental constraints; some work has indicated that membrane self-assembly, as well as RNA polymerization, is negatively affected by ionic solute concentrations much lower than those of the modern ocean, suggesting fresh-water environment for early life (Monnard et al. 2002; Deamer et al. 2002). While these authors suggest that this requires landmasses hosting freshwater bodies for life's earliest environments, we observe that ice melts to form freshwater, and that freshwater bodies can be found on, inside, and at the base of ocean ice or glaciers. Even in the absence of continental landmass, an early Earth might host volcanic islands with freshwater bodies and/or glaciers, and fairly long-lived ocean ice systems.

Concentration changes can also drive similar primitive homeostatic behavior (Engelhart et al. 2016). Eventually these tasks could be taken over by cellular mechanisms driven by metabolism as these early cells evolved. Selection for enzymatic mechanisms to facilitate these processes could be continually present if the temperature oscillations were variable in intensity, intermittent, or varied within gradients.

Genomic reconstructions. Reconstructing the gene content of LUCA also provides some hints as to the nature of life at the time of this entity. Such inferences are challenging and inherently limited, given LUCA's antiquity and the complications caused by horizontal gene transfer (HGT) and lineage-specific gene loss since LUCA. However, there is good evidence that LUCA's genome was small but functionally fairly comprehensive (Mirkin et al. 2003; Ranea et al. 2006; Koonin 2003). Conservative structural comparison of proteins across the tree of life has identified many ancestral protein superfamilies that were likely present in LUCA, and functional analysis of these superfamilies indicates that LUCA had nearly all the essential functional systems present in modern organisms (Ranea et al. 2006). Comparative analysis of RNA families indicates that, while most RNA families are domain-specific, several RNA families have patterns of vertical inheritance consistent with their origin pre-LUCA. This indicates a fairly complex protein synthesis system existed pre-LUCA (Hoeppner et al. 2012). Analysis of the distribution of conserved genes indicates that LUCA may have had a genome of as many as 600 genes, with the suggested set again nearly sufficient to sustain an organism (Mirkin et al. 2003). Since many genes present in LUCA could have been subsequently lost in descendant lineages, these genome size estimates based on comparative genomics may be best understood as lower-bound estimates. The complexity and comprehensiveness of LUCA's genome indicates that Darwinian evolution was in effect at an organismal scale (Lombard et al. 2012). Darwinian evolution at the organismal scale requires that both lineage and heredity—a specific line of evidence strongly suggesting compartmentation at the time of LUCA (Lombard et al. 2012; Ranea et al. 2006).

The nature of compartmentation. Archaea and Bacteria use unrelated pathways to synthesize chemically distinct membrane phospholipids; therefore, it has been proposed that LUCA did not use phospholipids in its membrane, and may have used mineral structure as a

means of compartmentation or been totally acellular (Koonin and Martin 2005, Koga et al. 1998). Mineral compartmentation is attractive in part because it could resolve the conundrum of Archaea and Bacteria's different membrane lipid biosynthesis mechanisms (Martin and Russell 2003). However, such a scenario requires a sessile progression from the origin of life to LUCA. Alternatively, the early appearance of membranes would imply that life was potentially motile very early in its history and physically independent of mineral structures.

The observation of the different membrane chemistries between Bacteria and Archaea could also be explained by takeover of analogous systems at the Domain level. Consistent with this, recent work suggests that a "hybrid" membrane of both bacterial- and archaeal-type lipids is stable and could have been used by LUCA, and thus a Domain level takeover event is at least biophysically plausible (Shimada and Yamagishi 2011). Additionally, such a takeover event is already hypothesized to have occurred at least once, as Eukarya, like Bacteria, use ether-linked phosopholipids, but are more closely related to Archaea. However, it remains challenging to distinguish between such takeover events and the independent origin of two systems at the base of the tree of life.

Hydrothermal vents, long regarded as a promising environment for the origin of life, produce minerals with compartment-like structures (eg., iron monosulphide precipitates), in addition to creating redox, pH, and temperature gradients that might be conducive to the origin of life (Martin and Russell 2003). Modern hydrothermal vent systems typically last between 1 and 10,000 years (Lombard et al. 2012). If life pre-LUCA was not free-living and was intrinsic to mineral structures within hydrothermal systems, all pre-LUCA evolution would have been tied to a specific site, and thus would have had to occur in a relatively short time (Lombard et al. 2012). It remains possible that hydrothermal vents on the early earth were longer-lived, or that

these proto-biological evolutionary processes did occur in a sufficiently short period of time. However, this remains a substantial constraint imposed by the model. More direct challenges to the mineral compartmentation model persist: namely, the observation that this model cannot couple membrane division with the replication of biological components, which is required for Darwinian evolution (Lombard et al. 2012), and the difficulty in explaining how ATP synthases and a component of the protein conducting channel emerged prior to LUCA if LUCA relied on mineral compartmentation (Gogarten and Taiz 1992; Kawamura et al. 2005; Jékely 2006). We propose that the inferred environmentally distinct states of a primordial RNA world and LUCA constitute an additional challenge to this scenario, as these infer environmental dispersal and diversification, necessitating motility that is not possible in highly localized mineral compartments dependent on a direct hydrothermal source for both compartmentation and energy.

With primordial vesicle motility, proto-organisms could disperse, encounter, and adapt to a variety of environments. Therefore, environmental adaptation could have been an important force even in life's earliest history; the thermostability of vesicles across a range of temperatures indicate that membrane stability would not limit environmental dispersal. Rather, dispersal and adaptation would be constrained by limitations on the other biological or pre-biological components of the system.

V. Evidence for a mesophilic LUCA

Mesophily of LUCA. Work by Woese and colleagues to sequence small-subunit rRNA genes from prokaryotes and eukaryotes culminated in the identification of Archaea, Bacteria, and Eukarya as the three Domains of life (eg., Woese 1987). This comprehensive view of the tree of life has given rise to speculation about characteristics of LUCA at the base of the tree. LUCA has

often been considered a thermophile or hyperthermophile (e.g., Woese 1987). The apparent thermophily of both the last Bacterial and last Archaeal ancestors do appear to support this hypothesis, but recent work suggests that the last Bacterial and the last Archaeal ancestors separately adapted to thermophilic environments, perhaps reflecting a global thermophilic bottlebeck (Boussau et al. 2008). Several compelling lines of evidence make the case for LUCA's mesophily, rather than thermophily or hyperthermophily.

G+C content of LUCA rRNA. G+C nucleotide pairs are more thermostable than A+T pairs due to an additional hydrogen bond. Due to their additional thermostability, G+C nucleotides are present more frequently in the rRNA of thermophiles and hyperthermophiles than in the rRNA of mesophiles (Galtier et al. 1999; Groussin et al. 2013). This "molecular thermometer" allows prediction of a prokaryote's optimal growth temperature based on the G+C content of its rRNA. Reconstructions of LUCA rRNA have shown a G+C content consistent with mesophily (Galtier et al. 1999; Groussin et al. 2013).

Amino acid composition of LUCA proteins. Reconstructed LUCA proteins also bear a signature of mesophily. Abundance of the amino acids I, V, Y, W, R, E, and L (denoted as IVYWREL) within protein sequences correlates strongly with an organism's optimal growth temperature (Zeldovich et al. 2007). The IVYWREL content of LUCA proteins, based on phylogenetic analysis, is consistent with an optimal growth temperature below 50 degrees C, indicating a mesophily.

Integrating these inferences with other biological records of environmental adaptation is a promising approach to constraining the environment of life's origin. If a cold origin to a mesophilic LUCA represents the path taken by life during its early evolution, then the earliest

metabolisms, peptides, and gene families should be compatible with a cool environment, with thermophilic and hyperthermophilic adaptations appearing later.

The case of reverse gyrase. Reverse gyrase is a topoisomerase unique to thermophiles and hyperthermophiles (Forterre 1996; Forterre 2002) that converts closed, circular DNA to a positively supercoiled form (Kikuchi and Asai 1984). Reverse gyrase is both critical and unique to hyperthermophilic life (Forterre 1996; Forterre 2002, Kikuchi and Asai 1984, Heine and Chandra 2009). While one experiment suggested that the thermophile *Thermococcus kodakarensis* can survive without reverse gyrase, growth in mutant strains was significantly inhibited and not observed at temperatures >90 degrees C (growth is observed in wild-type strains at up to 100 degrees C) (Atomi et al. 2004), supporting the conclusion that reverse gyrase and hyperthermophily are closely linked.

The evolutionary history of reverse gyrase is well constrained because of its structure: it includes two distinct regions, homologous to a topoisomerase and a helicase, respectively (Forterre 1996, Forterre et al. 1995). Either reverse gyrase was formed by the fusion of genes encoding of a topoisomerase and a helicase, or topoisomerases and helicases evolved through the splitting of a more ancient gene encoding reverse gyrase (Forterre et al. 1995).

The latter scenario presents a problematic evolutionary narrative, in which modern helicases and some topoisomerases are the descendants of reverse gyrase, requiring that reverse gyrase predated even more fundamental and essential cellular mechanisms like membrane transporters, V and F-ATPases, and elongation and initiation factors for protein synthesis (Forterre et al. 1995). In contrast, the evolutionary history revealed if reverse gyrase is the result of the merger of a pre-existing helicase and topoisomerase is parsimonious and compelling. This scenario requires that helicases and topoisomerases had already diversified to some degree prior

to the development of reverse gyrase. Therefore, the organisms that used these pre-existing helicases and topoisomerases, by necessity, did not use reverse gyrase. While it is possible that early hyperthermophiles may have used a different, primitive form of reverse gyrase, or not used reverse gyrase at all, there is no evidence for such a takeover.

This scenario is further borne out by the observation that reverse gyrase appears to have evolved in Archaea and spread into Bacteria through horizontal gene transfer (HGT) (Brochier-Armanet and Forterre 2006). This would mean that LUCA did not possess reverse gyrase. While the same caveats as above apply--perhaps there was a primitive form of reverse gyrase or LUCA was a hyperthermophile that did not require reverse gyrase—the simplest explanation is also the most consistent with a mesophilic LUCA.

VI. Observations on adaptions from a cold start to a mesophilic LUCA.

Modern psychrophily is taxonomically shallow. About 85% of the modern biosphere is permanently exposed to temperatures below 5 degrees C, including environments in terrestrial permafrost, glaciers, sea ice, cold water lakes, and marine sediments. Within these environments, psychrophiles are abundant and taxonomically diverse (Margesin and Miteva 2011). Yet there are no major microbial classes that have psychrophily as a shared character. In other words, unlike thermophily, psychrophily appears to be taxonomically shallow and evolutionarily young. Psychrophiles have several specific adaptations to low temperatures that have apparently occurred independently across lineages. While no work has yet dated the divergence times of known psychrophilic clades, it may be that the transience of the global cryosphere on geological timescales has played a major role in the history of psychrophily. For example, there may have been little or no cryosphere to speak of during the Paleocene-Eocene

Thermal Maximum (PETM) 55.5 million years ago (Sluijs et al. 2006) and extant psychrophile clades may all have diverged after this event. Nevertheless, observed adaptations of psychrophiles today illustrate the types of physiological constraints low temperatures pose for fundamental biological processes, and can serve as analogs for evaluating which of these processes could have emerged in low temperature conditions early in the history of life.

The RNA world works at low temperature—does anything else? As discussed above, prebiotic synthesis, the origin of life, and the emergence of the RNA world plausibly occurred at low temperatures, but it is important to assess how other elements of molecular biology would fare under similar conditions.

Due to decreased kinetic energy, chemical reactions generally proceed more slowly at cold temperatures. Slower metabolic and reproductive rates would not be problematic in an early, cold biosphere where all organisms were similarly limited, but warmer temperature regimes would provide the advantage of faster reaction rates, if organisms could tolerate the heat. This differential would provide selective advantage for being metabolically active at higher temperatures. The earliest ribozymes may have been short and simple, in part due to the availability of prebiotic chemicals (Akoopie and Muller 2016) as well as the fidelity of early RNA replication systems, which was probably lower (Poole et al. 1998). As RNA transcription fidelity improved, longer and more effective ribozymes, which would have more elaborate, stabilizing secondary and tertiary structures, could have emerged. If these longer ribozymes had higher temperature optimums, as in an experimental case by Akoopie and Muller (2016), this could have enabled organismal dispersal to warmer locations. Polypeptide-based enzymes with optimal activities at higher temperatures could have contributed to this transition. This proposed environmental dispersal of early life is illustrated in Figure 2.

There are few direct clues on how the transition from RNA to DNA could be related to temperature changes. Polymer stability and fidelity is perhaps the most important trait for the molecule of inheritance, independent of environmental temperature, and the transition from RNA to the more stable DNA can be understood primarily in these terms. It is unclear how increasing temperature could have driven this transition, as double-stranded DNA is not more thermally stable than double-stranded RNA (Wienken et al. 2011).

VII. Discussion and looking ahead.

Sequence and environmental reconstructions for studying life pre-LUCA. The species-level history of life prior to LUCA is obscure, because all lineages, by definition, coalesce at LUCA. We do not know what bottlenecks, mass extinctions, or radiations took place prior to LUCA's emergence. Much as is the case with biota across the fossil record, diversified groups could have coexisted for an extended period of time in the same ecosystems, with only eventual replacement. However, the pre-LUCA histories of some gene families can be examined (eg., Mirkin et al. 2003; Ranea et al. 2006; Gogarten and Taiz 1992; Fournier et al. 2015a; Fournier et al. 2015b). Some genes, present at the time of LUCA but not present within the genome of LUCA, were likely transferred to the ancestors of extant groups by lineages that existed alongside LUCA but became extinct more recently (Fournier et al. 2015b). Detection and characterization of these genes could provide an additional insight into the physiology of these lineages, and would further probe the history and environmental diversity of life pre-LUCA. If their deep topologies can be resolved and rooted with confidence, reconstructing their ancestral sequences would permit analysis of their likely environments using molecular thermometers or

other approaches. This could permit investigation of environment and lifestyle for pre-LUCA lineages, provided that enough conserved sites have endured to preserve a detectable signal.

It seems likely that population bottlenecks and adaptive radiations occurred following major innovations between the origin of life and LUCA. Developments such as the use of polypeptide-based enzymes over RNA world ribozymes may have offered such a comparative advantage that organisms with these innovations rapidly multiplied, replacing their progenitors. On the other hand, changing planetary conditions or sudden catastrophic events may have caused mass extinctions or tight bottlenecks through which life, reduced to a small number of survivors, narrowly survived. Both scenarios—successful adaptive radiation and survival of a bottleneck— create "winner takes all" scenarios in which survivors' characters and traits may be "locked in" for future generations, i.e., genetic drift. These events, perhaps in tandem with environmental adaptation, could have precipitated important changes in life between its origin and LUCA. Studies of the early Earth that not only identify and quantify probable events that would have led to these bottlenecks on the early Earth, but that also use these events to contextualize the early evolution of life or traits of life that may be the "locked in" residues of such events, may be productive.

Prebiotic synthesis: production versus accumulation. The model proposed here suggests that UV light is likely important for prebiotic chemistry, but that life, emerging from the products of these reactions, originated under UV-shielded conditions. The model calls for production and accumulation of prebiotically significant molecules in separate environments. Identifying plausible environments (e.g., an icy lake) where such environments could naturally occur and be connected by abiotic processes will help to contextualize where, how, and at what pace these processes may have occurred. Quantifying the potential fluxes of material produced,

transported, and stored in different environmental settings (e.g., the relative abilities of ice and clay minerals to concentrate chemicals at a range of temperature and salinity) will help to constrain probable environments. The role of abiotically emergent vesicles, which are likely to have emerged early and which may have played an important role in collecting and concentrating prebiotic chemicals, across these environments should be considered.

Studies of Europa, an icy moon of Jupiter, have suggested that products from its irradiated, oxidized surface are placed into contact with its liquid inner ocean through processes operating on geologically short timescales such as diapirism, cryovolcanism, fracturing, and reservoir collapse (Brown and Hand 2013; Sparks et al. 2017). Europa's dynamic, icy landscape and interior may provide a model for the processes that can assemble and concentrate prebiotic chemicals relevant to the origin of life. Ice sheet processes and dynamics may have played a role in the origin of life on Earth as well.

Environments on the early Earth: a checkerboard of habitability? Evidence that LUCA was separated in time, space, and environment from the origin of life requires an explanation. An actualistic approach—one that prefers explanations centered on processes that have been observed in modern systems—suggests that environmental dispersal and adaptation is consistent with the observed disjunction. It can also serve, at least in part, to explain the acquisition of complex biological characters that are firmly established by the time of LUCA. Given the range of possible habitats on the early Earth and the evidence for ancient lineages living alongside LUCA and its proximal descendants, such diversification seems probable. Whether or not this diversity represented the establishment of a true "ecology" that contributed to life's resilience and survival, and became extensive enough to be integrated with global geochemical cycles remains an open question. While our discussion here does not require that ecosystems and

ecology emerged prior to LUCA, this model is consistent with the possibility, which should be further considered.

Recent work suggesting that the early Earth was more temperate (Valley et al. 2002; Hren et al. 2009), and the flux of impactors smaller and less damaging than previously thought (Boehnke and Harrison 2016; Abramov and Mojzsis 2009) predicts that, as a whole, the early Earth could have provided a range of environmental niches stable for millions of years. Some models also indicate that the early Earth experienced a more extreme temperature gradient from equator to pole, due to Earth's faster rotation and/or a smaller land to ocean ratio, than it does now (Feulner 2012). Habitable environments may have been more common and spread across a significant portion of the early Earth's surface. These environments were perhaps not isolated oases, but a checkerboard of interconnected habitable zones. Even within a single location, a variety of environments, defined by differences in depth within the sediment, rock, or water column, would exist. Exploring habitability and diversity within a single location consisting of multiple environments, e.g., the layers of microbial mats or within ocean sediments, has been a rewarding approach in microbial ecology (e.g., Jangir et al. 2016; Stal 1995), and targeting environmental gradients for detection of has been promising (Cabrol et al. 2007). This framework may be useful in designing new approaches to and tests of prebiotic synthesis and early biological systems.

Implications for astrobiology. Recent findings suggest that Mars may have been habitable for hundreds of millions of years early in its geological history (Grotzinger et al. 2014). If this is indeed the case, environmental diversification may have occurred early and rapidly in a hypothetical history of life on Mars, as we propose it did on Earth. It would also follow that Martian life or evidence of Martian life may now exist only within refugia environments unlike

the one in which Martian life originated. Our search for evidence of life on Mars need not be constrained to probable environments of origin, but should focus on those environments most likely to capture and preserve traces of life.

Better understanding the impact of environmental limitations on the origin and early evolution of life, such as UV flux, can help determine the habitability of planetary bodies that are even outside our own solar system, such as planets around stars with high UV fluxes, e.g., the TRAPPIST-1 system (Gillon et al. 2017, O'Malley-James and Kaltenegger 2017).

Environmental adaptation as a preadaptation for the persistence of life. If environments on the early Earth were widespread, varied, and linked, it seems likely that first environmental adaptations could have been the result of life's expansion into adjacent and unoccupied environments along light, temperature, nutrient, or other gradients. In the absence of competition and with so much unexplored fitness landscape, this may have happened rapidly, and may parallel other rapid explosions of diversity in life's history (e.g., the Cambrian explosion). This diversification could allow life to weather changes in local or planetary conditions pre-LUCA, and could have allowed complex communities to develop. Rapid diversification and complex communities might be an important pre-adaptation for life's long-term persistence as part of a dynamic planetary system, in part because more diverse communities are more resilient to environmental change (Reusch et al. 2005; Hughes et al. 2008; Peterson et al. 1998). Life's ability to diversify and to adapt to new environments, which we suggest emerged very early in its history, has permitted the continual reworking of function and physiology for ~4 Ga on a changing planet.

VIII. Conclusion.

Between the origin of life and LUCA, life likely diversified into environments unlike the one where it emerged. Inferences based on the effects of, and adaptations to, temperature and UV radiation suggest these factors played critical roles, with RNA-based living systems originating in cold, UV-shielded environments. By the time of LUCA, organisms had diversified into environments including surface locations of moderate temperature. This diversification would have been facilitated by the early emergence of cellularization. Such a scenario reconciles sets of bottom-up (e.g., experimental prebiotic chemistry) and top-down (e.g., phylogenetics) observations that support conflicting narratives of life's origins, if constrained to a singular primordial location. This scenario also supports LUCA's identity as one population of cells among many cellular or protocellular systems that likely coexisted across diverse ecologies. This schema is consistent with a very early emergence of ecosystems as an important force in life's history. Early diversification could be a vital pre-adaptation for life's persistence on a changing planet.

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Figure captions.

Fig. 1 Earth's first two billion years saw dramatic planetary change alongside biological evolution. The early emergence of persistent liquid water on Earth's surface, and recent evidence suggesting that Earth remained habitable during the Late Heavy Bombardment, indicates that life may have emerged during the Hadean period, and Paleoarchean evidence suggests a diverse biota had developed at least 3.5 billion years ago. The complex and changing young Earth system may have been coupled with life early in their shared history

Fig. 2 Biological evolution permitted environmental expansion during life's earliest history, even as changing environmental conditions or unoccupied niche spaces in uninhabited environments drove biological evolution. Branches here illustrate hypothetical lineages and are not meant to represent the true phylogeny

Fig. 1

Planetary change

millions of years ago

Models of solar evolution predict a faint young sun for Earth's first two billion years

Oxygen isotopes from Jack Hills zircons suggest persistent liquid water on Earth's surface

Proposed Late Heavy Bombardment

Oldest metasedimentary rock preserved

Onset of plate tectonics with modern subduction style

Moon-forming impact within 60 million years of Earth's formation

Oldest continental crust preserved

Proposed "whiffs of oxygen"

Strong evidence for a geodynamo by 3.45 Ga

?

4567   4000   3500   3000   2500

Hadean	Archean

Eoarchean | Paleoarchean | Mesoarchean | Neoarchean

Biological evolution

Origin of life in RNA world

LUCA

Last Bacterial, Archaeal ancestors

Proposed isotopic evidence for metabolism

Putative microfossils from the Apex Chert

Transition to DNA-protein world

Isotopic evidence consistent with methanogenesis

The Strelley Pool Chert records a stromatolite reef

Fig. 1 Earth's first two billion years saw dramatic planetary change alongside biological evolution. The early emergence of persistent liquid water on Earth's surface, and recent evidence suggesting that Earth remained habitable during the Late Heavy Bombardment, indicates that life may have emerged during the Hadean period, and Paleoarchean evidence suggests a diverse biota had developed at least 3.5 billion years ago. The complex and changing young Earth system may have been coupled with life early in their shared history

Fig. 2

time

RNA world   RNA-protein world   RNA-protein-DNA world   DNA-protein world with modern cellularity   Present

UV-shielded environments

environment

UV exposure

1. Origin in a cold, UV-shielded environment

2. Biological evolution permits environmental expansion and is driven by environmental change. Temperature tolerance increases

3. Mesophilic, UV-tolerating LUCA emerges in DNA-protein world with modern cellularity

4. HGT occurs between lineages before, during, and after the time of LUCA, including extinct lineages

5. Last Bacterial and Archaeal Ancestors. Thermophilic bottleneck?

LUCA

HGT

Dotted lines represents the range of inhabited environments Overall environmental diversity increases with time

Fig. 2 Biological evolution permitted environmental expansion during life's earliest history, even as changing environmental conditions or unoccupied niche spaces in uninhabited environments drove biological evolution. Branches here illustrate hypothetical lineages and are 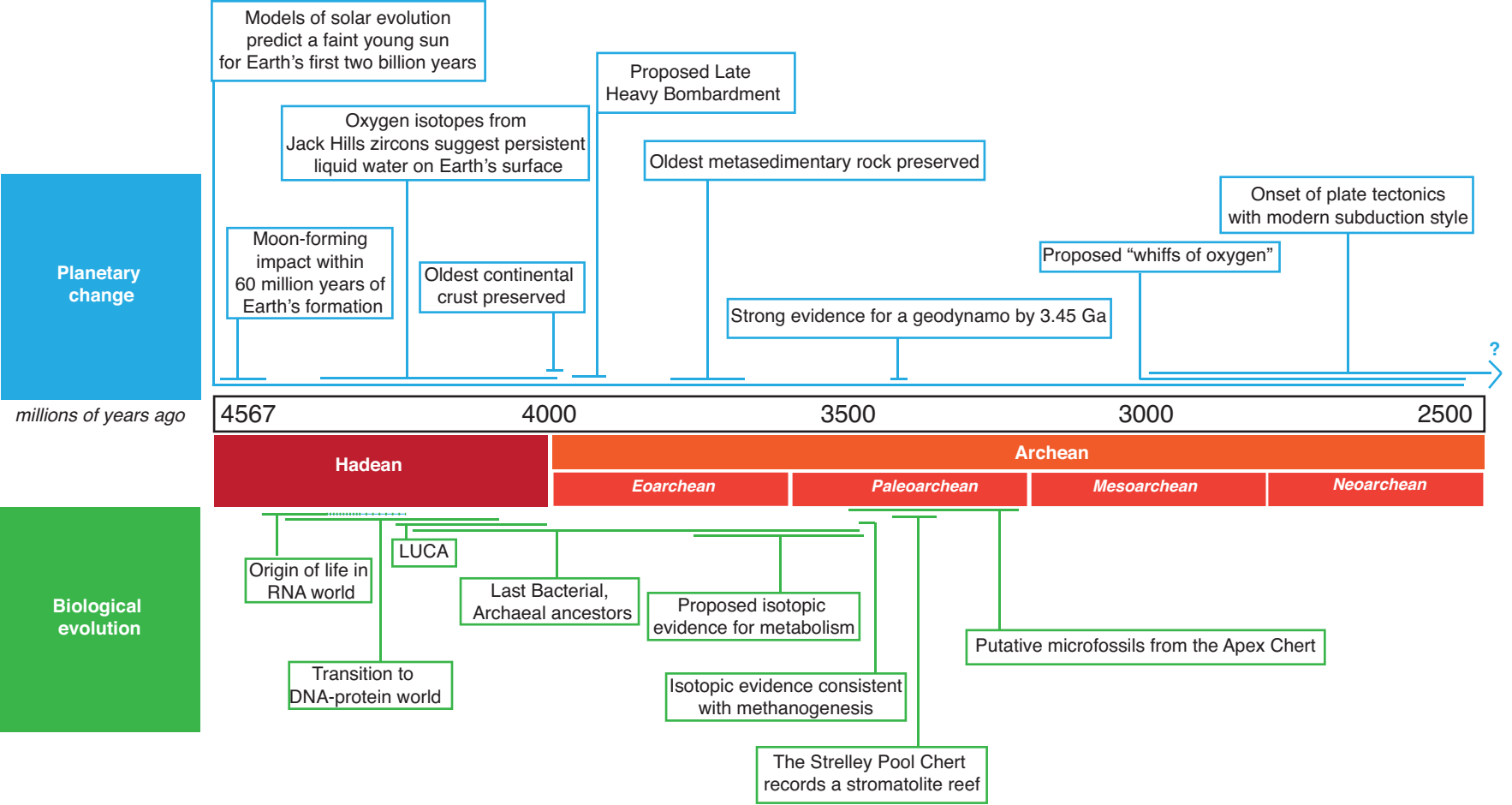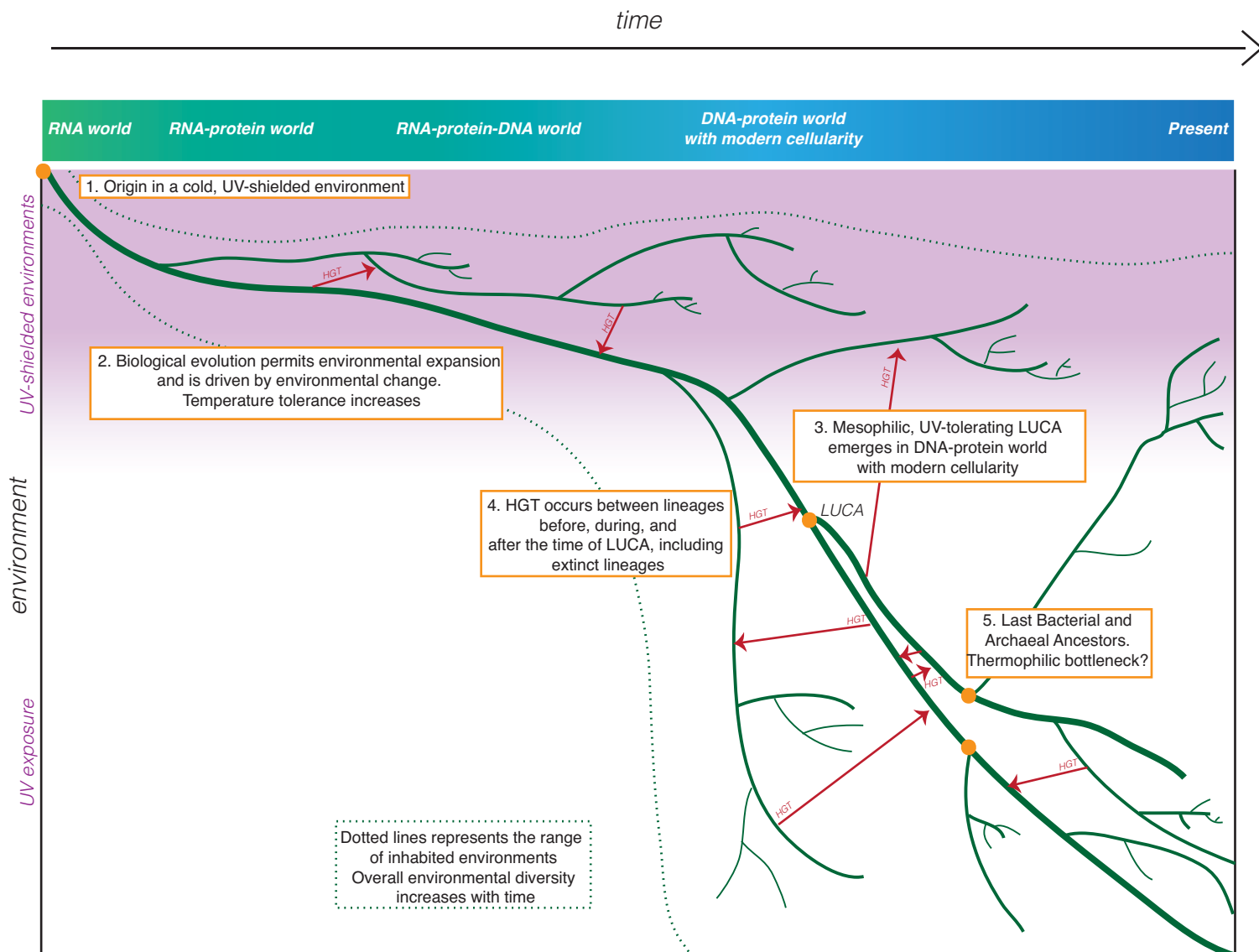not meant to represent the true phylogeny