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# The origins of life: novel perspectives over an old problem

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## Abstract

Life probably appeared on Earth around 4 billion years ago and was globally diffused within the next 500 million years. It is debated whether life emerged in a superficial terrestrial environment, as generally maintained by "primordial-soup" models, or in association with submarine hydrothermal vents. Simulation experiments show that abiotic formation of simple organic molecules from $CO_2$ and $H_2$, and of peptides from free amino acids is thermodynamically favoured under hydrothermal-vent conditions. In contrast, proposed pathways of abiotic synthesis of nucleotides and RNA fit better with superficial scenarios subject to wet/dry cycles. The "RNA World" hypothesis posits that a critical step towards life was the appearance of RNA enzymes (ribozymes) that catalysed RNA replication and random α-amino acid polymerization. The narrative presented here suggests that ribozyme interaction with peptides underpinned the emergence of populations of "protoribosomes" and virus-like RNA "protochromosomes" depending on each other for replication and subject to Darwinian evolution. The establishment of a genetic code coupled RNA and peptide evolution. RNA chaperoning of peptides positively selected self-folding peptide sequences, thus paving the way to the evolution of biologically active protein architectures. Association of informationally interlinked protoribosomes

and protochromosomes with liquid-crystal bilayers produced the first protocells, self-replicating structures that evolved an increasingly complex metabolism by replacing ancestral ribozymes with more efficient protein enzymes. The addition of the Sec translocon machinery and of integral lipid-synthesizing enzymes converted self-assembled protomembranes into hereditary encoded membranes. The transition to DNA as the repository of genetic information established the genotype-ribotype-phenotype tripartite organization of modern cells. Cell evolution was the first and most conspicuous expression of ecological inheritance in life history. Primordial-soup models favour a heterotrophic ancestral metabolism, whereas the alkaline-vent scenario points to a chemioautotrophic origin. In line with the latter, phylogenomic analysis suggests that the last universal common ancestor (LUCA) was a $CO_2$-fixing, $H_2$-dependent, $N_2$-fixing, thermophilic organism. Life utilized only a tiny part of the virtually limitless space potentially available in carbon chemistry, probably due to the need to control harmful spontaneous reactions in the overcrowded intracellular environment.

**Keyword:** Cell evolution, Genetic membranes, Genotype-Ribotype-Phenotype, Last Universal Common Ancestor, RNA World, Translation machinery

## Riassunto

La vita è probabilmente apparsa sulla Terra intorno a 4 miliardi di anni fa e si è globalmente diffusa nei successivi 500 milioni di anni. Non è chiaro se essa sia nata in un "brodo primordiale" sulla superficie del pianeta, o in associazione con fumarole alcaline nelle profondità marine. Simulazioni *in vitro* mostrano che la formazione abiotica di piccole molecole organiche da $CO_2$ e $H_2$, e di peptidi da aminoacidi liberi è termodinamicamente favorita nelle condizioni esistenti nelle fumarole alcaline. In contrasto, i meccanismi proposti di sintesi abiotica di nucleotidi e RNA sono meglio compatibili con ambienti di superficie soggetti a cicli di idratazione e disidratazione. L'ipotesi del "Mondo dell'RNA" assume che un evento cruciale nell'evoluzione della vita sia stata l'apparizione di ribozimi, molecole di RNA capaci di catalizzare la polimerizzazione casuale di aminoacidi in peptidi e la replicazione dell'RNA. La narrativa qui presentata suggerisce che l'interazione tra ribozimi e peptidi abbia prodotto popolazioni di "protoribosomi" e "protocromosomi" simili a virus a RNA, reciprocamente dipendenti per la replicazione e soggetti a evoluzione darwiniana. L'affermazione del codice genetico accoppiò poi l'evoluzione dell'RNA a quella dei peptidi. L'interazione peptidi-RNA favorì l'emergenza di sequenze peptidiche capaci di ripiegarsi in strutture regolari, dando così avvio all'evoluzione di architetture proteiche complesse. RNA e peptidi interconnessi dal codice genetico si associarono a protomembrane di origine abiotica, producendo protocellule, strutture autoreplicanti che svilupparono un metabolismo di crescente complessità rimpiazzando i ribozimi ancestrali con più efficienti enzimi proteici.

L'incorporazione di un sistema Sec di traslocazione e di complessi enzimatici per la sintesi di lipidi convertì le protomembrane in "membrane genetiche" trasmesse di generazione in generazione. L'adozione del DNA come veicolo dell'informazione genetica al posto dell'RNA introdusse l'organizzazione tripartita in genotipo, ribotipo e fenotipo che caratterizza le cellule moderne. L'evoluzione della cellula fu il primo e più cospicuo caso di eredità ecologica nella storia della vita. I modelli basati sul concetto di "brodo primordiale" favoriscono un metabolismo ancestrale di tipo eterotrofo, mentre lo scenario delle fumarole alcaline postula un'origine chemioautotrofa. In linea con l'ipotesi chemioautotrofa, l'analisi filogenomica suggerisce che l'ultimo progenitore universale comune (LUCA) fosse un organismo termofilo che fissava $N_2$ e $CO_2$ utilizzando $H_2$ di origine geochimica. La vita ha esplorato solo una minuscola parte dello spazio virtualmente illimitato della chimica del carbonio, probabilmente per la necessità di controllare dannose reazioni spontanee nel sovraffollato ambiente intracellulare.

**Parole chiave:** Evoluzione cellulare, Genotipo-Ribotipo-Fenotipo, Membrane genetiche, Mondo dell'RNA, Apparato di traduzione, Ultimo Progenitore Universale Comune

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## 1. Introduction

*Unraveling the origins of life is an appealing problem per se; nobody really cares, but everybody would like to know (Nicolas Galtier)*

The origins of life has been the central problem of biology since when Louis Pasteur and John Tyndall definitely disproved spontaneous generation in the mid-19th century. Panspermy hypotheses postulating an origin of life from extra-terrestrial spores do nothing more than transferring the problem to another place in the Universe (Chandra 2011). Panspermy cannot be rejected by principle but, in the almost complete absence of scientific evidence, it is generally assumed that terrestrial life originated on Earth.

Extant living beings, although as diverse as to warrant two or three Domains of life to categorize them (da Cunha et al. 2018; Williams et al. 2020), share a set of fundamental traits, most notably the translation apparatus, the genetic code and the use of DNA for storing genetic information. No less revelatory is the universality of many if not all existing protein folding domains and of fundamental protein architectures. The simplest explanation for this shared biology is that all living organisms inherited it from a Last Universal Common Ancestor (LUCA), implying that

essential hallmarks of extant life were most probably established long before the appearance of LUCA.

Direct and indirect evidence suggests that life was already present on Earth around 4 billion years ago (Ga), only about 600 million years after the formation of the Earth-Moon system (Dodd et al. 2017; Bell et al. 2015). The Earth surface was at the time almost entirely covered with an ocean because continental landmasses were scarcely developed and mostly submerged, with only volcanic edifices exposed to the atmosphere (Kamber 2015). It is estimated that the total volume of the ocean was initially about twice as much as today, the missing water having been transferred to the mantle by subduction of hydrated oceanic crust and in minor part lost by photodissociation (Genda 2016). The atmosphere consisted mainly of nitrogen, carbon dioxide and water, with minor amounts of hydrogen, methane and sulphur dioxide of geochemical origin, and traces of nitrogen oxides produced from nitrogen and water under the action of lightning. Because carbon dioxide was much more abundant than today, the ocean was acidic, with a pH in the range 5-6. Sodium chloride was directly added as vapour to the Earth atmosphere after the formation of the Moon and then, in cooler condition, dissolved in the ocean, which therefore was saline from the beginning (Camprubì et al. 2019).

The origins of life coincided with the origins of the cell, the essential unit of life. The cell is traditionally viewed as a duality of phenotype and genotype, the first providing material and energy for reproduction, the latter providing information. Ribosomes and RNA mediate the interaction between genotype and phenotype under the rules of the genetic code. The translation apparatus is generally considered as a part of the phenotype, yet in 1981 Barbieri described it as a third fundamental component of the cell, which he called the *ribotype*, and proposed that life ultimately derived from a self-replicating ancestral ribotype. Major steps in Barbieri's scenario of cell evolution were the emergence of self-replicating ribosome-like structures, or "ribosoids", their aggregation into nucleolus-like "nucleosoids", the acquisition of a bounding membrane, and the incorporation of DNA, initially as a parasite and then as the repository of genetic information. Based on his model, Barbieri described the cell as *a colony of ribonucleoproteins engaged in producing other colonies of ribonucleoproteins*. We will see in this review that recent work revives in part Barbieri's model.

Ribosomes are essential to protein synthesis and, indirectly, to RNA synthesis. Yet, although a pre-formed complement of ribosomes is transmitted to daughter cells at each cell division, ribosomes cannot be considered as hereditary structures because ribosomal RNA and proteins self-assemble into new ribosomes without requiring pre-extant ribosomes as templates. This is instead much the case for biological membranes, supramolecular architectures that carry essential information for making new membrane. Cavalier-Smith (2001, 2004) distinguished *hereditary* and *non-hereditary membranes*. The first are membranes

vertically transmitted from cell to cell and amplified upon request by addition of new components (lipids and proteins) in their pre-existing molecular framework. The process involves integral protein complexes including lipid-synthesizing enzymes and Sec translocons (du Plessis et al. 2011; Nyathi et al. 2013). A hereditary membrane can originate only from a membrane of the same type. The hypothetical loss of a hereditary membrane would be lethal because the cell could no longer recover it. Major instances of hereditary membranes are the cell membrane in all organisms, the outer membrane in gram-negative bacteria (negibacteria), the thylakoid membrane in cyanobacteria and chloroplasts, the ER, and probably the Golgi apparatus in eukaryotes. Non-hereditary membranes derive from a hereditary membrane and, if lost, can be recovered. Examples in eukaryotes are the nuclear envelope, which re-forms after mitosis from endoplasmic reticulum, and the bounding membrane of lysosomes, endosomes and vacuoles, deriving from the Golgi complex and continuously recycled. Akin to Cavalier-Smith's concept of hereditary membrane is the notion of "encoded membrane" introduced by Lane and Martin (2012) to denote a key step in pre-cellular evolution in which key components of membranes started being encoded by genes.

Membrane compartmentation is so central to the functioning of the cell that membrane-bound protocells and a rudimentary form of membrane heredity have been suggested to predate genetic heredity (West et al. 2017).

Compartmentation is, however, only one of the conditions required for the emergence of life. Living systems exist in a physical state that is extremely far from thermodynamic equilibrium or, equivalently, has extremely low entropy and thus low probability. To maintain this condition, living systems need coupling with an external disequilibrium, namely a supply of "negentropy" (Branscomb and Russell 2018a). The loss of thermodynamic coupling is what we call "death", dead organisms rapidly and irreversibly degrading to equilibrium. A third condition is a source of organic compounds providing the starting molecular framework for the construction of biological architectures. Last, living systems need a sink to get rid of waste without affecting chemical disequilibria.

## Glossary

**Annotation (of genes)**. The process of identifying the locations of coding regions (genes) in a genome (structural annotation) and determining what those sequences do (functional annotation). Once a genome is sequenced, it needs to be annotated to make sense of it.

**Asgards.** A recently proposed superphylum of archaea, resolved as the closest prokaryotic relative of eukaryotes in some phylogenetic trees (Williams et al. 2017, 2019)

**Autotrophy.** From Greek *autòs* "self" and *tròphein* "to nourish"). Organisms that obtain more than 50% of their carbon from $CO_2$ (or bicarbonate) are autotrophs. Organisms that obtain less than 50% of their cellular carbon from $CO_2$ are heterotrophs (Schönheit et al. 2016). This conventional definition takes considers that carboxylation reactions are universally present in all extant organisms, including humans.

**Chemioautotrophy.** A type of autotrophic metabolism that uses energy from the oxidation of inorganic compounds. Photoautotrophs instead use light energy.

**Darwinian evolution.** A population of self-reproducing systems evolves in a Darwinian way when the relative frequency of alternative hereditary traits arising from random mutation changes across generations, either in response to competition for resources or by chance.

**Hadean.** The geological eon preceding the appearance of the first known rocks (4.6-4.0 billion years ago).

**Heterotrophy.** By convention, heterotrophy is a form of metabolism in which less than 50% of total carbon is obtained from $CO_2$ or bicarbonate (cf. autotrophy).

**Homologous genes.** Genes derived by mutation from the same ancestral gene. Homologous genes are recognizable from sequence similarities.

**Horizontal gene transfer (HGT)**. Transfer of genetic material from organism to organism independent of kinship relationships. HGT is distinct from vertical inheritance, viz. parent-to-offspring gene transfer. Genes acquired by HGT do not reflect common ancestry and need to be excluded from phylogenetic analysis. HGT frequency is relatively high in prokaryotes, much lower in eukaryotes.

**Orthologous genes (orthologs).** Genes derived from a common ancestral gene by a speciation event. Orthologous genes control the same function in related species.

**Paralogous genes (paralogs).** Genes derived by duplication of an ancestral gene in the same organism and diverged for novel, sometimes subtly different functions. Genes coding for different forms of tubulin in eukaryotes, or of collagen in animals are examples of paralogous genes. Molecular phylogeny compares the sequences of orthologous genes in separate lineages whilst excluding paralogous versions.

**Protein domain.** A segment of a polypeptide chain that folds autonomously from the rest of the filament. A domain may have a length of 40 to 350 amino acid residues. Single polypeptide chains may be comprised of one to several domains. Domains in multidomain proteins may affect each other's folding. Domain shuffling, viz. random recombination of gene sequences coding for domains belonging to different proteins, is an important mechanism of biological innovation. Most existing proteins are mosaics of domains from other proteins.

**Secondary (protein) structures.** Three-dimensional arrays of local peptide segments deriving from the formation of hydrogen bonds between the carbonyl oxygen and amide nitrogen of the peptide backbone. The most common secondary structures found in native peptides are alfa-helices and beta-sheets. Amino acids vary in their ability to form secondary structure elements. Proline and glycine are sometimes known as "helix breakers" because they disrupt the regularity of the alfa helical backbone.

## 2. The environmental context

The debate around the origin of life has long revolved around "primordial-soup" scenarios originally proposed by Alexander Oparin and J.B.S. Haldane and supported by experimental work of Stanley Miller (Miller 1953). Traditional models postulate abiotic formation of simple organic molecules from carbon dioxide and reducing compounds under the action of energy sources such as UV light, lightning, volcanism, or meteoritic impacts. Although differing in details, most primordial-soup models share the view that abiotic organic compounds accumulated in shallow superficial basins and, interacting with catalytic minerals, polymerized into molecules of increasing complexity. Under appropriate conditions, abiotic polymers in turn aggregated into self-replicating complexes that primed the evolution of living cells. This kind of narrative has been subject to extensive criticism from thermodynamic, biochemical and geochemical perspectives. Branscomb and Russel (2018a) view it as a "Frankenstein" scenario that fails to provide a steady source of disequilibrium to support pre-cellular systems.

Life is an emerging property of systems of linear polymers (nucleic acids and proteins) with specific monomer sequences. The spontaneous polymerization of monomers in solution proceeds up to an equilibrium characterized by small amounts of polymers with random sequences, and a majority of free monomers. The polymerization of monomeric units is generally a thermodynamically disfavoured reaction because it involves an increase of free energy ($\Delta G>0$); for the equilibrium to favour polymer formation, polymerization must be coupled with a reaction that has a negative G, as occurs in living systems.

The submarine hydrothermal scenario has been proposed relatively recently as an alternative to primordial-soup models (Martin and Russell 2007; Russell et al. 2010, 2013; Sleep et al. 2011; Sojo et al. 2016; Branscomb and Russell 2018b). Discovered at the onset of the current millennium (Kelley 2001), submarine hydrothermal vents are solid chimneys deriving from the interaction of marine water with peridotite rock along mid-ocean ridges. These edifices consist of silicates, clays, carbonates, and sulphides, and have a porous structure in which mineral-enriched water flows slowly across myriads of apertures, with prolonged interaction with ocean water (Cardoso and Cartwright 2017; Fig. 1).

Alkaline vents have several properties considered favourable to the emergence of life:

- A finely compartmented scaffold rich in catalytic minerals such as mackinawite (iron and nickel sulphide), greigite ($Fe_3S_4$), silica ($SiO_2$) and fougerite (a complex of ferrous-ferric iron, hydroxide ions and carbonate).
- A continuous supply of reactive compounds, in particular hydrogen, methane, hydrogen sulphide, ammonia, formate ($HCOO^-$), cianide ($CN^-$).
- A constant source of disequilibrium in the form of temperature, pH and redox potential gradients.
- Temperatures high enough to accelerate chemical reactions but compatible with the stability of organic molecules involved in biology.
- The possibility to discharge waste in the ocean, thus preventing the establishment of unfavourable equilibria. This is an

important aspect, usually neglected in "primordial-soup" models.

$CO_2$ and $H_2$, and the polymerization of free amino acids into peptides under

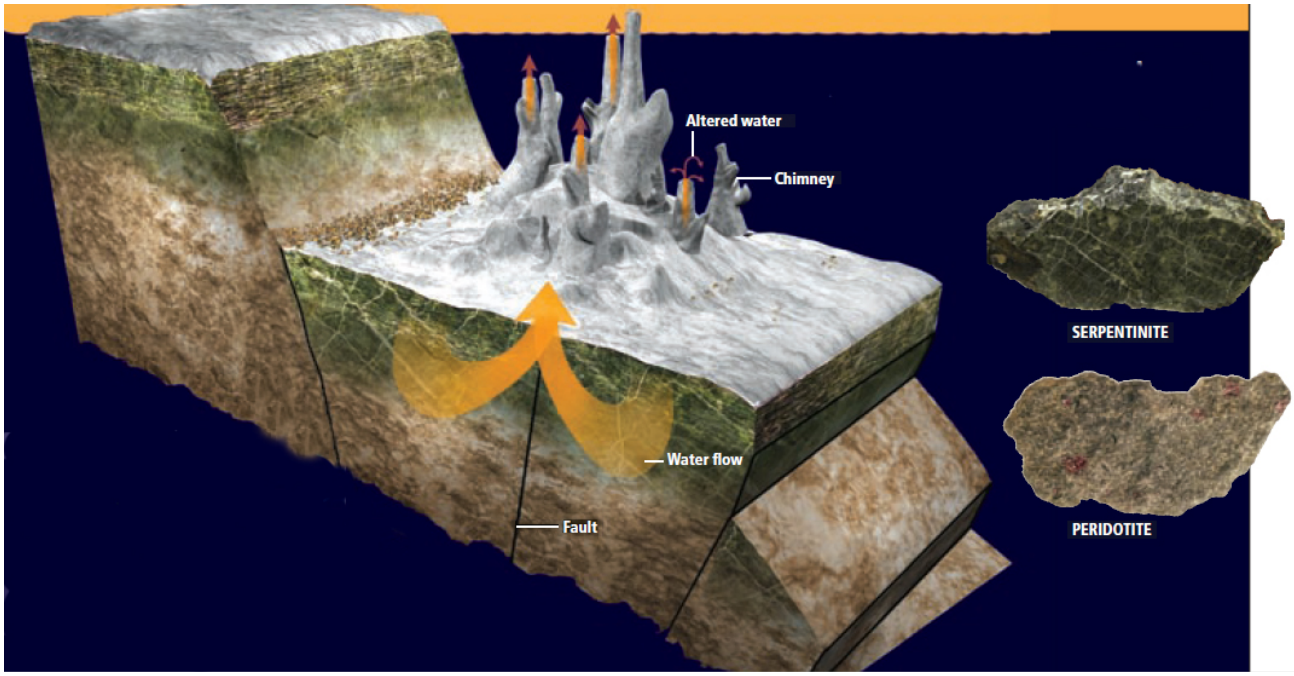


**Figure 1:** Alkaline hydrothermal vents develop along mid-ocean ridges from the interaction of marine water with newly formed oceanic crust rich in reactive peridotite rock. The process, known as serpentinization, converts peridotite into serpentinite; the reactions involved are exothermic and impart water an alkaline pH. Marine water penetrates the crust across faults. Circulating across the rock, the water charges with a diversity of minerals some of which contain biologically important inorganic catalysts such as iron, nickel, manganese, molybdenum, tungsten, and emerges at emission points at a temperature in the range 40-90 °C. In contact with cold oceanic water, dissolved minerals precipitate into solid chimneys that can attain heights of tens of meters. Alkaline vents depend on newly formed oceanic crust for activity; when the reactive minerals are exhausted, the vents die out, but others develop on new oceanic crust. The average duration in activity of alkaline hydrothermal vents is in the order of $10^4$ years.

Field observations (Proskurowski et al. 2008; Lang et al. 2010) and simulation experiments (reviewed in Colín-García et al. 2016) have shown that the conditions existing in alkaline vents are conducive to spontaneous formation of organic molecules from carbon dioxide and hydrogen or other inorganic reductants. Abiotic organic synthesis may involve not only simple molecules such as formate and acetate, but also aminoacids and a diversity of sugars including ribose and deoxyribose. Most remarkably, the formation of simple organic molecules from hydrothermal vent conditions are exergonic, thermodynamically favoured reactions (Lemke et al. 2009; Amend et al. 2013).

Less straightforward are the results of research on abiotic synthesis of nucleic acids. Geochemically plausible pathways have been described for pyrimidine but not purine nucleotides (Sutherland 2010). Formamide generated from ammonia and formate was found to be a precursor for abiotic synthesis of nucleic acid bases and purine acyclonucleosides in conditions simulating alkaline vents (Saladino et al.

2012a,b). A major problem with the alkaline vent scenario is the tendency of RNA to hydrolyse spontaneously into free nucleotides in alkaline solution, which would prevent accumulation of abiotic RNA (Bernhardt 2012). In fact, experiments simulating conditions in alkaline vents using circular ribonucleotides, imidazole-activated ribonucleotides with montmorillonite catalyst, or ribonucleotides in the presence of lipids, only managed to produce RNA oligomers up to four units in length (Burcar et al. 2015). In contrast, Da Silva et al. (2015) reported mononucleoside polymerization into RNA chains of 20-100 units, upon exposition to multiple cycles of hydration-dehydration at elevated temperatures and in presence of monovalent salts, suggesting the involvement of molecular alignment at water/air interfaces. Higgs (2016) developed a theoretical model accounting for the effect of repeated wet/dry cycles on spontaneous monomer polymerization. In thermodynamics terms, the entropy increase associated with water evaporation compensates the loss of entropy due to monomer polymerization. Nam et al. (2018) reported spontaneous synthesis of purine and pyrimidine ribonucleosides within aqueous microdroplets containing phosphoric acid, ribose, nucleobases and magnesium ions as a catalyser. The authors suggested that alignment at the air–water interface of microdroplet surfaces permitted the reactants to overcome the thermodynamic barrier for condensation reactions. The demonstration that wet/dry cycles may provide conditions favourable to spontaneous polymerization favours terrestrial models in opposition to the submarine scenario. Taking on board the novel information, Pearce et al. (2017) suggest that the bulk of nucleotide precursors of pre-biotic RNAs arrived from space with meteorites and interplanetary dust and polymerized under the action of wet/dry fluctuations in superficial ponds.

Based on similar premises as those underlying the alkaline vent hypothesis, Mulkidjanian et al. (2012) have proposed terrestrial anaerobic geothermal fields as the possible set for early life evolution (Fig. 2). These environments may provide essential conditions for the emergence of life including a supply of organic compounds, catalytic minerals, compartmentation, and steady chemical disequilibria. Moreover, unlike submarine vents, terrestrial geothermal fields might have benefited from exposition to wet/dry cycles. Geothermal systems like those proposed by Mulkidjanian et al (2012) still exist today and may have been in existence on Hadean Earth, despite the likely low extension of exposed landmass at that time (Camprubì et al. 2019). The hypothesis suggests that the high K/Na ratio that characterizes the living cytoplasm is a legacy of the special conditions that characterize terrestrial geothermal fields. Based on the assumption that ancestral cells, or protocells, probably had leaky membranes, Mulkidjanian et al. (2012) infer that the universal preference of extant life for potassium is incompatible with an origin in a marine set, where sodium largely predominates over potassium. Moreover, the frequency of Zn-dependent enzymes in modern life does not fit well with extremely low estimates of Zn concentrations ($10^{-12}$-$10^{-16}$ M) in the Hadean ocean. The geothermal terrestrial scenario incorporates most features of submarine hydrothermal vents considered favourable to the emergence of life and adds more, notably

exposition to wet/dry cycles. The model proposes that $CO_2$ reduction in light-mediated reactions provided a steady source of organic compounds whilst zinc and manganese sulfide afforded early biological systems protection from UV. Becker et al. (2018) propose a plausible abiotic pathway from simple inorganic precursors to nucleotides driven by wet/dry cycles and fluctuations of physicochemical

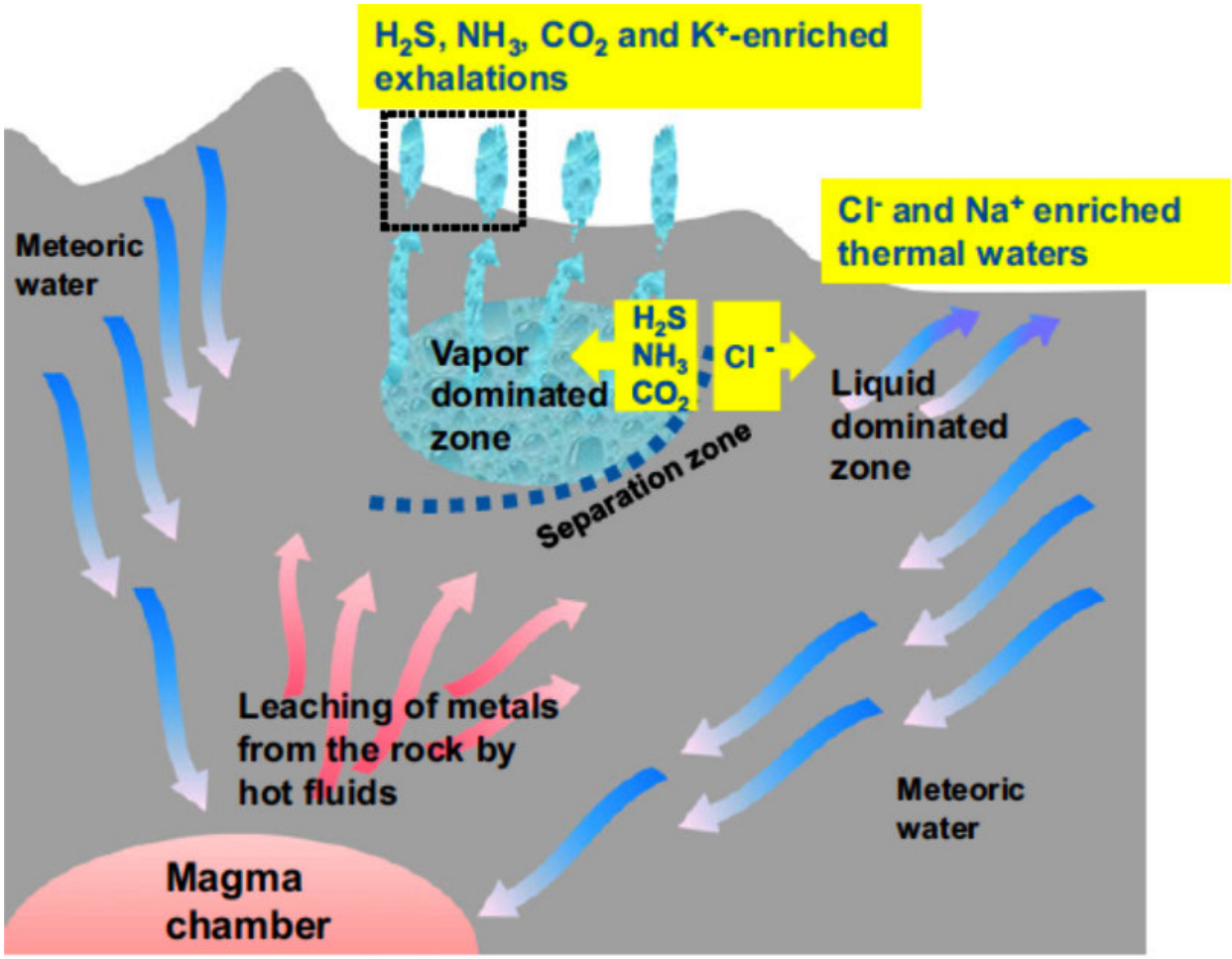


**Figure 2:** A terrestrial geothermal system proposed as the possible set for the birth of life on Hadean Earth. The system is essentially a lake or a system of lakes lying above a volcanic magmatic chamber and fed mostly by water from rain and snow (meteoric water). In the deep underground, water mixes with cation- and anion-enriched magmatic fluids and is heated to 300-500 °C. Ascending toward the surface, hot water interacts with the rock and is enriched in metal cations and anions such as $Cl^-$, $HS^-$, and $CO_3^{-2}$. At shallower depths, the rising hot water starts to boil because of lower pressure. Separation of a vapor phase from the liquid phase causes chemical separation of dissolved compounds, with some (e.g. chloride ions) mostly remaining in the liquid phase, and others such as the gaseous compounds $CO_2$, $NH_3$, and $H_2S$ redistributing into vapor. The liquid phase emerges to the surface following the crevices within the rock. The vapor rises upward and spreads within the rock; the subsurface area that is filled by steam and gas is called the vapor-dominated zone. Part of the steam condenses near the surface and is ejected at thermal springs, and the rest of the steam reaches the surface through fissures of the rock to form fumaroles. Metal cations are carried by both the liquid and vapor phases, although the $K^+/Na^+$ ratio is higher in the vapor phase. From Mulkidjanian et al. (2012) under conditions granted by PNAS licence to publish.

parameters in a terrestrial geochemical scenario. Crucially, terrestrial geothermal fields like those considered by Mulkidjanian and co-workers might remain active for several million years, which is a more plausible time frame for the transition from molecules to life than the relatively short lifetime of submarine alkaline vents.

## 3. The RNA World hypothesis

In living organisms, proteins, RNA and DNA mutually interact in a closed circuit in which each type of molecule depends on the others for replication. Proteins catalyse the synthesis of RNA and DNA from nucleotides; RNA provides the information required for amino acid polymerization in cognate sequences; DNA provides the information for the synthesis of preordained sequences of RNA and for its own replication.

It is extremely unlikely that the three types of polymers and their complex interactions emerged together from scrap. Unlike DNA, RNA is a flexible polymer that can produce a diversity of molecular architectures made of alternating single- and double-filament regions and interconnecting loops (Hiller and Strober 2011). The discovery that RNA is not only able to carry genetic information (as in RNA viruses) but can also perform "non-canonical" functions, led to the hypothesis that RNA preceded proteins and DNA in a pre-biotic evolutionary phase dubbed the "RNA World " (Robertson and Joyce 2012).

Research has revealed several instances of RNA sequences that might be heritages of the putative "RNA World". Among these are riboswitches, non-coding traits of mRNA that bind specific metabolites and may affect gene expression at the transcription or translation level (Breaker 2010). Even more interesting in the present context are ribozymes, RNA sequences that perform enzymatic functions. The best-known example of a ribozyme is the peptidyl-transferase activity responsible for the formation of the peptide bond between the amino acid and elongating peptide during protein synthesis. This catalytic activity lies in the large ribosome subunit and depends on a region of rRNA known as the peptidyl transferase complex (PTC), with no protein directly participating in the process. With a turnover rate of 10-20 peptide bonds per second and an error frequency of $10^{-4}$, the PTC has a catalytic efficiency comparable to that of many protein enzymes (Fox 2010; Moore and Steitz 2010).

A second example of ribozymes is from "self-splicing" sequences that catalyse their own excision from longer RNA filaments. These include type-I and type-II introns present in mRNA, tRNA and rRNA precursors in all three domains, as well as hairpin and hammerhead ribozymes common in viruses but also found in plants and animals (Woodson 2005; Ferré D'Amaré and Scott 2010; Lambowitz and Zimmerly 2011). A further type of ribozyme is RNA in spliceosomes, which directly participates in the excision of spliceosomal introns in precursors of messenger RNAs, a type of introns unique to eukaryotes (Will and Lührmann 2011). Mono and di-nucleotides such as ATP, GTP, coenzyme-A, NAD and FAD, which perform essential functions in all extant organisms, might be vestigial remnants of a "RNA World" (Yarus 2011).

Although RNA may have enzymatic properties, no RNA is known that can self-replicate autonomously, namely without the assistance of a protein enzyme (e.g. an RNA-dependent RNA polymerase). Several

research groups have tried to produce self-replicating RNA molecules by natural selection. Random RNA sequences were copied through numerous replication cycles mediated by a protein RNA-dependent RNA polymerase; with time, many variant copies were produced due to the accumulation of copying errors. The products were tested at intervals and molecules with catalytic properties were selected for further cycles of replication and selection. This process led to the identification of RNA sequences that were able to function as an RNA ligase, i.e. to bind together short RNA sequences by phosphodiester bonds. A self-replicating system was assembled using two complementary ribozymes, each able to produce copies of the other by linking together two shorter sequences (Lincoln and Yoice 2009; Yoice 2009). More recently, an experimental protocol devised by Wochner and co-workers permitted the selection of a novel ribozyme family capable of accurately replicating templates with a length up to 206 nucleotides (Wochner et al. 2011; Attwater et al. 2013). These ribozymes, however, were extremely slow and depended on primers with a specific sequence; no instance of self-replicating RNA sequence has been reported to date.

Besides phosphoryl transfer (the cleavage or ligation of the RNA phosphodiester backbone) and peptide bond formation, the catalytic repertoire of known ribozymes includes RNA aminoacylation, a reaction analogous to amino acid activation by aminoacyl~tRNA synthetases (Chumachenko et al. 2009; Yarus 2011).

The RNA World hypothesis assumes that life started from RNA polymers. The possibility that primordial nucleic acids were not identical to RNA ("XNA"), and that RNA took over at a later stage has been considered (Schrum et al. 2010; Robertson and Joyce 2012). Interestingly, ribose and its 2′-deoxyribose analogue in DNA perform better than almost any other scaffolding molecules for supporting Watson-Crick pairing (Benner 2010). Moreover, ribose and deoxyribose appear to be particularly easy carbohydrates to synthesize prebiotically (Herschy et al. 2014).

A polymer required to perform catalysis as well as to carry biological information faces contrasting demands. In fact, the two functions are not easily coupled, because catalysis requires three-dimensional folding, whereas genetics requires the polymer not to fold to function as a template for its complement. In addition, an ancestral ribozyme replicase should have been able to replicate itself into a complementary filament and then copy this into the original, functional polymer. The co-existence of complementary filaments might have resulted into the formation of reverse double-stranded filaments scarcely prone to replication. In modern cells, this does not cause any difficulty, as the nucleic acid is either double-stranded or protected from hybridization by single-strand-binding proteins. Taylor (2006) suggests that the high temperatures postulated in hydrothermal/geothermal pre-biotic scenarios favoured the formation of parallel-stranded duplexes (made of filaments with the same 3′ to 5′ orientation) that were more prone to replication than antiparallel strands. RNA appears to be quite special among alternative polymeric systems in its ability to strike a balance between the contradicting needs of catalysis and genetics. Attempts at replacing the ribose-phosphate backbone in RNA with alternative backbones more

plausible in a "prebiotic" scenario have shown that RNA performs much better than alternative polymers, thus supporting the RNA-first hypothesis (Neveu et al. 2013).

Why did life choose polymers with repeated negative charges on their backbone for storing genetic information? In general, even modest changes in the structure of an organic molecule significantly affect its physical properties. For transmitting and expressing genetic information, however, living systems need molecules that tolerate minor structural changes without significantly modifying their physical properties. The negative charges on the backbone of nucleic acids impart these polymers a strong polyanionic character that is barely affected by changes in the base sequence on which the biological properties closely depend. A repeating charge might be a general rule for any genetic molecule acting in water (Bennet 2010).

## 4. The Genetic Code

There could be no cell, however rudimentary, without a genetic code, so a code must have evolved at a pre-biotic stage. The origin of the genetic code is one of the most perplexing problems in evolutionary biology, so much so that little novel insight has emerged in the last decades, despite dramatic progress in molecular biology (Wolf and Koonin 2007; Koonin and Novozhilov 2009, 2017).

In living organisms, the amino acid sequence of proteins assembled by ribosomes mirrors the nucleotide sequence of messenger RNAs (mRNAs) under the rules of the 64-triplet code. The link is established by "transfer" RNAs (tRNAs) that function as adaptors. One of the most critical steps in this process is the binding of free amino acids at the CCA end of cognate tRNAs, namely tRNAs bearing a nucleotide triplet (anticodon) complementary to a triplet (codon) for the same amino acid. Aminoacylation of tRNA is catalysed by aminoacyl~tRNA synthetases, a family of enzymes with medium specificity (the rate of mis-aminoacylation is one in $10^4$ cases, implying an average frequency of error of 0.03 amino acids for a peptide of 300 amino acids). The code is virtually universal among extant life forms and is therefore known as the *Standard Genetic Code* (SGC); although many deviations from the SGC exist, particularly in organelles and prokaryotes with small genomes, these are limited in scope and obviously secondary in origin (Maynard Smith and Szathmáry 1995; Koonin and Novozhilov 2017).

The design of the translation system in even the simplest modern cells is extremely complex. At the heart of the system is the ribosome, a large assemblage of at least three RNA molecules and 60-80 proteins arranged in a precise spatial architecture and interacting with other components of the translation system in a most finely choreographed fashion (Section 5). Other essential components include the complete set of tRNAs for the 20 amino acids (only about 40 tRNA species, due to the general occurrence of isoacceptor tRNAs), a set of aminoacyl~tRNA synthetases, and a complement of at least seven-eight translation factors (Smith et al. 2008; Fox 2010; Moore and Steitz 2010; Opron and Burton 2019).

Simpler, albeit less efficient solutions must have preceded such a hugely complex biosynthetic system. The observation that aminoacyl~CCA complexes (CCA is the

three-nucleotide tail shared by all tRNAs) can participate in peptide bond formation in ribosomes suggests that protein synthesis began as a non-coded process and that the tRNA adaptors were a late addition introduced when the system was able to produce coupling enzymes (aminoacyl~tRNA synthetases) with the necessary specificity (Fox 2010). Maynard Smith and Szathmáry (1995) suggested that the ancestral aminoacyl~tRNA synthetases were ribozymes, later replaced by protein enzymes; this hypothesis received support from the observation that *in-vitro* evolved short RNA molecules were able to catalyse RNA aminoacylation (Chumachenko et al. 2009; Yarus 2011) and is consistent with recently proposed scenarios of ribosome and protein evolution (Sections 5 and 6).

The structure of the code is non-random and ensures high robustness to mutational and translational errors. For example, for most codons, the third base may be one of the two purines (adenine and guanine) or one of the two pyrimidines (uracil and cytosine) without changing the meaning. This suggests that the primordial genetic code was based on couples of nucleotides, which allowed for 16 possible codons (the number of combinations with repetition of four elements in groups of two is $4^2$). Thus, the amino acids initially used in peptide synthesis might have been less than twenty, the rest having been co-opted later, when a more complex metabolic network had developed and the translation system had been refined. Ten amino acids are consistently produced in prebiotic chemistry experiments, in the following order of relative abundance: glycine, alanine, aspartic acid, glutamic acid, valine, serine, isoleucine, leucine, proline, tyrosine. This order reflects the free energies of their synthesis, the first being those thermodynamically more stable. The same amino acids, with the same relative abundances, also occur in meteorites. Several lines of evidence suggest that these ten amino acids are older than the others, in the sense that they were probably present in the first proteins whereas the others were not (Koonin and Novozhilov 2017).

With the addition of a third nucleotide, stereospecificity and the accuracy of synthesis increased, but the possible combinations became 64 ($4^3$). The sharp increase in the number of combinations available allowed the recruitment of novel amino acids. Because the number of amino acids deployed in protein synthesis was ancestrally set to twenty, the genetic code underwent "degeneracy", with most amino acids being assigned two or more codons. Code degeneracy had the positive effect of alleviating the consequences of point mutations (mutations that substitute a single nucleotide for another). Increasing the number of encoded amino acids above twenty would have enhanced the diversity and structural versatility of peptides chains but probably would also have reduced the accuracy of translation. Setting the number to twenty was probably the optimal solution between the two opposite effects (Maynard Smith and Szathmáry 1995). Codes based on codons of four or more nucleotides require a greater level of degeneracy, therefore affording enhanced resilience to point mutations and greater translation accuracy, but arguably they would be slower and more expensive than shorter codification systems. A code based on 64 triplets succeeded as the best solution among a suite of alternatives tested by evolution, thus becoming universal.

The debate on the origin and evolution of the genetic code currently revolves around four competing perspectives. (i) The *stereochemical hypothesis* suggests that direct affinity between single amino acids and codons (or anticodons) played a pivotal role in primordial translation before being replaced by the extant indirect mechanism. (ii) The *coevolution model* proposes that the code structure coevolved with amino acid biosynthesis pathways. (iii) The *error minimization* scenario assumes that the code emerged from selection to minimize adverse effects of point mutations and translation errors. (iv) The *frozen accident* idea holds that the standard code has no special properties but was fixed simply because all extant life forms share a common ancestor, with subsequent re-assignment of codons generally precluded by deleterious effects on protein structure. These four perspectives are not mutually exclusive, thus adding further complexity to the problem. For example, mathematical analysis has shown that the SGC is more robust than approximately every million randomly chosen codes. Nevertheless, the SGC is far from being the best possible code: given the astronomical number of codes that are theoretically possible with three-base codons and four bases ($>10^{84}$), there are billions of variants more robust than the actual universal code. We can infer that, once attained a sufficient level of reliability, the SGC was irreversibly fixed in evolution, becoming in part a "frozen accident" (Koonin and Novozhilov 2009, 2017; Facchiano and Di Giulio 2018).

Modern rRNAs and tRNAs are chiral molecules containing D ribose, and during translation they work together to make chiral proteins exclusively with α-L-amino acids. This is highly advantageous to modern organisms because mixed chirality would interfere with self-organization of structural motifs such as α-helices and β-sheets, which are fundamental in modern proteins. Structural analysis of the ribosomal peptidyl transferase complex indicates that the chirality of the sugar ring in RNA is well paired with the choice of α-L-amino acids, this possibly being another instance of "frozen accident" (Fox 2010). As already observed, homochirality is not only a property of proteins but virtually applies to any biological compound that may exist in isomeric forms. In modern living systems, biological molecules are the products of enzyme-catalysed reactions, and homochirality is maintained by stereospecific interaction with the active site of enzymes. There is no reason to exclude that enzymes working with different stereoisomers of the same compound may appear by mutation. Mutant enzymes of this sort, however, are quickly eliminated by selection because their products cannot interact with other enzymes in the metabolic network. Was a similar constrain present in pre-biotic systems? The answer is "*probably yes*" because selection favoured the emergence of well-integrated metabolic networks. Initially, the choice between enzyme/substrate variants was probably random; yet selection had to choose one working isoform and eliminate the other(s) to avoid competition and futile cycles, thus making homochirality a general property of the living world. It is important to notice that this did not involve a sort of hindsight: alternative competing solutions disappeared because of lower efficiency or simply by chance.

## 5. Ribosome evolution and the roots of life

Modern ribosomes consist of small (SSU) and large (LSU) subunits that associate together during protein synthesis and separate again in conjunction with the release of the finished protein. Each subunit is an RNA/protein complex. In bacteria and archaea, the LSU typically contains a 23S rRNA and a 5S rRNA, whereas the SSU contains a 16S rRNA. In eukaryotes, the LSU contains three rRNA molecules (28, 5.8 and 5 S), the SSU contains a 23S rRNA. Associated with rRNAs are a number of ribosomal proteins (r-proteins). There are 31 and 21 r-proteins in the LSU and SSU of *E. coli*, and 49 and 33 in the LSU and SSU of mammals, respectively. Prokaryotic ribosomes have a molecular mass of about 2.5 MDa; eukaryotic ribosomes vary around 4.5 MDa, most of the variability being due to LSU sizes. The secondary structure of the LSU rRNA reveals the presence of six domains named with the roman numbers *I* to *VI*, with the PTC being located in Domain V. The SSU rRNA is comprised of four domains named with roman numbers *I* to *IV* or, based on the position, *3'm, 3'M, C* and *5'*. The SSU domains can be assembled independently, whereas the LSU is monolythic. Cells contain from thousands to millions of ribosomes, depending on their sizes and metabolic activity, ribosomal mass typically accounting for about one third of cellular dry mass. Because of this, rRNAs and r-proteins are the most abundant macromolecules in the biological world (Bowman et al. 2020).

The functional core of the SSU is the decoding centre (DCC) and the functional core of the LSU is the peptidyl transferase centre (PTC). The DCC recognizes codons on mRNA by coupling them with the cognate tRNA. The PTC catalyses peptide bond synthesis and forms a nanopore that permits the elongating peptide to enter an exit tunnel extending through the LSU and ultimately be released from the ribosome. Every coded protein ever produced by life on Earth has passed through the exit tunnel, which could therefore be viewed as the "birth canal of biology" (Bowman et al. 2020). The LSU is able to catalyse peptide bond formation in vitro, in the absence of the SSU. For further details on ribosome functioning, see Opron and Burton (2019).

Prokaryotic and eukaryotic ribosomes share a common structural core made of parts belonging to both the LSU and SSU. The common core comprises 34 conserved proteins and about 4,400 RNA bases, and harbours the major functional sites, i.e. the PTC, the tRNA-binding sites and the decoding site. Superimposed to the common core is a set of moieties specific to each domain: expansion segments of rRNAs, domain-specific r-proteins, and insertions and extensions of shared r-proteins. Interaction between ribosomal subunits depends on several contact points at the interface, called bridges (Melnikov et al. 2012).

Ribosomal RNA and r-proteins have been sequenced in an expanding range of organisms. Concurrently, x-ray crystallography and cryo-electron microscopy have provided atomic-resolution structures of ribosomes in representatives of all three domains of life. Integrated analysis of the data has produced a wealth of novel insight (Fox 2010; Melnikov 2012; Petrov et al 2014, 2015; Bernier et al. 2018; Bowman et al. 2020).

Of special interest in the present context are the following points.

- The common core, encompassing 2800 nucleotides, 19 LSU r-proteins and 15 SSU r-proteins to a total mass of nearly 2 million Daltons, is conserved over the entire tree of life in sequence and especially in three-dimensional structure.
- The common core was finalized around 3.8-4.2 GYA, preceding the emergence of LUCA.
- Bacterial ribosomes are almost entirely composed of the common core (around 90% of total rRNA), with only minor deviations between different lineages, suggesting that the rRNA of bacteria has remained essentially static in size from divergence from LUCA
- Archaeal ribosomes are slightly larger and more variable than bacterial ribosomes. For example, the LSU rRNA of *P. furiosus* is 248 nucleotides larger than the common core rRNA. The largest archaeal rRNA expansions are found in some Asgards.
- Eukaryotic ribosomes have expanded much beyond the common core by addition of novel rRNA segments and novel r-proteins, most of which lie at the periphery of the ribosome.
- The common core rRNAs of bacterial and archaeal ribosomes are most similar, followed by those of archaeal and eukaryotic ribosomes. The common cores of bacterial and eukaryotic ribosomes are the most divergent.

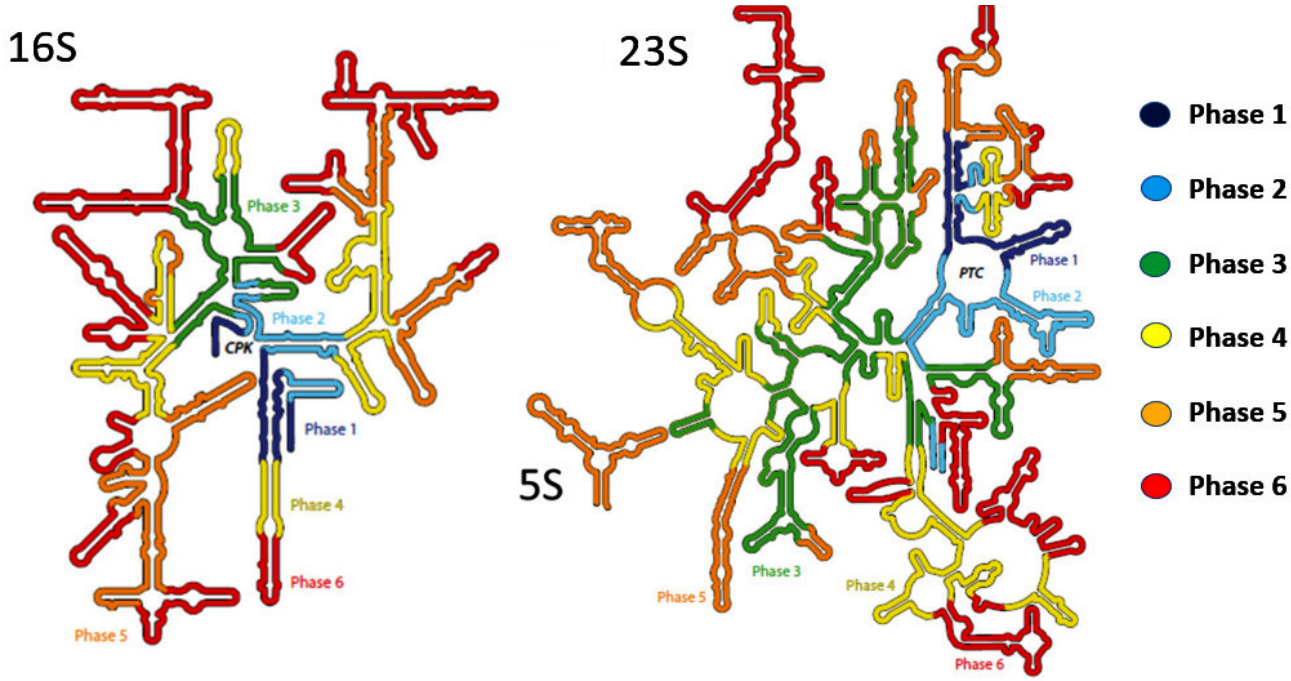


**Figure 3:** Six-phase accretion model mapped on small- and large-subunit rRNAs of the bacterium Escherichia coli. 16S and 23S rRNAs evolved by accretion of "expansion segments" to originally much smaller molecules (dark-blue segments). The small and large ribosome subunit evolved independently from phase 1 to phase 3 and started working cooperatively in phase 4. The 5S rRNA was recruited in the large subunit in phase 5. The ribosomal core attained the final size before the appearance of LUCA. Modified from Petrov et al. (2015) under conditions granted by PNAS licence to publish.

- rRNA expansion segments have been added without perturbing the molecular architecture of the pre-existing core.
- rRNA size in eukaryotes generally increases with organismal complexity.

Comparative analysis of two- and three-dimensional reconstructions of LSU and SSU rRNAs from prokaryotes and eukaryotes has enabled researchers to infer the order of addition of individual RNA segments and create a hierarchical map of rRNA structure. The evidence thus obtained suggests that ribosome evolution has proceeded by superimposing new layers over pre-existing parts, building up an onion-like molecular edifice made of an ancient core and progressively more recent parts outwardly. LUCA's ribosomes were most likely not radically different from ribosomes in present-living prokaryotes. Early in eukaryote evolution, ribosomes acquired an extra shell of eukaryote-specific rRNA extensions and r-proteins. Further ribosome elaboration occurred in complex animals, with the addition of tentacle-like rRNA projections (Bokov and Steinberg 2009; Petrov et al. 2014, 2015; Bowman et al. 2020).

Petrov et al. (2015) identified six major phases in ribosome evolution from the beginning to the emergence of the first cells (Fig. 3).

- **Phase 1:** *appearance of self-folding RNA oligomers* forming a double-helix stem and a single-strand loop. Folding made RNA molecules less prone to chemical degradation, thus elongating their average life, and favoured the emergence of catalytic properties.
- **Phase 2:** *emergence of RNA replicase ribozymes and of ancestral forms of LSU* and *SSU rRNA, tRNA* and *mRNA*. The emergence of ribozymes with RNA replicase activity not only ensured the maintenance of a steady RNA population, replacing RNA lost by degradation or leakage, but also gave the start to Darwinian selection for more efficient self-replicating ribozymes, The primordial LSU rRNA was able to catalyse peptide bond formation, producing uncoded oligopeptides. Primordial mRNA was a random population of single-stranded oligomers. Primordial tRNAs consisted of a simple CCA tail and subsequently acquired the double-helix amino acid acceptor stem.
- **Phase 3:** *SSU and peptide recruitment into pre-proto-ribosomes*. The LSU expanded to form a short exit tunnel and an embryonic subunit interface, by which it bound to SSU, which therefore became a component of the emerging peptide-synthesizing complex. LSU–SSU association was mediated by interaction with proto-mRNAs and tRNAs. The proto-tRNA minihelix was extended by an insertion to form prototypes of modern L-shaped tRNAs. Catalytic efficiency of the PTC and product length increased. Uncoded peptides started being incorporated into pre-proto-ribosomes. Darwinian selection started operating on proto-rRNAs, proto-tRNAs, and proto-mRNAs in a coordinated way.
- **Phase 4**: *birth of proto-ribosomes*. A primitive genetic code started coupling RNA and peptide evolution (Sections 3 and 4). The evolutionary trajectories of LSU, SSU, proto-mRNAs, and proto-tRNAs were more strongly integrated. The addition of novel RNA segments expanded the subunit interface, elongated the exit tunnel, and formed well-defined pockets for tRNA binding; proto-tRNAs

were optimized to form base-pair triplets with proto-mRNAs, which remained a population of single-stranded oligomers.

- **Phase 5**: *addition of a GTP-dependent ratcheting system* responsible for the coordinated movement of the ribosome and mRNA. This innovation marked the transition from a catalytic system driving spontaneous reactions to a decoding machine driving coupled reactions. The genetic code improved.
- **Phase 6**: *the rRNA common core was finalized and the genetic code froze in the modern form*. The addition of novel protein-binding RNA segments expanded the set of ribosomal proteins.

The SSU rRNA might have derived from a pre-existing RNA replicase ribozyme that was secondarily co-opted in peptide synthesis (Smith et al. 2008; Fox 2010). This hypothesis gains support from evidence suggesting that large and small subunit rRNAs initially evolved independently (Petrov et al. 2014, 2015). An ancestral RNA replicase activity of the small subunit rRNA is consistent with the fact that here lies the decoding site responsible for codon-anticodon interaction in modern ribosomes. A hybrid dimer, half RNA replicase and half peptide polymerase, could perform mRNA translocation before the advent of protein elongation factors (Taylor 2006). Putative ribozymes extinguished, or were reduced to simple cofactors such as NAD, FAD and CoA, when more efficient protein enzymes replaced them at a later stage of evolution. With the emergence of a genetic code, mRNAs that had passed initial selection aggregated into longer molecules, thus reducing the rate of dispersion of functional sequences. Association of these enlarged RNAs with peptides might have produced virus-like "protochromosomes" that on phase 5 took control of peptide synthesis and of their own replication, thus establishing a separation between catalytic and informational functions.

Analysis of the six reading frames in the rRNA of the bacterium *Escherichia coli* (three in the 5′ to 3′ direction, and three in the opposite direction) has demonstrated the occurrence of segments with high sequence homology with tRNAs for all twenty amino acids (Root-Bernstein and Root-Bernstein 2015). The entire set of tRNAs is represented in the 16S rRNA, whereas 23S rRNA directly encodes only six tRNAs. Its complementary sequence, however, contain segments with high levels of homology with all tRNAs. This result points to a strong evolutionary link between rRNA and tRNA, although there is no clue to establish whether tRNAs derived from rRNA or vice versa. The same research also showed that rRNA contains sequences encoding for segments of ribosomal proteins and of proteins involved in ribosomal function such as RNA polymerase and amino acyl-tRNA synthetases, thus suggesting the existence of an ancestral link also between rRNA and mRNA.

The transition from short oligonucleotides to functional ribosomes working under the rules of a fully developed genetic code (phase 1 to 6) most likely covered a long time interval. This does not easily conciliate with "primordial-soup" scenarios linked to transient sets such as volcanic eruptions, lightning, meteoritic impacts. Indeed, the same also applies to the alkaline vent hypothesis, the average lifetime of single vents in the order of $10^4$ years being probably insufficient to foster such a complex succession of events. The problem might be easier to handle under the

assumption that RNA-peptide complexes were able to migrate and "colonize" new favourable locations, thus substantially elongating their evolutionary trajectory. In the alkaline vent scenario, for example, populations of RNA-peptide complexes bound to mineral fragments might have moved from old to young vents following ocean currents. On the other hand, terrestrial geothermal fields like those considered as the possible cradle of life by Mulkidjanian et al. (2012) may remain active for several million years independently of local climatic conditions. In addition, geothermal pools in the same field may exchange materials through rains or overflowing, thus increasing the chances for evolution. It should also be considered that, in a lifeless world, organic molecules probably had longer lifespans than today, at least in protected environments, being not subject to biological decomposition.

## 6. How did life discover folding peptides in a universe of unstructured sequences?

Nucleic acids and proteins must assume defined three-dimensional structures for biological activity, yet their ability to do so is starkly different. Nucleic acids fold spontaneously, based primarily on simple base-pairing rules, and can in general be denatured and renatured reversibly by chemical agents or warming/cooling cycles. Protein folding into complex architectures is much more a delicate process subject to severe constraints. For example, secondary folding into structures such as α-helices and β-sheets requires the regular formation of hydrogen bonds between the amino hydrogen and carboxyl oxygen atoms in the peptide backbone, which is possible only under certain conditions. Likewise, peptide folding into tertiary structures depends on the formation of a hydrophobic core isolated within a shell of secondary structures. Proteins require stable folds, robust to environmental fluctuations, but they must also find solutions that are flexible enough for allostery and complex interactions. This is not an easy task. The number of sequence possibilities for a random polypeptide chain exceeds the number of atoms in the known universe already at a chain length of around 60 residues. Yet only an extremely small fraction of these sequences exhibit folding competence, thus precluding the possibility for proteins to evolve by random substitution of single amino acids. Biological proteins, despite their apparent limitless diversity, are in fact combinations of "only" about 10,000 basic domains, indicating that the main mechanism underpinning protein evolution is domain shuffling rather than elaboration of novel sequences. Although relatively numerous, however, these domains still account for only an infinitesimal fraction of possible peptide sequences. How did evolution manage to find folding motifs in an almost limitless universe of unstructured sequences?

Combined sequence and structural analysis of a diversity of proteins across the whole spectrum of life has permitted the discovery of a much more circumscribed number of structural motifs (possibly around 100) with an average length of 24 amino acids, which are universally spread in nature and particularly abundant in iron-sulfur- and nucleic acid-binding proteins. It has been suggested that these motifs emerged in a pre-biotic RNA-Peptide World and were

fixed in evolution as structural subunits of protein domains (Alva et al. 2014).

Because of its onion-like structure, the ribosome provides a window that looks back to the very beginning of peptide evolution. Molecular dissection of prokaryotic ribosomes has revealed that the inner sphere with a radius of 20 Å from the geometrical centre contains no protein structure. Because this area coincides with the peptidyl transferase ribozyme, the absence of protein is viewed as a legacy from the putative RNA World. The area immediately outside, 20 to 50 Å from the centre, contains few r-protein segments that form random coils with little or no secondary structures. The ribosomal sphere between 50 and 70 Å from the centre is more protein-rich; moreover, the protein chains located here have an increased content of secondary structures. Ribosomal proteins located between 70 and 90 Å from the centre are organized into secondary structures that may also form super secondary arrangements (https://en.wikipedia.org/wiki/Supersecondary_structure). These proteins, however, still lack a tertiary structure. Globular proteins resembling cytoplasmic proteins are only found in the more peripheral shell of the ribosome. When these data are mapped against the relative ages of rRNA, they provide a reconstruction of natural protein evolution (Lupas and Alva 2017; Kovacs et al. 2017). The first abiotic peptides, either formed by spontaneous polymerization or produced by RNA ribozymes without a genetic code, had random sequences and were most likely unstructured. Peptide association with RNA probably increased the chemical stability of both polymers. Besides using non-specific acid base interactions, peptides may stereospecifically bind to RNA by hydrogen bonds. Specific binding to folded RNA may have guided, and even forced unstructured peptides into more complex conformations with novel properties. This might have favoured the emergence of a primordial code and most likely exerted a strong selection pressure for better-structured peptides after the code was established. The broad diversity of protein domains in nature might have evolved by accretion of a limited number of motifs that emerged in ribosomal proteins in response to interaction with rRNA. At some stage, this led to the appearance of proteins no longer dependent on RNA for folding, which therefore could work outside of the ribosome. In this perspective, cytoplasmic proteins might be viewed as the outermost shell built by ribosomes under selection pressure for more effective self-replication (Alva et al. 2015; Lupas and Alva 2017).

The appearance of complex three-dimensional protein architectures permitted the evolution of a diversity of enzymes with superior catalytic properties, which replaced pre-existing analogous ribozymes. The emergence of protein enzymes paved the way to two further innovations needed for making a cell: a self-replicating bounding membrane and an autonomous metabolism.

## 7. From self-assembling bilayers to encoded membranes

Far from being a simple bag for living matter, the cell membrane is a highly selective barrier that controls the inflow and outflow of a diversity of molecules. Most importantly, the cell membrane can build electrochemical gradients and use them for making ATP or for transporting solutes

against their electrochemical gradient. Because of this, any attempt at reconstructing the evolution of life should address not only the emergence of a genetic system and a metabolism, but also the origin of cell membranes.

The basic components of membranes are amphipathic lipids, molecules consisting of a hydrophilic (polar) and hydrophobic (non-polar) part. Interacting with water, these molecules spontaneously arrange into bilayers that isolate the hydrophobic parts inside, exposing the hydrophilic parts on either side (Chen and Walde 2010). Cells do not make new membrane by assembling together single molecules from naught, as a bricklayer does in building a wall. The cells can only expand pre-existing membranes by adding new molecules one by one into the pre-existing framework, and then pinch off them to form separate compartments. This is the way membranes are transmitted to daughter cells during cell division. Thus, much like chromosomes, biological membranes are hereditary structures (Cavalier-Smith 2001, 2004). If membranes are essential to life and cells can make them only from pre-existing membranes, how did membranes evolve?

Supporters of the alkaline vent scenario suggest that at an early stage of pre-cellular evolution, peptides of abiotic origin bound to mineral microcompartment walls and modified their properties. Under the repulsive force of water, peptide-mineral pellicles might have incorporated amphipathic lipids, isolating their hydrophobic tails from water circulating on either side. This model receives support from the observation that the addition of peptides and RNA to the solution circulating in a simulated hydrothermal vent modified the elemental stoichiometry of the chimneys, perhaps epitomizing the putative takeover of abiotic metabolism by organic polymers (McGlynn et al. 2012). It has been suggested that organic-mineral protomembranes were able to harness chemiosmotic energy of geochemical origin (Lane and Martin. 2012; Russell et al. 2013; Sousa et al. 2013). Ancestral membranes did not require lipids as complex as those forming modern membranes, simple long-chain organic acids or monoglycerides being more likely precursors in a prebiotic world. Peptides with a hydrophobic tail of glycine residues and a hydrophilic head of aspartic acid are also able to form stable bilayers in an aqueous environment subject to wet/dry cycles (Smith et al. 2008; Mulkidjanian et al. 2009; Mansy 2010; Schrum et al. 2010; West et al. 2017).

To generate self-replicating protocells, ancestral membranes needed three further innovations: (i) a stable association with cognate (viz. informationally interlinked) protoribosomes and protochromosomes; (ii) mechanisms mediating the incorporation of amphiphilic peptides and lipids for membrane growth; (iii) a chemiosmotic machinery ( Section 8). The evolution of the Sec protein insertion machinery (du Plessis et al. 2011; Nyathi et al. 2013) and integral lipid-synthesising complexes converted protomembranes into "encoded membranes" (Lane and Martin 2012), hereditary structures vertically transmitted during replication.

Phylogenomic analysis indicates that LUCA had a bounding membrane containing all fundamental components of modern membranes (Jekely 2006), thus the innovations outlined above probably predated LUCA. Reiterating Virchow's classic aphorism "omnis cellula e cellula" (every cell

from a cell), cell biologist Günter Blobel (Blobel 1980) wrote "omnis membrana e membrana", every membrane from a membrane. Because life is almost certainly monophyletic, this implies that all membranes existing today, however modified and specialized, arise from one ancestral membrane. Yet, because of major divergences in membrane architecture between the archaea and all the other extant organisms, some researchers have proposed a dual origin ( Section 10).

## 8. Early metabolic pathways

Metabolism (from Greek metabolè, change) is the set of life-sustaining chemical transformations that enable living organisms to produce building materials and to obtain energy. It is generally agreed that a primitive form of metabolism preceded the emergence of life. Among the scenarios proposed, however, only the submarine hydrothermal model analyses in some extent the likely mechanisms involved. Active oceanic vents present today on the Earth are rich with life, thus it is extremely difficult to verify the existence of abiotic sources of organic compounds. Radiometric investigation in the field, data from artificial systems and theoretical models suggests that reductants of geochemical origin such as hydrogen, hydrogen sulphide and ferrous iron, could spontaneously react with carbon dioxide, producing a diversity of organic molecules (Proskurowski et al. 2008; Lang et al. 2010). Moreover, experimental evidence suggests that, in the conditions presumably existing in alkaline vents in the Hadean Earth, the pH gradient across mineral pellicles supported the synthesis of energy-rich molecules such as pyrophosphate ($HP_2O_7^{-3}$) and trimetaphosfate ($H_3P_3O_{10}^{-3}$). These compounds could accumulate in pre-biotic microcompartments by thermophoresis (Baaske et al. 2007) and/or by binding to mineral surfaces (Martin and Russell 2007; Russell et al. 2013; Sojo et al. 2016). Proponents of the terrestrial geothermal model assume similar properties for their geochemical scenario, with wet/dry cycles driving thermodynamically unfavoured reactions (Mulkidjanian et al. 2012).

In a context characterized by the presence of reactive chemical species and stable sources of disequilibrium, ribozymes could work co-operatively with peptides and inorganic catalysers, generating metabolic networks of increasing complexity. With time, proteins took on enzymatic functions, whilst RNA role as a catalyser progressively lessened. The replacement of ancestral ribozymes with limited catalytic performances by more versatile protein enzymes vastly increased the diversity, abundance and complexity of the proto-biotic organic pool. Catalytic metals possibly played a major role in this fundamental transition. The suite of metal-enzymes in extant organisms, such as ferredoxin (iron-sulphur), cytochromes (iron), plastocyanin (copper), carbonic anhydrase (zinc), nitrogenase (molybdenum), urease and hydrogenase (nickel), are probably modern descendants of pre-biotic inorganic catalysers (Nitschke et al. 2013).

A fundamental step towards the emergence of an autonomous metabolism was the appearance of a form of bioenergetics, namely a mechanism that that utilized environmental disequilibria to make ATP or its likely precursor acetyl-phosphate (Martin and Russell 2007). Modern cells have two ways of producing ATP. The first is substrate-level phosphorylation (SLP), the second is

chemiosmosis. SLP directly couples exergonic redox reactions to ATP synthesis. As an example, the oxidation of 3-phosphoglyceraldehyde to 3-phosphoglycerate and subsequent conversion of the latter to pyruvate are sequentially coupled to the synthesis of two ATP molecules. Chemiosmosis instead couples redox reactions to the building of an electrochemical gradient across a membrane, generally redistributing protons. Proton flow down their electrochemical gradient is coupled to ATP synthesis by ATP synthase, an enzymatic complex inserted within the membrane (Schoepp-Cothenet et al. 2013). SLP is a biochemically simple and extremely fast way of making ATP; in contrast, chemiosmosis is a relatively complex and slow process, yet it is much more efficient that SLP in extracting energy from reactions including those with extremely low energy yields.

It is debated whether early life was heterotrophic or autotrophic. Primordial-soup models favour a heterotrophic early life, whereas the submarine vent hypothesis points to a chemioautotrophic ancestral metabolism (Martin and Sousa 2016). If heterotrophic, early life most likely employed SLP to produce ATP; if autotrophic, instead, it probably employed chemiosmosis. Having detected no enzymes involved in autotrophic pathways in a set of universal proteins, Mulkidjanian et al. (2012) assumed that early life was heterotrophic, probably obtaining energy from anaerobic oxidation of organic compounds of abiotic origin. By analogy with metabolic pathways in extant acetogenic bacteria and methanogenic archaea, supporters of the submarine scenario suggest that early life used hydrogen of geochemical origin to reduce carbon dioxide to acetate (eq. 1) or methane (eq. 2) through the acetyl-CoA (or Wood-Ljungdahl) pathway (Lane and Martin 2012; Sousa et al. 2013; Schönheit et al. 2016).

$$8H_2 + 4HCO_3^- + 2H^+ \rightarrow 2CH_3COO^- + 8H_2O$$

(eq. 1)

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

(eq. 2)

A deep ancestry of the acetyl-CoA pathway is supported by phylogenetic analysis of the enzymes involved, such as the bifunctional enzyme carbon monoxide dehydrogenase/acetyl-CoA synthase reducing $CO_2$ to CO, the molybdo/tungstopterin proteins reducing $CO_2$ to a formyl moiety, and [NiFe] hydrogenases extracting electrons from hydrogen (Sousa et al. 2013; Schoepp-Cothenet et al. 2013; Weiss et al. 2016, 2018).

Alternatively, Russell and Nitsche (2017) proposed that early life could make a live from the oxidation of abiotic methane from serpentinization (Section 2), using nitrogen oxides (eq. 3) or ferric ions (eq. 4) as electron acceptors. Putative methanotrophic life could use metabolic intermediates of methanotrophy as a source of organic carbon.

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 6H_2O + 4N_2$$

(eq. 3)

$$CH_4 + 8Fe^{+3} + 2H_2O \rightarrow CO_2 + 8Fe^{+2} + 8H^+$$

(eq. 4)

Paleogeochemical inference suggests that the reaction of atmospheric molecular nitrogen with carbon dioxide under the effect of lightning or meteorite impacts could generate substantial amounts of nitrogen oxides despite the lack of free oxygen on the Hadean Earth (Ducluzeau et al. 2009; Wong et al. 2017). Likewise, photo-oxidation of ferrous iron ($Fe^{+2}$) by solar

ultraviolet could produce ferric iron ($Fe^{+3}$) that precipitated to the ocean bottom, becoming accessible to early life for redox processes (Nitschke and Russell 2013; Russell et al. 2013).

## 9. DNA recruitment gave the start to modern biology

A network of entangled metabolic pathways and the genetic system evolved together. Evolutionary pressure for novel and more efficient protein catalysts promoted the diversification of RNA templates, or "replicators". The persistence of numerous independent replicators enhanced recombination, as occurs still today in some viruses, but also increased the risk that cooperative sequences were separated and potential synergies dispersed (Maynard Smith and Szathmáry 1995). Aggregation of smaller replicators into larger molecules was positively selected because it favoured (a) the synthesis of novel, larger and more versatile peptides, and (b) coordinate expression, replication, and transmission of functionally related sequences (Koonin and Martin 2005). These expanded replicators might have been similar to negative-strand RNA viruses, consisting of a long peptide-bound RNA filament functioning as a template for more transient mRNAs.

At a point, the replicators attained sizes that raised problems of instability: being the single-strand RNA filament highly flexible, it tended to spontaneously break and/or to fold into structures that interfered with transcription, translation or replication. Thanks to its double-helix structure and the absence of the oxygen atom in the position 2 of the sugar (deoxyribose, instead of ribose), DNA is much more stable that RNA. A further advantage of the double helix structure is that the two strands carry the same information, yet only one is decoded, the other being used for correcting errors during replication, thus affording the genetic system enhanced stability. DNA is only one of several possible forms of double-strand nucleic acid that could have evolved in a RNA-based biological world. The fact that all extant life uses DNA implies that the transition from a single-strand to double-strand nucleic acid occurred only once or, more likely, that only the contemporary DNA form survived early selection (Benner 2010).

As for other major innovations, the transition from RNA to DNA occurred gradually, for example first utilizing double-filament RNAs for information storage and replication, and single filament RNAs for expression, and then separating the two functions completely with the use of different nucleotide precursors: deoxyribonucleotides for double-filament replicators and ribonucleotides for single filament "messengers". The separation also involved replacement of uracil with thymine in the replicator, thus reducing the risk of mutation due to the tendency of cytosine to convert into uracil by deamination; a misplaced uracil cannot be recognized in RNA, whereas it can be pinpointed as an alien base in DNA and removed by repair systems (Forterre et al. 2004).

RNA replacement by DNA as the repository of biological information established the genotype-ribotype-phenotype tripartite organization typical of modern cells. This transition increased the efficiency of storage, replication and transmission of genetic information and activated a strong evolutionary pressure towards functional

aggregation of genes. Less "co-operative" genes were unavoidably suppressed, favouring the emergence of highly integrated gene communities, or genomes. Gene aggregation into large genomes enhanced combinatorial sequence shuffling, fostering the emergence of an expanding diversity of multidomain proteins with novel biological functions (Alva and Lupas 2018). Surprisingly, the DNA polymerases involved in DNA replication in bacteria, archaea, and eukaryotes display no appreciable homology. The apparent diversity of the replication machineries among the three Domains sharply contrasts with the conservation of proteins involved in transcription and translation. The lack of homology among DNA polymerases in the three lineages precludes the reconstruction of the ancestral state, suggesting multiple origins for DNA replication and even the possibility that LUCA was an RNA-based cell. The discovery of sequence homology between the catalytic core of the archaeal DNA polymerase PolD and that of the large subunit of the RNA polymerases responsible for DNA transcription in all three Domains fostered a more parsimonious scenario. Koonin et al. (2020) suggested that RNA polymerases and replicative DNA polymerases in the three Domains evolved from a common ancestor that functioned as an RNA-dependent RNA polymerase in the RNA-protein world before the advent of DNA.

## 10. The membrane gap: ancestral or derived?

Barring the prokaryote/eukaryote divide, the largest gap in the biological world is in the molecular architecture of membranes. The two gaps do not coincide, the latter setting the bacteria and eukaryotes into an assemblage separate from the archaea. In all three domains of life, the main membrane lipids are glycerophospholipids, amphipathic molecules made of glycerol bound to two long hydrophobic "tails" and to a phosphate group. Apart from this commonality, glycerophospholipids could not be more different in the two assemblages (Fig. 4). In bacteria and eukaryotes, glycerol is in the L form, the hydrophobic tails are linear aliphatic chains bound to C1 and C2 of glycerol by ester bonds, and phosphate is bound at the C3 position. In the archaea, glycerol is in the D form, the hydrophobic molecules are isoprenoid chains bound at C2 and C3 positions by ether bonds, and phosphate is bound at the C1 position (Shimada and Yamagishi 2011).

Isoprenoid chains are produced by the mevalonate (MVA) pathway in eukaryotes and a modified form of the same in archaea (Boucher et al. 2004), whereas the bacteria mostly employ the non-homologous methylerythritol phosphate (MEP) pathway. Photosynthetic eukaryotes acquired the MEP pathway through the chloroplast endosymbiosis. The occurrence of the MVA pathway in some bacteria has traditionally been interpreted as a trait acquired by horizontal gene transfer from an eukaryotic or archaeal donor. At odd with this claim, more recent phylogenomic evidence suggests that the MVA pathway ancestrally occurs in all three domains and was probably present in the last common ancestor (Lombard and Moreira 2011).

The evolutionary significance of the membrane divide between G3P lipid stereochemistry in bacteria and eukaryotes

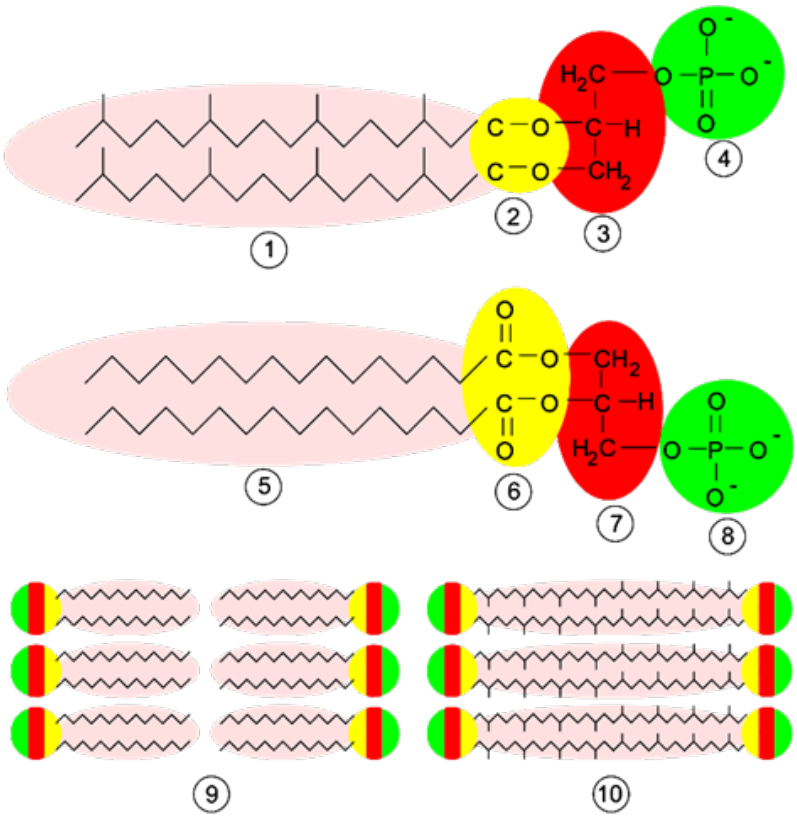


**Figure 4:** (1-5) Glycerolipid structure in the archaea: (1) isoprenoid chains, (2) ether bonds, (3) D-glycerol, (4) phosphate group. The isoprenoid chains are bound to glycerol at the C2 and C3 positions by ether bonds, phosphate is bound at the C1 position. (5-8) Glycerolipid structure in bacteria and eukaryotes. (5) Linear fatty acid chains, (6) ester bonds, (7) L-glycerol, (8) phosphate. The fatty acid molecules are bound to glycerol at C1 and C2 positions by ester bonds, phosphate is bound at the C3 position. (9) Bilayered membrane organization as typical of bacteria and eukaryotes. (10) Unilayered membrane of tetraether lipids in hyperthermophilic archaea. Non-hyperthermophylic archaea have bilayered membranes as in bacteria and eukaryotes, but their glycerolipids have archaeal stereochemistry. From: https://commons.wikimedia.org/wiki/File:Archaea_membrane.svg

and G1P in archaea is controversial. The current mainstream debate is focused on two competing scenarios. The first holds that the bacteria and archaea diverged from a common ancestor that either lacked true membranes (Martin and Russell 2003, 2007) or possessed heterochiral membranes made of both G3P and G1P lipids (Wächtershäuser 2003; Peretó et al. 2004; Lombard 2012; Koga 2014). After splitting, the bacteria evolved or retained a G3P membrane stereochemistry, whereas the archaea evolved or retained a G1P stereochemistry, the choice depending on adaption to different environmental conditions. Under this scenario, the eukaryotes derived by the symbiosis of an archaeon and a bacterium, the latter having been converted into the mitochondrion (Lòpez-Garcìa and Moreira 2019; Spahn et al. 2019). It is proposed that cellular integration of the two symbionts involved the loss of the archaeal G1P lipid biosynthetic machinery and its replacement with the symbiont's G3P machinery, thus explaining why eukaryotes have bacterial-type G3P membranes. Importantly, the Sec protein translocon is universal in the three domains, indicating that the last common ancestor did have a true membrane (Jekely 2006). The hypothesis of a heterochiral common ancestor for archaea and bacteria receives support from the demonstration that vesicles (liposomes) made of heterochiral membranes not only are stable, but are even more resistant to high temperature (in terms of proton permeability) than homochiral G3P vesicles (Shimada and Yamagishi 2011). Caforio et al (2018) have demonstrated that the same also applies to living cells. They engineered a strain of the bacterium *Escherichia coli* to produce archaeal lipids in addition to its

normal lipids; the transformed cells produced hybrid membranes made of lipids of ether-linked isoprenoids and ester-linked fatty acids bound to a G1P or a G3P backbone, respectively. The cells were perfectly viable, with growth rates comparable to the wild type, and showed a higher tolerance to heat treatment compared with control strains. Overexpression of archaeal lipids above 30% of the total lipid content, however, was associated with disturbances including the release of membranous vesicles in the growth medium and asymmetric cell division.

The alternative scenario, sponsored by Forterre (2013) and Cavalier-Smith (2014), posits that the three Domains derive from a common ancestor with G3P stereochemistry. In this perspective, the bacteria and eukaryotes retained ancestral G3P membranes, whereas the archaea secondarily evolved G1P membranes by adapting to hyperthermal acidic habitats. Like the competing hypothesis, this scenario implies an intermediate phase in which G3P and G1P lipids co-existed but provides a strong selection pressure to explain the transition.

The archaeal domain typically encompasses hyperthermophilic and acidophilic forms with optimum temperature above 80 °C and optimum pH below 3, yet mesophilic (optimum temperature in the 20-45 °C range) and even psychrophilic archaea (-20 to +10 °C) are also known. The paradox is only apparent, as archaeal membranes are stable at high temperature but remain fluid and functional also at low temperatures. A unique trait of hyperthermophilic archaea is that their membranes consist of only one layer of bipolar lipids with a tetraether structure (Fig. 4.10). These special membranes are more stable and less permeable to protons that two-layered membranes in conditions of high temperature and low pH. Molecular evidence points to hyperthermophyly and underpinning tetraether G1P membranes as the ancestral condition in archaea (Gribaldo et al. 2006). This suggests that two-layered membranes secondarily evolved in derived archaeal lineages that lost tetraether lipids by adapting to less extreme habitats but retained the ancestral isoprenoid G1P stereochemistry (Cavalier-Smith 2014). This scenario has received support from recent phylogenomic work (Williams et al. 2017).

Hyperthermophyly also occurs among bacteria, and at least two bacterial lineages (the Thermotogales and Aquificales) possess tetraether lipids; these lipids, however, have G3P stereochemistry and are made with non-isoprenoid fatty chains, thus they most likely evolved independently of archaeal tetraether lipids (Schouten et al. 2007; Glansdorff et al. 2008). Hyperthermophyly has been suggested to be an ancestral trait of the Bacteria domain, possibly inherited from LUCA (Di Giulio 2003). Phylogenetic analysis by Lake et al. (2009) does not support a hyperthermophilic root of the tree of life. A phylogenetic analysis based on ribosomal proteins resolves the Thermotogales and Aquificales as sister groups and places them in a derived position in the bacterial tree (Yutin et al 2012). In contrast, a more recent analysis based on a large dataset from metagenomic sequencing places the Thermotogales and Aquificales in a basal position, thus supporting the view that hyperthermophyly is an ancient trait in bacteria (Schulz et al. 2017). Some archaea possess a G3P

dehydrogenase besides a G1P dehydrogenase, whereas some bacteria have a G1P besides G3P dehydrogenase, both occurrences being interpreted as the likely outcome of horizontal gene transfer (Peretó et al. 2004). The Firmicutes, a gram-positive group of bacteria, possess a geranylgeranylglyceryl phosphatase (the enzyme responsible for the formation of the ether bond in archaeal lipids), probably acquired from an archaeon by horizontal gene transfer (Valas and Bourne 2011).

Spontaneous racemization (*viz.* the conversion of the L isoform into the D isoform) of aspartic acid residues in proteins increases exponentially with temperature, thus obliging microorganisms living at high temperatures to continuously replace their proteins to maintain enzymatic activity (Onstott et al. 2014). Such a major constraint over life at high temperature provides robust evidence against hyperthermophyly being an ancestral trait of life.

From the analysis of a large dataset of prokaryotic sequences, Weiss et al. (2016, 2018) traced back to LUCA 355 protein families. The properties of these proteins depict LUCA as an anaerobic, $CO_2$-fixing, $H_2$-dependent with the acetyl-CoA (or Wood-Ljungdahl) pathway (Section 8), nitrogen-fixing and thermophilic (not hyperthermophilic) organism. The hypothesis of ancestral chemioautotrophy receives support from evidence for $^{13}C$-depleted organic carbon in extremely ancient sedimentary rocks (Ueno et al. 2006; Dodd et al. 2017; Tashiro et al. 2017). It is important, however, to emphasize that LUCA might have been the last microbial population preceding the divergence of bacteria and archaea, yet it was not the first organism or the first cell and certainly does not represent the origin of life.

## 11. Concluding remarks

Life is a chemical system that exists in a condition of disequilibrium and evolves in a Darwinian way. Access to an external source of disequilibrium is an essential condition for the emergence of life from "inanimate" matter. Among a diversity of sources potentially available on the Hadean Earth, modern models almost unanimously point to geochemical disequilibria inherent to volcanic activity, a conclusion in line with evidence of a chemioautotrophic ancestral metabolism. The ultimate driver of volcanic activity on Earth is gravitational separation of an iron-nickel core and a silicate-rich mantle exposed to the surface (viz. not segregated under massive amounts of hydrogen/helium as in gaseous giant planets). Because this is the natural fate of "terrestrial" planets in the Solar system and most likely also elsewhere in the Universe, it is tempting to infer that (i) terrestrial planets have an intrinsic propensity to generate life, (ii) life is probably widespread in the Universe, and (iii) the ultimate source of the chemical disequilibria that started life on Earth and possibly on other planets is gravitational energy stored during Universe expansion. This position shares the view that life as defined above is *a likely, almost necessary occurrence whenever and wherever the right conditions happen to develop* (Russell et al. 2013; Hazen 2017), rather than *a most rare occurrence if not a unique trait of our planet* (Monod 1971).

Terrestrial life as we see it today is a highly refined product of billions of years of evolution, yet it retains traits that probably appeared at the very beginning of its history.

The peptidyl transferase ribozyme, unstructured protein chains in the ribosome core, universal use of proton gradients in energy transduction and of transition metals in catalysis are likely legacies of a pre-cellular stage intermediate between the living and non-living world. The narrative presented in this review is compatible in essential points with both deep-ocean and terrestrial geothermal scenarios. Partnership between RNA and protein has probably dominated biology since the very beginning. Inherited from LUCA and retained virtually unchanged for several billion years, the translation machinery is the fundamental hallmark that defines the universal tree of life (Bowmann et al. 2018).

The appearance of the first cells - self-reproducing systems with at least one hereditary membrane, a genetic system, and a metabolism - was a crucial, irreversible step that put an end to pre-biotic evolution, because cells were immensely more efficient than any pre-cellular system in exploiting environmental resources. From then on, the chance for independent emergence of alternative life forms on Earth was virtually nil, as pre-existing life would immediately out-compete them. The same probably applies for hypothetical extra-terrestrial life reaching the Earth in the form of resting cells.

It is generally assumed that self-replicating molecules and a rudimentary metabolism appeared independently, and that life emerged when a translation system based on a genetic code linked them together. The notion of living organisms as mortal vehicles for immortal genes as popularized by Richard Dawkins (Dawkins 1976) is a useful metaphor that helps catching the way natural selection works, yet biological inheritance implies not only the transmission of genes but also of the biochemical machinery necessary for their functioning. To replicate, genes must fit in a cell, a structured and predictable environment that depends on genes for maintenance and reproduction but cannot be built by genes from scratch. A long phase of pre-biotic evolution was necessary not only to produce self-replicating molecules but also to accommodate them in a reproducible house - the cell - made of inter-linked genotype, ribotype and phenotype. The transgenerational transmission of a cellular organization is thus the most ancient and conspicuous expression of *ecological inheritance*, or *niche construction*, namely the ability of life to modify the environment and transmit the changes across generations (Laland et al. 2016).

A further point worth of attention is the relatively low chemical diversity of living systems. Estimates of the total number of carbon-based compounds with molecular masses in the same range as those of living systems, *i.e.* below 500 Da, give numbers in excess of $10^{60}$. Life uses only a tiny fraction of this potential "chemical space" (Dobson 2004). The first explanation that comes to mind is that life chemistry is still focused on ancestral molecular diversity, namely the few types of compounds of abiotic origin wherefrom life started plus their close derivatives. This is unlikely. Under positive selection, billions of years of evolution would have certainly produced much a greater chemical diversity. The most likely explanation stems from the consideration that, for thermodynamic reasons, cells are crowded systems containing high concentrations of molecules, either free or in the form of polymers. This condition is

conducive to spontaneous, uncontrolled reactions that may interfere with metabolic pathways and/or damage key cellular components. Thus, life has evolved under strong chemical constraints, being obliged to exclude potentially noxious molecular architectures. Even keeping chemical diversity under tight control, life had to accept compromises, a most prominent example being that of glucose. This is a six-carbon sugar with an aldehyde functional group, universally employed as a substrate in respiration and as a source of carbon chains in most biosynthetic pathways. The highly reactive aldehyde group can easily be blocked by glucose polymerization into glycogen or another polysaccharide, but the free form tends to react with amino groups in proteins, causing structural damage. Presumably, glucose emerged as the most abundant and widespread natural monosaccharide because it is less reactive with proteins than other monosaccharides. Damage to proteins exposed on cellular surfaces from excessive glucose concentration in the blood is at the origin of diabetes in humans.

Life restriction to a limited range of carbon compounds does in no way imply that forms of life arisen independently in the Universe are obliged to explore the same molecular space. Even without considering chirality, the overall chemical space available to life is most likely much wider than that exploited on Earth. This means that, in contrast with commonplace science-fiction, no alien creature could feed on terrestrial organisms, nor could humans feed on non-terrestrial life. If ever possible, therefore, colonization of other planets will require that humans take with them a large complement of organisms, both prokaryotic and eukaryotic, to establish self-supporting "terrestrial" ecosystems. Great job opportunities for "terraforming" ecologists in a possible remote future.

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