LUCA and the Origins of Cellular Life

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Key Points

- LUCA (the Last Universal Common Ancestor) is an inferred transitional state between the origin of life and the first microbial fossils.
- LUCA is the common ancestor of bacteria and archaea, which means it is the common ancestor of all extant life.
- Universal traits are widespread and conserved across life, but they cannot provide information about some aspects of LUCA's physiology.
- Lateral gene transfer between archaea and bacteria makes tracing specific genes to LUCA challenging.
- According to recent reconstructions, LUCA had a genetic code and was a chemolithoautotroph, but was half-alive, dependent on geochemistry.
- Present data suggest that LUCA existed in serpentinizing hydrothermal vents that provided H_2 as reductant, CO_2 as carbon source for the acetyl-CoA pathway, transition metals as catalysts, methyl groups and gradients.

Glossary

(Anti)Codon A Codon is a sequence of three nucleotides in mRNA that encodes a specific amino acid. An anti-codon is a sequence of three nucleotides in an amino acid-carrying tRNA that pairs with a codon during protein synthesis, translating the genetic code into amino acid sequence.

(Poly)Peptide A chain of 2–50 amino acids. A polypeptide has at least 51 amino acids.

Acetogens Strictly anaerobic bacteria that obtain their carbon and energy from the reaction of H_2 and CO_2 to form acetate using the acetyl CoA pathway.

Acetyl-CoA pathway The most ancient pathway of CO_2 fixation, present in bacteria and archaea. When it functions autotrophically, it generates acetyl-CoA or pyruvate from CO_2 and H_2. Widespread in many obligate anaerobes such as methanogens, acetogens and others.

Adenosine triphosphate (ATP) The universal energy currency. It can be employed to promote metabolic reactions that require energy input. ATP is a nucleotide with two high-energy phosphoanhydride bonds.

Aminoacylation The attachment of an amino acid to a tRNA.

Archaea One of the two domains of prokaryotes. They differ from bacteria in many ways, most notably in the composition of their lipid membranes, which contain isoprene ethers of glycerol-1-phosphate.

Autotrophy Autotrophic organisms have the ability to produce organic compounds using CO_2 as the source of carbon. The energy can come from light or chemical reactions.

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Bacteria One of the two domains of prokaryotes. They differ from archaea in many ways, most notably in the composition of their lipid membranes, which contain fatty acid esters of glycerol-3-phosphate.

Carbon fixation The process by which some organisms transform CO_2 to organic compounds.

Catalyst A substance that increases the rate of a chemical reaction without being changed in the process.

Cell wall A structural layer present outside the cytoplasmic membrane. It gives structural strength and protection from osmotic lysis to the cell. The composition of cell walls is significantly different between bacteria, archaea and eukaryotes.

Chemolithoautotroph An organism that is at the same time a chemotroph (obtains energy from chemical reactions, not light), a lithotroph (a chemotroph that gets energy from inorganic compounds, not organics) and an autotroph (uses CO_2 as the source of carbon, not organics).

Chemotroph An organism that gets its energy from chemical compounds, not light.

Class One of the classifications of taxonomy. The highest taxon is a domain. Then in descending order there are: kingdom, phylum, class, order, family, genus and species.

Cofactor An additional chemical component bound by an enzyme and required for its activity. Cofactors can be metal ions or more complex organic molecules (coenzymes).

Differential gene loss An evolutionary process which results in a certain gene being lost in some lineages, but retained in others. This means that a gene originating in an ancestral lineage is not present in all of its descendents.

DNA Deoxyribonucleic acid, the molecule that contains the genetic material (genes) of all cells and some viruses. The universal code of life.

Domain Highest taxonomic classification in biology.

Electron transfer Electrons are atomic particles that orbit around the atomic nucleus, and that can get transferred during redox reactions from atoms of an electron donor to atoms of an electron acceptor.

Enzyme A biological catalyst made up of proteins. Each enzyme increases the rate of a specific reaction or group of reactions.

Eukaryote A cell or organism whose cells have a nucleus and organelles. The nucleus and organelles have their own membranes. All true multicellular organisms are eukaryotes.

Family One of the classifications of taxonomy. See "Class".

Gene family A group of genes that evolved by duplication from one original gene. All genes in a gene family are therefore evolutionarily related, and so are the proteins in the corresponding protein family.

Gene A segment of DNA (or RNA in some viruses) that encodes a specific protein.

Genome The total of all genetic information of a cell or virus.

Geochemistry The study of the chemistry of Earth.

GTP Guanosine triphosphate, an energy currency used in translation and ribosome biogenesis.

Habitat An environment within a larger ecosystem in which an organism lives.

Heterotrophy Heterotrophic organisms need an organic substance as their carbon source.

Homologous Similar by common ancestry. Proteins in different species that are similar in sequence and/or structure because they have a common ancestor are homologous proteins.

Hydrogenase An enzyme capable of using H_2 as the electron donor for another compound, almost always the small protein ferredoxin. Found in anaerobic organisms.

Hydrothermal vents Springs at the bottom of the ocean that emit warm or hot water and usually other compounds that have seeped out of the rocks because of the hot water venting through them. They are usually close to crustal spreading zones on the sea floor.

Intra- and inter-domain Intra = within/inside, inter = between/among. Intra-domain LGT denotes transfers of genes between different bacteria, or between different archaea. Inter-domain LGT denotes gene transfers between bacterial and archaeal cells.

Ion A positively or negatively charged molecule or atom.

Lateral gene transfer (LGT) A process by which prokaryotes transfer genes from one cell (organism) to the other independently of vertical parent-to-offspring inheritance. LGT can occur by conjugation (the transfer of regions of DNA from one organism to the other via specialized plasmids), transformation (the direct uptake of environmental DNA) or transduction (the transfer of DNA segments via viral vectors).

Lineage A temporal sequence of individuals of given taxonomic rank that have a continuous line of common descent. Examples are the primate lineage, the insect lineage, the bacterial lineage, etc.

Membrane In generic terms, a thin sheet or layer. In biological or biochemical terms, a membrane refers specifically to a lipid bilayer. Membranes separate the contents of a cell from the environment. They sustain ion gradients and harbor transmembrane proteins.

Metabolism The sum of biochemical reactions in a cell. These reactions can be involved in breaking down compounds and harnessing energy (catabolism) or in the synthesis of cell parts (anabolism). Acetogens and methanogens have the unusual property of harnessing energy from the synthesis of organic compounds from H_2 and CO_2.

Methanogens Strictly anaerobic archaea that obtain their carbon and energy from the reaction of H_2 and CO_2 to form methane using the acetyl-CoA pathway.

Monophyly Descent from a single common ancestor.

MtrA-H A membrane-integral enzyme (a methyltransferase) in methanogens. It transfers a nitrogen bound methyl group to CoM (coenzyme-M) and simultaneously pumps Na^+ out of the cell. It generates the ion gradient that methanogens use to drive their ATP synthase.

Nucleobase A chemical compound made up of one or two rings containing C, O, H and N. There are 5 nucleobases in the genetic code (including DNA and RNA): Adenosine (A), Guanine (G), Cytosine (C), Thymine (T) and Uracil (U). A forms a basepair with T/U and C forms a basepair with G.

Nucleotide A monomeric unit of a nucleic acid made up of a sugar (ribose in RNA, deoxyribose in DNA), a phosphate group and a nucleobase.

Organelle A membrane-enclosed structure within a eukaryotic cell. Examples are the mitochondrion and the chloroplast.

Organic compound A chemical molecule that contains at least one carbon-hydrogen or carbon-carbon bond.

Phototroph An organism that gets energy from light.

Phylogenetic tree Phylogeny is the history of evolution of all life on Earth. A phylogenetic tree is the most common way in which phylogeny can be portrayed. Because it consists of nodes and branches, it is called a tree.

Phylum One of the classifications of taxonomy. See "Class".

Prokaryote A single-cell organism that lacks organelles and usually has its genome in a single circular molecule instead of multiple nucleus-enclosed chromosomes like eukaryotes. Bacteria and Archaea are prokaryotes.

Proton gradient A spacial difference between the concentration of protons (a positively charged hydrogen atom), often generated by a combination of proton pumps and membrane separation. The protons can spontaneously (without energy input) move only in one direction, from higher to lower concentration.

Radicals Highly reactive chemical compounds with unpaired electrons. They are formed in some chemical reactions, including some enzymatic reactions.

Redox reactions Reactions where the number of electrons in one or more atoms effectively changes. An electron transfer between an electron donor and an electron acceptor is a redox reaction.

Replication DNA synthesis from an existing DNA strand.

Reverse Krebs cycle A cyclic metabolic pathway of CO_2 fixation that produces organic compounds from carbon dioxide by using electron donors such as ferredoxin or NADPH. It requires energy input (ATP hydrolysis). It is used by some bacteria. Also known as the reverse tricarboxylic acid cycle (rTCA).

Ribosome Component of the cell that is made up of proteins and ribosomal RNA (rRNA). It produces proteins by linking amino acids together by reading the codons provided by the mRNA with the aid of tRNAs. They are responsible for the translation process in cells.

Ribozyme Short for ribonucleic acid enzyme. An RNA molecule capable of catalysis.

RNA Ribonucleic acid. There are multiple forms of RNA, the most well known ones are messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). It is essential for protein synthesis.

Rnf Short for *Rhodobacter* nitrogen fixation. A membrane protein (oxidoreductase) in acetogens that transfers electrons from reduced ferredoxin to NAD^+ and pumps Na^+ out of the cell. It generates the ion gradient that acetogens use to drive their ATP synthase.

Serpentinization A reaction of metal minerals in the Earth's crust with ocean water that produces H_2 and alters the composition of the minerals.

Species In eukaryotes that reproduce sexually a species is roughly defined as all organisms that can reproduce with one another and produce viable and fertile offspring. In prokaryotes the definition is more challenging. It is usually a group of strains that share major traits and differ from other groups of strains in one or more major traits. All members of a species have a relatively recent common ancestor.

Substrate-level phosphorylation A mechanism that involves the direct synthesis of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and phosphate, with the energy to carry out this reaction coming from the hydrolysis of a high-energy bond in a high-energy compound such as 1,3-bisphosphoglycerate or acetyl-phosphate, not from a chemiosmotic transmembrane gradient.

Trait A very broad term to define the characteristics of an individual. This can refer to anything, from morphological characteristics such as shape, to metabolic adaptations. Most traits are determined by genes and/or the habitat the organism lives in.

Transcription mRNA synthesis from a complementary DNA strand.

Transition metals Metals from groups 3−12 of the periodic table, such as copper, iron, nickel and cobalt. They have unpaired electrons in their d-orbitals, which determines their reactivity.

Translation Protein synthesis on the ribosome using mRNA as a template.

Tree of Life The phylogenetic tree of all life on Earth.

Abstract

The last universal common ancestor (LUCA) is the ancestor of all life on Earth. It is an inferred intermediate state between the origin of life and the first free living cells. Lateral gene transfer being common among prokaryotes makes it difficult to determine what LUCA looked like and because of these challenges, many hypotheses exist about the nature of LUCA and the universal tree of life. Recent studies suggest that LUCA most likely lived from chemical reactions (not sunlight), obtained its carbon from CO_2 and used H_2 as a source of energy and electrons along a reaction sequence that strongly resembles the modern acetyl-CoA pathway. It probably arose and lived in a serpentinizing (H_2-producing) hydrothermal vent which provided CO_2 as its carbon source and H_2 as its reductant, transition metals as catalysts and cofactors as well as ion- and temperature-gradients.

Introduction: What is LUCA?

The Earth is 4.55 billion years old, liquid water existed on Earth roughly 4.2 billion years ago and the first traces of life appear in rocks that are 3.9–3.5 billion years of age (Arndt and Nisbet, 2012). The last universal common ancestor, LUCA, is the inferred link between geochemical reactions on an uninhabited planet and a young Earth teeming with microbial life. All chemical reactions of life take place in water, or in the water-free active site of enzymes that are dissolved in water. Considering that LUCA is a water-based organism, it must have existed sometime between the first appearance of water and the origin of the two prokaryotic domains: Bacteria and Archaea. There is no direct evidence in fossils or rocks that would reveal what LUCA looked like or where it lived. But we can explore the attributes, the biology and the lifestyle of LUCA using evolutionary inference and phylogenetic reconstructions. Based on what we know about modern and ancient lineages, we can also explore the kind of habitats in which LUCA might have existed.

An early concept of LUCA was called the progenote (Woese and Fox, 1977). The progenote (from Greek: "*pro*", before, and "*genēs*", of specified kind) is a hypothetical primitive entity that had not yet evolved a link between genotype and phenotype. Modern prokaryotes are complex free-living cells and are assigned either to Bacteria or Archaea in classification systems. In primordial evolution, there had to be intermediate states en route to cellular organization that were not yet free-living, hence not assignable either to Bacteria or Archaea. The progenote had smaller and less complex proteins and genomes and was just in general far less complex than the cells we know today. Prokaryotes then evolved from the progenote. Today, the term progenote has largely been replaced by the term LUCA.

There are many different theories for the origin of life and how LUCA ultimately came to be. There are those focused on the genetic material and the concept of self-replicating RNA molecules (Joyce, 2009; Cech, 2012). These give rise to a concept of LUCA that existed more or less as a kind of "living" nucleic acid. RNA can be synthesized in laboratory experiments but under conditions that most geochemists would deem unlikely to have ever existed on the primitive Earth (Pahlevan et al., 2019). Furthermore, RNA-centric views of LUCA focus on the origin of genetic material rather than metabolism and therefore do not readily connect to metabolism or the environment. There is also a biological approach to LUCA from a comparative viewpoint. Traits that are common to all cells should be present in LUCA, and this is where newer findings have led to major changes in how LUCA is reconstructed (Weiss et al., 2016, 2018).

Main Sets of Ideas on the Nature of LUCA

The idea that all life arose from a common ancestry can be traced back to Darwin who first proposed the Tree of Life (TOL). Different kinds of trees that try to relate the different species to each other have since been proposed. Until recently the three-domain system suggested by Woese and colleagues (Woese and Fox, 1977; Woese et al., 1990) has been the most widely accepted tree of life. They suggest that LUCA is the common ancestor of Bacteria, Archaea and Eukaryotes with Archaea and Eukaryotes branching off later and having a common ancestor. However, in recent years more and more research suggests that we are actually dealing with a two domain system, with LUCA being the common ancestor of Bacteria and Archaea and Eukaryotes then either branching off from a phylum of Archaea (Williams et al., 2013) or coming from endosymbiotic events of Archaea and Bacteria (Weiss et al., 2018).

The question of what LUCA actually looked like, or to which modern cells it was most similar in terms of physiology, is a strongly debated topic. There is the bacterial root case, with Archaea branching off at a later point (Cavalier-Smith, 2006) (**Fig. 1**i). The reverse case was also suggested (Caetano-Anollés et al., 2014) (**Fig. 1**ii). There was also the suggestion that LUCA was very complex, having for example different kinds of lipids in the cell membrane, and Bacteria and Archaea eventually branched off from this complex LUCA (Wächtershäuser, 2003) (**Fig. 1**iii). Newer studies have pointed to a geochemical, half-alive LUCA, which was more primitive than either Bacteria or Archaea and was not yet capable of being a free-living cell, requiring chemical input from its geochemical environment (**Fig. 1**iv), but already had many functions inherent to life (Weiss et al., 2016, 2018).

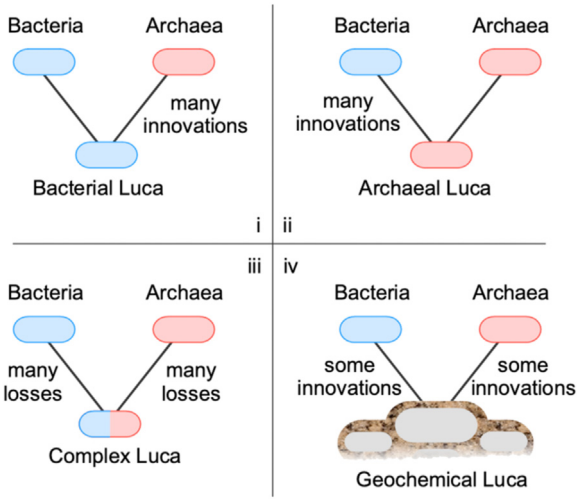

Fig. 1 Different views of the relationship of the last universal common ancestor LUCA to modern cells. See text for details.

LUCA's Universal and Non-universal Traits

Universal traits are widespread across all organisms of the tree of life, which makes them naturally traceable to LUCA. In other words, if all living organisms, from bacteria to human, have genes stored in their DNA, it is clear-cut to surmise that their common ancestor had genes stored in its DNA. This trait was then simply inherited from one generation to the next, over hundreds of millions of years and even billions of years. New species, families, classes, phyla of organisms emerged, always inheriting the trait. However, an inference of the characteristics of LUCA based on universal traits paints too narrow a picture, because many biological properties cannot be inferred by looking at what is universal across the tree of life. Even though all prokaryotes are water-based unicellular organisms with no organelles, they are very diverse, in that they exhibit a wide range of ecological, physiological and metabolic adaptations (Madigan et al., 2018), the driving forces behind their evolutionary success.

DNA, RNA and Metabolism

All life on Earth has DNA and a transcription, translation and replication apparatus, and while the transcription and translation machinery has a highly conserved, homologous core in all domains of life, the DNA replication enzymes are not homologous. This seems to imply LUCA could not replicate its DNA in the same way modern cells do.

LUCA also had a core metabolism for the synthesis of amino acids, cofactors and nucleotide bases, with 402 core reactions required to synthesize those compounds from H_2, CO_2, NH_3, H_2S, phosphate and mineral salts (Wimmer et al., 2021). But not every prokaryote on Earth actually uses these 402 core reactions, because many organisms obtain important nutrients from their environment. For example, humans (and all animals) obtain their B vitamins and half of their amino acids from their diet. In early stages of evolution, before LUCA had a fully developed metabolism, LUCA also must have acquired many essential building blocks of life from the environment.

All cells are surrounded by lipid membranes, but the chemical constituents of cell membranes in bacteria and archaea are different: bacteria synthesize fatty acid esters via enzymatic pathways, archaea synthesize isoprene ethers via enzymatic pathways (Koga et al., 1998). As lipids, LUCA probably used hydrophobic compounds provided by the environment before the enzymatic pathways of bacteria and archaea evolved (Martin and Russell, 2003).

The components that are responsible for performing cellular functions sometimes differ across bacteria and archaea but the underlying function they perform is for the same purpose. To account for these differences, which impact the ribosome (the central protein-synthesizing machine of the cell) (Fox et al., 1980), the lipid membrane, the cell wall (Albers and Meyer, 2011) and more, biologists typically resort to one of the four basic schemes outlined in **Fig. 1**. Either (i) archaea underwent many transitions and evolved from a bacterial ancestor, or (ii) bacteria underwent many transitions and evolved from an archaeal ancestor, or (iii) LUCA had all such traits and bacteria and archaea evolved via loss rather than invention, or (iv) LUCA was simple, not free living, chemically supported by the environment and the ancestors of both lineages evolved the differences that distinguish bacteria from archaea today. Although there is no fossil LUCA in existence to provide final answers, the simplest explanation is that LUCA was a primitive organism that had primordial or geochemically supplied versions of many of these functions. All theories for the origin of life require that chemical components had to be provided by the environment before cells could synthesize all of their constituents. In that sense, LUCA was likely a progenote, a not-yet free-living cell, because of the significant differences between Bacteria and Archaea, even though many of the functions and (proto-) proteins common among cells were already present in LUCA (Di Giulio, 2023).

Because all cells use the same genetic code, we can infer that LUCA had a functional (possible primitive) ribosome that could form peptide bonds and synthesize proteins. Despite there being differences between ribosomes of bacteria and archaea, parts of the ribosome are remarkably conserved, including the overall 3D structure and several ribosomal proteins and ribosomal RNAs. In terms of structure and function, the most strongly conserved portion of the ribosome is a 70 nucleotide stretch containing the peptidyl-transferase site (the site that makes peptide bonds during translation) called the protoribosome (Yonath, 2009). Although the protoribosome corresponds to only about 1% of a modern ribosome's total mass, it can bind aminoacyl-tRNAs and form peptide bonds from them (Bose et al., 2022). But the protoribosome can also bind aminoacyl minihelices, 7−11 nucleotide long RNA molecules that correspond to the stem of tRNA where amino acids are bound. Minihelices are possibly the simpler evolutionary precursors of tRNAs, they can also be aminoacylated by aminoacyl-tRNA synthetases (Tamura, 2015; Schimmel, 2018).

Lateral Gene Transfer

Inheritance in prokaryotes is not always vertical. That is, prokaryotes can, and do, readily transfer genes across lineages without regard for lineage affiliation. Lateral gene transfer was discovered in the 1950s and 1960s in hospitals, as antibiotic resistance spread rapidly from one pathogen to the next, making antibiotic treatments increasingly difficult. The reason turned out to be lateral gene transfer: the antibiotic resistance genes were encoded on small extrachromosomal DNA circles (plasmids) that were passed from donor to recipient regardless of species boundaries (Freeman, 1951; Akiba et al., 1960). Prokaryotes are capable of harnessing chemical energy or light energy and evolved the ability to use a wide variety of chemical compounds as electron donors and acceptors. Considering the diversity of prokaryotic metabolism, universality is clearly not a good indicator of the metabolic traits of the last universal common ancestor. Most of the metabolic adaptations in prokaryotes have originated long after LUCA and reflect the flexibility of prokaryotes to adapt to different habitats and lifestyles. Their evolution cannot be easily traced on a phylogenetic tree because of the prevalence of intra− and interdomain LGT (lateral gene transfer) in prokaryotes (Nelson-Sathi et al., 2015). LGT makes it extremely challenging to distinguish whether the presence of a trait in two evolutionarily distant lineages is the result of lateral gene transfer between members of these lineages, or of the presence of the trait in a common ancestor, with differential loss of the trait in the lineages that do not exhibit it. Coupled with gene loss, these are main reasons why determining which traits were present in LUCA and in what form, and which were not, remains difficult to this day. Note that LGT can only explain the phylogenetic distribution of shared traits, it does not explain the origin of novel traits.

LUCA's Carbon Source

Cells are made up of roughly 50% carbon by dry weight. In order to obtain the carbon to build up their cells, prokaryotes can metabolize preexisting organic compounds (heterotrophs) or reduce environmental CO_2 to organic carbon (autotrophs). The only carbon compound that was continuously bioavailable on the early Earth was CO_2 (Sossi et al., 2020; Mrnjavac et al., 2023), the carbon source of all modern ecosystems. What about organisms that cannot fix CO_2 (heterotrophs)? Even today, all cells acquire carbon either from CO_2 or from organic compounds that were at some point produced by autotrophs from CO_2. This all points to heterotrophs appearing late in evolution by inventing mechanisms to tap the organic compounds that autotrophs were producing and releasing into the environment. Under this premise, LUCA was an autotroph capable of carbon fixation. It was once thought that glycolysis, the breakdown of glucose (C6) to pyruvate (C3) in heterotrophs, was the first pathway of carbon metabolism (Degani and Halmann, 1967). However, the early Earth lacked substrates from which cells could extract energy for heterotrophic growth (Schönheit et al., 2016) while pathways that supply energy from reactions of H_2 and CO_2 were abundant on the primordial Earth and probably provided the energy for the origin of LUCA (Weiss et al., 2016).

LUCA's Energy Source and Energy currencies

To obtain the energy necessary to fuel the reactions that sustain life, modern prokaryotes conserve energy from energy-releasing (exergonic) reactions in chemical compounds (chemotrophs) or from photons emitted either by the sun or by thermal light emitted from hydrothermal vents (phototrophs) (Martin et al., 2018). In most cases energy is released in a series of electron transfer reactions (redox reactions) in which electrons move from the initial electron donor (reductant) to an electron acceptor (oxidant), which can either be an environmental compound such as SO_2 (respiration) or produced during metabolism such as pyruvate (fermentation). Phototrophy originated post-LUCA in the domain bacteria, while chemotrophy is considered to be the ancestral mechanism of energy conservation. Few of these compounds were available on the early Earth, but CO_2 and H_2 were both abundant in serpentinizing hydrothermal vents (Schwander et al., 2023).

Modern theories for the origin of life assume the existence of a LUCA that was a chemolithoautotroph, meaning it used CO_2 as a carbon source and relied on environmental inorganic electron donors for biosynthesis and chemical reactions to satisfy its energy needs. In all modern cells (hence in LUCA), energy is conserved in the form of adenosine triphosphate (ATP). ATP is the most well-known energy currency, but it is not the only one. In all modern cells (hence in LUCA) GTP is employed by enzymes that are involved in the assembly and maturation of the ribosome, and by proteins that move the ribosome along the mRNA during translation. GTP at the ribosome is equally universal as ATP in biochemical pathways. Because all proteins are synthesized by the ribosome, including the main enzyme that catalyzes ATP synthesis—the ATP synthase—and because translation is GTP dependent, it would appear that GTP (which powers ribosome biogenesis and function) is the more ancient energy currency, with ATP appearing

after the origin of the ATP synthase. During the early evolution of life before LUCA, GTP became established as the universal energy currency of translation, while ATP became the main energy currency of metabolism (Mrnjavac and Martin, 2024).

Extrapolating From Genomes: A Geochemical LUCA

As outlines above, LGT can hinder the inference of which proteins were present in LUCA because genes present in bacteria and archaea can either be the result of vertical inheritance from LUCA or the result of LGT. How to distinguish between the two? One approach is to identify and remove cases of lateral gene transfer between bacteria and archaea. One such study identified from over 250,000 gene families present in bacteria and archaea 355 gene families that, in their respective phylogenetic tree, showed a clear separation of bacteria and archaea (the domains are monophyletic), and that were present in at least two distinct lineages of each domain (Weiss et al., 2016). These 355 genes were probably not the result of LGT and hence were likely present in the last universal common ancestor. The LUCA reconstructed from those genes was not a free-living cell, which require about 1000 genes to survive. Rather, it was half-alive and was heavily dependent upon geochemistry (Fig. 2). It had a DNA-based genetic code and ribosomal translation. In order to satisfy its energetic requirements, it harnessed geochemically generated ion gradients via an ATP synthase, but also used substrate-level phosphorylation (SLP).

Out of the 7 known CO_2 fixation pathways (Sánchez-Andrea et al., 2020), only genes for the acetyl-CoA pathway turned up in the LUCA reconstruction. The acetyl-CoA pathway was previously proposed to be the most ancient metabolic pathway of CO_2 fixation (Fuchs and Stupperich, 1985), it is the only one present in both archaea and bacteria, and it can release energy instead of consuming it. The acetyl-CoA pathway is at the core of modern chemolithoautotrophic theories for the origin of life (Martin and Russell, 2007). The electron donor for the pathway is H_2, and hydrogenases required to harness H_2 gas by prokaryotes were also present in the LUCA gene set. The pathway is highly dependent on transition metals, in that they make up the metal clusters in the active sites of several enzymes of the pathway and are bound by several cofactors. It also relies on organic cofactors such as flavins, pterins, corrins (which also require cobalt), and others. Genes for the synthesis of several cofactors were found in the LUCA set.

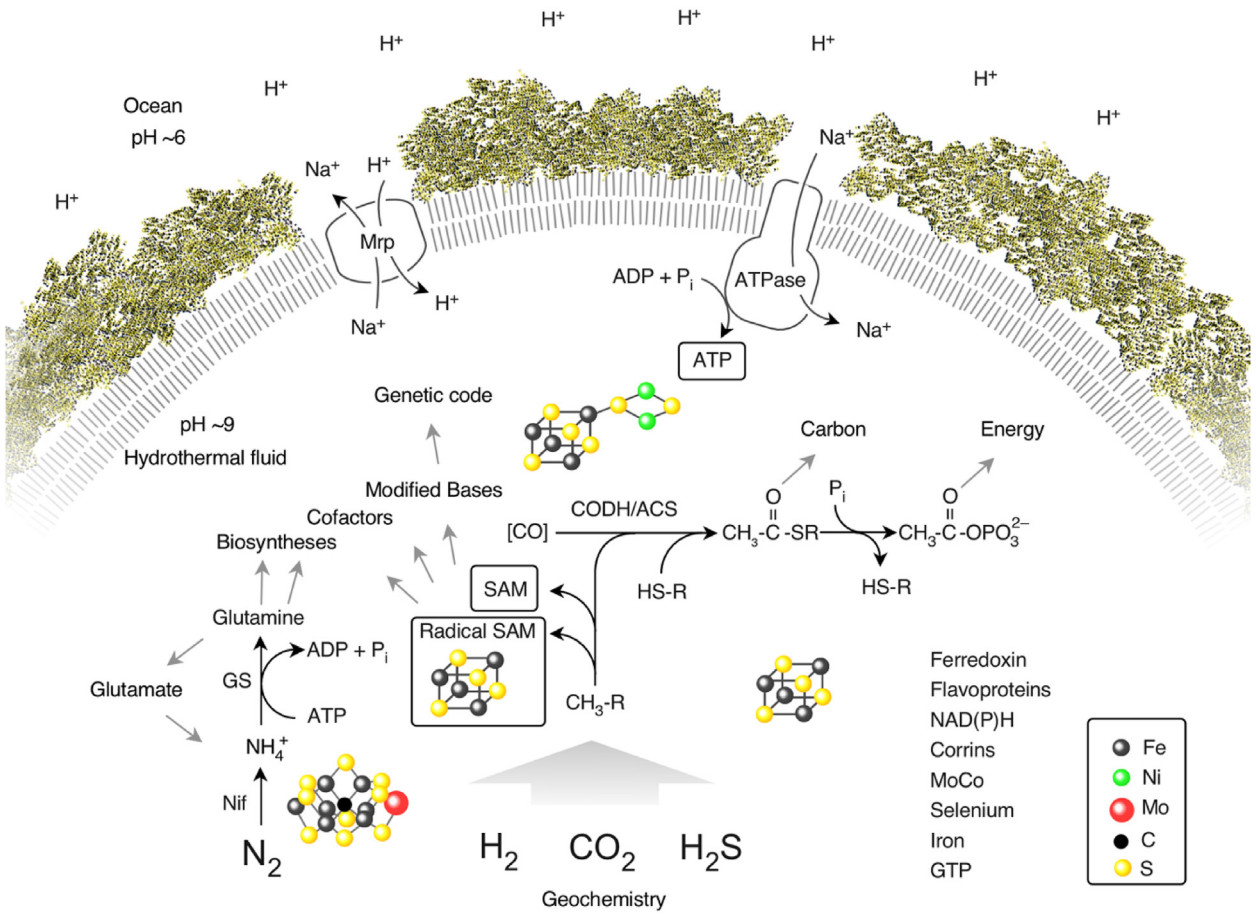

Fig. 2 A geochemical LUCA, as reconstructed in Weiss et al. (2016). LUCA was not a free-living cell; it existed enclosed in pores in hydrothermal vent chimneys. According to this reconstruction, it was a half-alive transition state between the onset of metabolism and free-living cells (Bacteria and Archaea). Reproduced from Weiss et al. (2018) under a creative commons CC BY 4.0 license. © 2018 Weiss et al.

Some common cofactors bound by the proteins of LUCA include iron-sulfur clusters, which are known for their role in electron transfer in proteins, and the organic cofactor *S*-adenosyl methionine (SAM) which enables the transfer of methyl groups, $-CH_3$, between compounds, sometimes involving a radical mechanism. Several enzymes that catalyze the introduction of chemical modifications (with methyl group additions being the most common) on nucleobases that make up RNA nucleotides were also identified. This points to the antiquity of RNA base modifications, which is not surprising, as RNA modifications are required for codon-anticodon recognition, i.e. for the functioning of the genetic code. It is also possible that direct chemical interactions between the chemical ligands of modified bases preceded the genetic code in primitive translation processes (Müller et al., 2022).

Habitat and Physiology

Hydrothermal Vents and Ancient Microbes

At the bottom of the ocean there are hydrothermal vents that undergo a process called serpentinization. Serpentinization is a geochemical process in which water circulating through the Earth's crust reacts with metal-containing minerals and produces hydrogen (Schrenk et al., 2013). Serpentinizing hydrothermal vents exist today but also existed on the early Earth (Tamblyn and Hermann, 2023). This hydrogen can then be used as an electron donor for reactions that produce simple organic compounds that are identical to intermediates and end products of the acetyl-CoA pathway (Schwander et al., 2023). Serpentinizing systems in deep-sea hydrothermal vents are an environment that provides CO_2 and H_2 for the acetyl-CoA pathway and transition metals for the enzymes' metal centers and cofactors. At present, they are most likely the habitat in which LUCA could have arisen. The ubiquity of methylations in LUCA's RNA and metabolism suggests that the genetic code and protometabolism arose in an environment where reduced C1 intermediates (such as methyl groups) were readily available. RNA methylations in LUCA (and modern cells) reflect the chemical nature of the environment where the genetic code arose. This is in agreement with previous proposals of deep-sea vents as sites for life's origin and early evolution (Baross and Hoffman, 1985).

Serpentinizing systems provide all essential components for life: carbon, hydrogen, nitrogen, oxygen, phosphorus, sulfur, metals and minerals, as well as energy, temperature, ion and pH gradients (Schwander et al., 2023). Even compounds that were thought difficult to abiotically synthesize such as a nitrogen source for amino acid production and a phosphorus source have been synthesized in a simulated hydrothermal environment through serpentinization by Shang et al. (2023) (ammonia) and Pasek et al. (2013) (phosphite). Under the conditions of serpentinizing hydrothermal vents, the synthesis of amino acids takes place readily (Kaur et al., 2024).

Another strong indicator for serpentinizing hydrothermal vents being the habitat that hosted LUCA is the identification of bacterial acetogens (Clostridia) and archaeal methanogens as the bacterial and archaeal lineages that appeared to be the most ancient based on their basal branching in phylogenetic trees (Weiss et al., 2016), in line with previous proposals (Decker et al., 1970). They are part of microbial communities that inhabit hydrothermal vents today (Colman et al., 2022; Nobu et al., 2023) and are able to employ the acetyl-CoA pathway for both carbon fixation and energy conservation (Schöne et al., 2022). They live off CO_2 and H_2, like LUCA did, and under chemolithoautotrophic theories for the origin of life they represent living fossils, reflecting ancestral metabolic traits.

Geochemical Origins

One can occasionally read that all life depends on energy from the sun, but that is not true because life in serpentinizing hydrothermal vents thrives in total darkness, powered by pure chemical energy, the reaction of H_2 with CO_2. It is true, however, is that all life on Earth depends on organisms that fix CO_2 (also called primary producers). It is therefore likely that life has also started out that way.

CO_2 was made widely available on the early Earth through the Moon-forming impact (Mrnjavac et al., 2023). This planetary collision between the young Earth and a Mars-sized body (Theia) gave origin to the Moon, but also significantly modified the Earth. It melted all of the Earth's mantle, and vaporized ~20% of it, which resulted in a large amount of carbon dioxide degassing into the atmosphere. When the planet cooled down, the water vapor from the atmosphere condensed and rained down to form the oceans. CO_2 from the atmosphere dissolved in the oceans, the same way gasses from the modern atmosphere, such as N_2 and O_2, dissolve in the oceans today. The CO_2 dissolved in the early ocean was made available to react with H_2 on metal catalysts in the Earth's crust. Such reactions have been studied in laboratory experiments, using reactors to simulate temperature and pressure conditions at deep-sea hydrothermal vents. Results show that in the presence of H_2 and metal catalysts (native transition metals Ni^0, Fe^0 or Co^0 or their alloys) that naturally occur in serpentinizing systems, CO_2 is rapidly reduced to formate (an organic acid with one C atom, $HCOO^-$), acetate (an organic acid with 2 C atoms, CH_3COO^-) and pyruvate (an organic acid with 3 C atoms, CH_3COCOO^-) in aqueous solution by hydrogen-driven reactions (Varma et al., 2018; Preiner et al., 2020; Belthle et al., 2022; Beyazay et al., 2023a,b). The reactants (CO_2 and H_2) and organic products derived from CO_2 in the experiments coincide with the substrates, intermediates and products of the acetyl-CoA pathway employed by modern acetogens and methanogens. In addition, the enzymes and cofactors of the acetyl-CoA pathway harbor Fe, Co, and Ni in their active sites. This resemblance implies that the experimentally investigated CO_2 reduction with H_2 might be a true prebiotic analog of the acetyl-CoA pathway. Going one step further, recent work has shown that H_2 in the presence of nickel drives a series of reactions analogous to the microbial reverse Krebs cycle, the metabolic pathway that follows the acetyl-CoA pathway and is fed by its products or their derivatives. The reverse Krebs cycle precursor

generates ketoacids, from which several amino acids are synthesized in the presence of ammonia (Kaur et al., 2024). These reactions occur in one pot under the same reaction conditions, which makes them a natural reaction sequence in a prebiotic setting, as they could have occurred in the same chimney pore of a hydrothermal vent.

Evolving Metabolic Complexity

The first step in the origin of life was generating the first organics from the available inorganic material. As the diversity of organic molecules increased, more chemical transformations became possible, and some small organic molecules started catalyzing reactions, in concert with the metal surfaces, or independently from them. The proposal that surfaces and cofactors predate enzymes is not new (Eakin, 1963). In some cases, metal ions and cofactors act in concert to accelerate chemical reactions, as in the case of non-enzymatic transaminations catalyzed by pyridoxal phosphate, a cofactor that catalyzes the transfer of amino groups, $-NH_2$, to generate amino acids (Dherbassy et al., 2023).

In addition to pyridoxal phosphate, several other biological cofactors have been studied in a prebiotic setting. The redox cofactor NAD is a hydride (H^-) carrier in cells and can be reduced to NADH without enzymes, by hydrogen gas on a surface of solid-state iron, nickel or cobalt (Henriques Pereira et al., 2022). Metals in serpentinizing hydrothermal vents could have catalyzed NAD reduction by H_2 before the origin of enzymes that catalyze this reaction in modern cells.

Another ancient redox carrier in cells is a small protein named ferredoxin. It contains one or more iron-sulfur clusters which can be reduced or oxidized, and it acts as a soluble electron donor, transferring electrons to cellular proteins and their substrates. In acetogens and methanogens it is reduced enzymatically with electrons from H_2, but this reaction cannot proceed directly, because under physiological conditions H_2 is not a strong enough reductant. Cells are forced to employ an intricate mechanism that involves separating the electron pair from hydrogen, with one electron going to a better electron acceptor, which allows the other electron to reduce ferredoxin (Herrmann et al., 2008). It was found, however, that at conditions resembling alkaline hydrothermal vents, ferredoxin can be reduced without enzymes by adding metallic iron and H_2 to the reaction mixture (Brabender et al., 2024). Why is H_2 capable of reducing ferredoxin directly under these conditions, but not in cells? The ability of H_2 to donate electrons depends on temperature and pH. In alkaline hydrothermal vents, the ability of H_2 to donate electrons increases (in chemical terms: its midpoint potential becomes more negative), which makes ferredoxin reduction with H_2 possible (Boyd et al., 2020). This reduction of ferredoxin by H_2 is a (so far rare) example of interaction between a protein and a solid-state metal surface, pointing to a transition phase in early evolution when proteins were present, but were still in contact with metal surfaces, before LUCA's metabolism evolved to become fully independent of the solid state.

At the onset of metabolism, prebiotic chemical reactions were catalyzed by environmental metal surfaces. Such reactions likely brought forth LUCA to start, and as LUCA progressed in complexity, the system slowly transitioned to include organic catalysts generated by the reaction network (Sousa et al., 2015; Xavier et al., 2020), although organic cofactors could not always replace metal catalysts. Metal clusters in the active sites of modern enzymes of the acetyl CoA pathway are likely relics of a metal-catalyzed protometabolism. The increase in the number and complexity of reactions and their products was followed by a similar increase in the diversity and complexity of catalysts. Before LUCA had evolved a protoribosome, amino acids could still be polymerized into oligopeptides of reproducible sequence by non-ribosomal protein synthesis (Lipmann, 1973). Genetic information was probably first encoded in RNA. The origin of a replicating genetic code and protein synthesis on the ribosome allowed standard Darwinian evolution and selection to set in.

The core missing elements for cellularity are cellular membranes. There are indications that LUCA was able to harness ion gradients via the universally conserved rotor-stator ATP synthase (Lane et al., 2010), but no indications that LUCA was able to synthesize lipids (Weiss et al., 2016). As outlined in **Fig. 1**, membranes of archaea and bacteria are chemically different, and are synthesized by different, non-related enzymes. This means that modern membranes likely originated after the two domains separated, in other words, after LUCA. However, simpler membranes separating the system from the surrounding environment before domain separation could have been present. These membranes might have been made up of hydrocarbons or simpler lipids such as fatty acids, which have been shown to form abiotically under alkaline hydrothermal conditions (He et al., 2021; Purvis et al., 2024). These ancestral membranes could have served to compartmentalize reactants and products, maintain ion gradients and embed early membrane proteins. LUCA probably did not harbor fully functional membrane electron transfer chains made up of multiple intricate protein complexes, because these are not conserved across that bacterial-archaeal divide, and LUCA likely powered its ATP synthase with environmental ion gradients. The protein that H_2-dependent chemolithoautotrophic archaea (methanogens) use to generate ion gradients (a methyltransferase called MtrA-H) is not related to the protein that H_2-dependent chemolithoautotrophic bacteria—acetogens—use to generate ion gradients (a NADH:ferredoxin oxidoreductase called Rnf) (Martin and Kleinermanns, 2024). This circumstance, in addition to the independent origins of bacterial and archaeal membrane synthesis, indicates that the process of cellularization and escape occurred independently in the ancestors of bacteria and archaea, the free-living descendants of LUCA.

Conclusion

Defining LUCA and the origins of cells remains a complex but actively studied topic. Approaches to studying the nature of LUCA using gene phylogenies alone are impacted by lateral gene transfer and gene loss, which make it difficult to determine which genes

really originated in LUCA and which were invented later. Part of these problems are rooted in the circumstance that protein sequences are not well conserved for many gene families. There is hope that the use of protein structural information will provide more insights. Genome-based studies point to LUCA's reliance on the acetyl-CoA pathway, a H_2-dependent, chemolithoautotrophic lifestyle and its use of a genetic code that included modified bases in tRNA. Approaches to LUCA that rely on strict conservation of traits in all prokaryotes indicate that LUCA had ribosomes that used GTP during translation, relied upon GTP for ribosome biogenesis and had a rotor-stator ATP synthase that operated in membranes of hydrophobic compounds provided by the environment. LUCA also had the 20 amino acids, the eight main nucleotides of DNA and RNA and the roughly 18 organic cofactors that are universal to modern metabolism. Approaches to studying the nature of LUCA using chemical reactions that simulate the conditions of H_2-producing hydrothermal vents converge on a H_2-dependent chemolithoautotrophic lifestyle and the acetyl-CoA pathway, as suggested by genomic inferences. These studies suggest that LUCA was not a free-living cell, but was half-alive instead in that it had protein synthesis and genes and hence could evolve, but was confined to inorganic compartments and was dependent upon nutrients and energy provided by a serpentinizing hydrothermal system, the catalysts and chemistry of which supported the origin and early evolution of LUCA's metabolism. Ion and pH gradients provided an energy source that early life learned to harness, while temperature gradients could locally increase the concentrations of reactants via a process called thermophoresis (Matreux et al., 2024). These conditions could make some reactions possible that would otherwise be unlikely to occur and could impose a certain degree of specificity and directionality to protometabolic reactions. Serpentinizing systems provide the core elements present in all life, with CO_2 as the main carbon source, as well as metal catalysts and H_2, a strong electron donor. The physiology of LUCA aligns well with laboratory simulations of prebiotic reactions and with the geochemistry of serpentinizing vents.

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