Trends in Biochemical Sciences

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Review

Carbon–Metal Bonds: Rare and Primordial in Metabolism

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Submarine hydrothermal vents are rich in hydrogen (H_2), an ancient source of electrons and chemical energy for life. Geochemical H_2 stems from serpentinization, a process in which rock-bound iron reduces water to H_2. Reactions involving H_2 and carbon dioxide (CO_2) in hydrothermal systems generate abiotic methane and formate; these reactions resemble the core energy metabolism of methanogens and acetogens. These organisms are strict anaerobic autotrophs that inhabit hydrothermal vents and harness energy via H_2-dependent CO_2 reduction. Serpentinization also generates native metals, which can reduce CO_2 to formate and acetate in the laboratory. The enzymes that channel H_2, CO_2, and dinitrogen (N_2) into methanogen and acetogen metabolism are the backbone of the most ancient metabolic pathways. Their active sites share carbon–metal bonds which, although rare in biology, are conserved relics of primordial biochemistry present at the origin of life.

Highlights

The active sites of ancient enzymes that channel CO_2, N_2, and H_2 into ancient metabolism share conserved chemical components: carbon–metal bonds.

Although rare in biology, carbon–metal bonds are common in industrial catalysts and are in everyday materials, such as steel.

In enzymes and cofactors, carbon forms covalent bonds with iron, nickel, and cobalt, transition metals characterized by unfilled *d* electron orbitals.

Of nine carbon–metal bonds in primary metabolism, six can be traced to the last universal common ancestor (LUCA).

Residing at the interface of the environment and metabolism, carbon–metal bonds are relics of primordial chemistry that existed on the early Earth, before the advent of enzymes.

Metabolism Emerged from Geochemical Reactions

Intuition has it that there are some traces of ancient chemical evolution preserved in modern metabolism. This idea is germane to the **continuity thesis** (see Glossary) that unites theories viewing the origin of life as inherently probable because physical and chemical constraints apply uniformly across the transition from inanimate to living matter [1]. As such, continuity is inherent to theories about ancient metabolism that address transitions from inorganic geochemical settings to biochemical processes, because they embrace justified supposition that reactants and catalysts in the former gave rise to reactions in the latter. Life is a chemical reaction. It started out under anaerobic conditions [2] because molecular oxygen is a product of microbial metabolism. If we look for chemical continuity between the geochemical setting where life arose and modern microbial metabolism, there are two places to look: energetics and catalysts. The pursuit of chemical continuity in energetics leads directly to the main exergonic (energy-releasing) reactions (the core **bioenergetic reactions**) that cells harness to conserve energy. There are hundreds of core bioenergetic reactions that anaerobes tap to conserve energy [2,3], but the only environments known that harbor naturally occurring reactions with *bona fide* similarity to bioenergetic reactions are hydrothermal vents. These vents harbor rock–water–carbon interactions that take place deep in the crust of the Earth in the strict absence of oxygen [4–9]. Those geochemical reactions generate large amounts of H_2 that in turn reduces CO_2 to generate formate and methane, which emerge in the vent effluent [4–7]. The CO_2-reducing geochemical reactions share in turn conspicuous similarity to the core bioenergetic reactions of some modern H_2-dependent anaerobes [8,9], the **acetogens** [10] and **methanogens** [11], strictly anaerobic **autotrophs** that satisfy both their carbon and their energy needs from H_2 and CO_2.

Ideas about the continuity of catalysts that promote the chemical reactions of life center around organic cofactors, thioesters, and iron sulfide (FeS) clusters, all of which are presumed to have preceded enzymes in evolution. Organic cofactors are essential to biochemistry [12]. In many metabolic reactions, the cofactor provides the catalysis, the enzyme just holds it in place

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and provides a hydrophobic pocket where cofactor and substrate can react [13]. The popular idea of an RNA world [14] arose around the concept that cofactors preceded enzymes in evolution and were originally connected to RNA molecules as the precursors of proteins [15]. Thioesters have long been recognized as being central to metabolism [8,16]. The thioester bond is long and easily cleaved [17], making them highly reactive [18]. Thioester bonds have a high free energy of hydrolysis (–43 kJ/mol), higher than that of ATP (–31 kJ/mol) [8], which is often generated from thioesters in metabolism. Thioesters are thought to have preceded phosphates as energy currencies in evolution [16,19] and they can be synthesized in the laboratory from carbon monoxide (CO) and methyl sulfide in the presence of FeS [20], compounds that were likely present on the primordial Earth [8]. FeS clusters are traditionally viewed as primitive catalysts in metabolism because they are completely inorganic and because metal sulfides would have been common on the early Earth [21,22]. Reduced FeS clusters are also a currency of chemical energy [23,24], similar to thioesters and ATP. They are also highly enriched in genomic reconstructions of the metabolism of the **last universal common ancestor (LUCA)** [25], which used the acetyl-CoA pathway and lived off gases, harnessing energy by reducing CO_2 with H_2 [26]. Physiology is rich in continuity because some 4 billion years after the origin of life [27] ,the same chemical energy that powered LUCA still fuels the growth of modern methanogens and acetogens that inhabit the crust today [28–32].

If the continuity principle holds at the active sites of enzymes, then enzymes with which anaerobes access CO_2, N_2, and H_2 at the interface between the environment and biology can provide insights into the nature of primordial metabolism. Here, I discuss ancient enzymes at the core of metabolism in autotrophs that harness carbon and energy via the reduction of CO_2 with H_2. I make the case that their shared common structural feature that is otherwise very rare in biology (carbon–metal bonds) reflects chemical reactions and catalysts that were significant in the evolutionary context of the origin of life.

Carbon on the Early Earth: The Starting Point

Any discussion about carbon chemistry before enzymes or biochemistry at the origin of life is aided by considering benchmarks concerning the chemistry of the early Earth, which provides many helpful constraints. First, it constrains the times at which these reactions could have started. The moon-forming impact approximately 4.5 billion years ago is a crucial benchmark because it turned the Earth into a ball of boiling magma of at least 1500°C [33], too hot for any organic compounds or anything resembling life. It is certain that the Earth was completely molten because it is spherical; the moon-forming impact left no crater. Second, it constricts what compounds were initially available. Magma converted carbon on Earth into atmospheric CO_2 [33–35]. With the Earth in a molten state, gravity caused dense material-like **native metals,** such as iron and nickel, to sink to the core, with lighter material, such as silicates, differentiating to the surface [34,35]. By approximately 4.2 billion years ago, the surface had cooled, rock had formed, and water vapor had condensed to oceans [33–35]. Water was drawn into cracks in the crust, became heated at depth and circulated back into the ocean, giving rise to hydrothermal systems. Atmospheric CO_2 became dissolved in the ocean, was sequestered in the crust as carbonates via hydrothermal convection, and then transferred to the mantle via subduction [33–35]. At depths of several kilometers within the crust, water circulating in hydrothermal convective currents began reacting with inexhaustible reserves of iron II [Fe(II)]-containing minerals to initiate an important geochemical process: **serpentinization** [34–36].

During serpentinization, Fe(II) minerals are oxidized by water (H_2O) to generate Fe(III) and H_2 gas. Serpentinization is exergonic in that it releases energy [4,5] in a process that continues to this day [37]. The effluent of modern hydrothermal vents often contains ~10 mM H_2 [38], orders of magnitude more than H_2-dependent microbes require for growth [11]. At some sites, hydrothermal

Glossary

Acetogens: organisms that generate acetate (and water) as the sole end-product of their main bioenergetic reaction (sometimes also called homoacetogens). All acetogens characterized so far belong to the bacteria.

Autotrophy: a trophic (nutritional) mode in which the cell satisfies its carbon needs from CO_2. The opposite of autotrophy is heterotrophy, a trophic mode in which the cell satisfies its carbon needs from reduced organic compounds.

Awaruite: a naturally occurring nickel iron alloy, typically Ni_3Fe, that is formed from the divalent metals in serpentinizing hydrothermal systems, probably during phases of high H_2 production.

Bioenergetic reactions: energy-releasing reactions that cells use to conserve chemical energy. The most common currency of biochemical energy is ATP, but there are other energy currencies used in cell metabolism, including acyl phosphates, thioesters, reduced ferredoxin, or ion gradients.

Continuity thesis: a philosophical construct that separates scientific from vitalistic thought on the nature of self-organization. It unites theories viewing the origin of life as inherently probable based on their shared premise that physical and chemical constraints apply uniformly across the transition from inanimate to living matter. It distinguishes them from theories viewing the origin of life as so improbable that supernatural influence was required for life to arise from the elements on the early Earth.

Electron bifurcation (flavin based): a soluble mechanism of energy conservation that generates reduced ferredoxin as the energy currency. In flavin-based electron bifurcation, an electron pair is split at a flavoprotein. One electron is transferred energetically 'downhill' to an acceptor with a more positive midpoint potential than the donor, the other is transferred energetically 'uphill' to an acceptor with a more negative midpoint potential, a low potential ferredoxin in cases studied so far.

Last universal common ancestor (LUCA): a hypothetical entity, envisaged by some as an organism, envisaged by others as a geochemically supported chemical reaction, that had

effluent also contains abiotic methane and other reduced carbon compounds that result from H_2 interacting with inorganic carbon, such as CO_2, in the crust [4–7,34–38]. Whether with or without enzymes, in the reaction of H_2 with CO_2, the equilibrium lies on the side of reduced carbon compounds [3]. Among the many core bioenergetic reactions known [2], only acetogens [10] and methanogens [11] are known to harness energy solely from the reduction of CO_2 with H_2. The deep biosphere of the Earth is replete with acetogens and methanogens [28–32]. Considering the origins of ancient metabolism from the standpoint of geochemical constraints, the exergonic reduction of CO_2 with H_2 comes into focus as an ancient bioenergetic route [4–7,34–38].

Carbon–Metal Bonds in CO_2 Fixation Pathways

CO_2 fixation also constrains biochemical origins, because, as hinted at earlier, CO_2 was the starting point for biological carbon. Autotrophs, whether they obtain their electrons from H_2 like acetogens and methanogens do, or from other electron donors with the help of chlorophyll-based photosynthesis, comprise the basis of all food chains [39], Among modern microbes, there are six known pathways of biological CO_2 fixation in nature [40] the Calvin cycle, the reverse citric acid (TCA) cycle, and the acetyl-CoA (or Wood–Ljungdahl) pathway [41,42], as well as three pathways described by Georg Fuchs and colleagues: the dicarboxylate/4-hydroxybutyrate cycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, and the 3-hydroxypropionate bi-cycle [43]. Among those six, the acetyl-CoA pathway is the only one that occurs in both archaea and bacteria [40,43], suggesting that it is the most ancient CO_2 fixation pathway. Furthermore, its distribution among acetogens (bacteria) and methanogens (archaea) is not the result of **lateral gene transfer (LGT)**; different C1 carriers are used in their pathways, and the enzymes of the methyl synthesis branch in bacteria are unrelated to those of archaea [44].

Thermodynamics also constrain biochemical origins. The acetyl-CoA pathway is exergonic [41,43] and is used by acetogens and methanogens to generate ion gradients that are harnessed by ATPases to satisfy the core ATP needs of the cell while simultaneously supplying reduced carbon [43]. Although the acetyl-CoA pathway as it occurs in acetogens entails consumption of one ATP at the formyltetrahydrofolate synthase reaction [10], one ATP is generated at the acetate kinase reaction [10], such that acetate synthesis from CO_2 involves no net ATP input, while ions pumped during acetate synthesis reduction fuel ATP synthesis via the F_1F_0 ATP synthase at the plasma membrane [10]. Put simply, the acetyl-CoA pathway is carbon and energy metabolism in one, although its main role in acetogens and methanogens is energy. Approximately 95% of the CO_2 that the acetyl-CoA pathway reduces in acetogens and methanogens leaves the cell in the form of acetate or methane as the end-product of energy harnessing, with CO_2 incorporation as cell mass having a quantitatively lesser role [11,45]. For example, in acetogens, ~24 molecules of CO_2 are excreted as acetate during ATP synthesis for each CO_2 that is incorporated as cell mass [45], whereas, in methanogens, ~20 molecules of methane are synthesized for each CO_2 fixed [11]. By contrast, the other CO_2 fixation pathways require net ATP input, meaning that some independent form of energy metabolism is required to support CO_2 fixation [8,46]; the amounts of energy required by other CO_2 fixation pathways can be found in [46].

The key enzymes of the acetyl-CoA pathway are CO dehydrogenase (CODH) and acetyl-CoA synthase (ACS) [47]. Although there are over 400 known reactions in metabolism that involve the assimilation or dissimilation of CO_2, there is only one portal for CO entry into metabolism: the CODH reaction [48]. CODH synthesizes CO from CO_2 [42,43]. The role of CO in metabolism is specific; energy is released by the acetyl-CoA pathway during the conversion of a CO-derived carbonyl group to a carboxylate [48], while the other CO_2 fixation routes reduce carboxylate groups to carbonyls [40,41,43,46].

the genetic code that gave rise to all life on Earth.

Lateral gene transfer (LGT): inheritance of genes by mechanisms other than simple cell division-coupled chromosome replication, for example by plasmids (conjugation), phage (transduction), or naked DNA uptake (transformation).

Methanogens: organisms that generate methane as the end-product of their main bioenergetic reaction. All methanogens known so far belong to the Archaea.

Native metals: zero valent metals, that is, metals in the elemental state.

Organometallic compounds: substances with one or more covalent bonds between a carbon atom and a metal atom. The corresponding bonds are called organometallic bonds, metal–carbon bonds, or carbon–metal bonds.

Serpentinization: a spontaneous, abiotic, geochemical process in which water circulating through hydrothermal systems is reduced to H_2 by Fe(II)-containing minerals in the crust.

ACS condenses a methyl group and CO to an acetyl group that is covalently bound to the enzyme and removed by the thiol of coenzyme A to yield the energy-rich thioester acetyl-CoA [49,50]. The methyl group is donated to ACS via methylcobalamin in the corrinoid FeS protein, CoFeS [51–54], which performs an unusual metal-to-metal methyl transfer reaction. The teams of Steve Ragsdale and Holger Dobbek have generated high-resolution structures of the active sites of CODH [55–57], ACS [56,58–60], and CoFeS [51,52,54]. Importantly, all three active sites are ancient, tracing to the genome of the LUCA [25]. More importantly, in the context of this paper, all three active sites represent **organometallic** compounds; that is, they contain carbon–metal bonds (Figure 1). The acetyl-CoA pathway can even be emulated by reactions between native metals and CO_2 in laboratory experiments, narrowing the gaps between the geochemical and biochemical reactions of carbon (Box 1).

Ancient Metabolic Networks

The acetyl-CoA pathway requires high concentrations of reduced low potential ferredoxin to operate [10,23,42,43,61–63]. The acetyl-CoA pathway and ferredoxin are both replete with metals, and both figure prominently in studies of biochemical pathway evolution. Metabolic pathways themselves should contain information about how they interacted with, and emerged from, the ancient environment [12]. Each genome encodes a collection of enzymes that supports life, and those enzymes catalyze reactions that connect substrates, generating a metabolic network for each organism. Goldford *et al.* [16] recently probed metabolic networks to see whether there

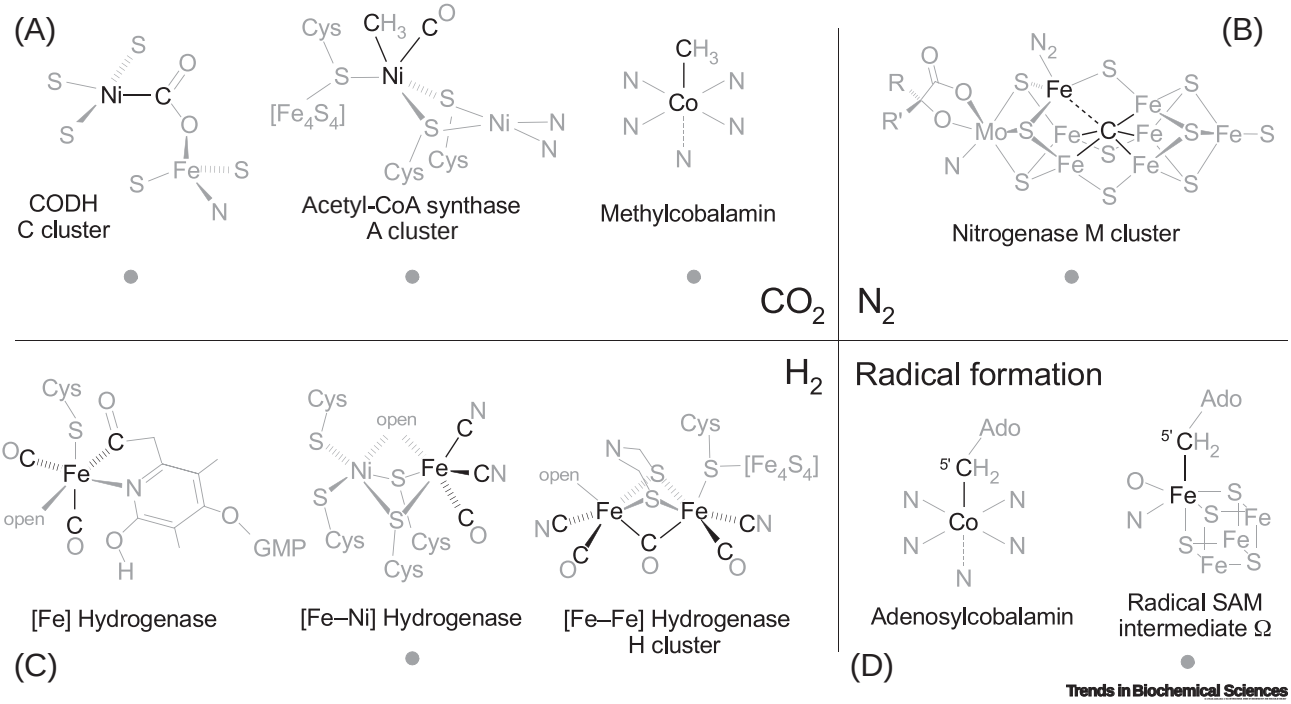

Figure 1. Carbon–Metal Bonds. (A) Carbon dioxide (CO_2) fixation: the structure of the carbon–nickel bond in the carbon monoxide dehydrogenase (CODH) reaction intermediate shown is according to Dobbek *et al.* [55], Jeoung and Dobbeck [59], and Can *et al.* [49,50]. The structure of carbon–nickel bonds in the acetyl-CoA synthase (ACS) A-cluster is redrawn from Svetlitchnyi *et al.* [58] and Can *et al.* [49]. Methylcobalamin, a cofactor in the CO_2 fixation pathway, performs metal-to-metal methyl transfer from corrinoid iron sulfur protein (CoFeS) to the A cluster of ACS [51,52]. (B) N_2 fixation: the structure of the carbon–iron bond in the nitrogenase M cluster is redrawn from Hu and Ribbe [83], Anderson *et al.* [77], Raugei *et al.* [85], and Cao *et al.* [86]. (C) H_2 oxidation: the structures of carbon–iron bonds in [Fe–Ni] hydrogenase [87,88], [Fe] hydrogenase [88,89], and [Fe–Fe] hydrogenase [88,90,91]. (D) Radical formation: the structures of adenosylcobalamin and the radical *S*-adenosyl methionine (SAM) intermediate omega are redrawn from [93,99,100]. Active sites and cofactors that trace to the last universal common ancestor (LUCA) [25] are indicated with a dot. Carbon–metal bonds are highlighted in black.

Box 1. Native Metals in Ancient Metabolism

If microbial physiology arose from inorganically catalyzed acetate and methane-forming reactions at hydrothermal vents [7,8], then inorganically catalyzed acetate and methane synthesis reactions should take place in the laboratory without the help of cells. Although hydrothermal vents naturally generate methane, formate [4,5], and other organic compounds [37] under conditions where geochemical H_2 synthesis is active, laboratory simulations of such reactions using iron minerals have traditionally delivered slow rates and steep challenges [7].

However, in the presence of native metals, the corresponding organic syntheses in the laboratory become facile. Native iron, a pure metal, efficiently generates acetate and formate in laboratory experiments [111,112]. Moreover, Varma *et al.* recently showed that both native nickel and native iron catalyze the synthesis of formate, methyl groups, acetate, and pyruvate in micromolar to millimolar concentrations, rather exactly retracing the steps of the acetyl-CoA pathway [113]. When Varma *et al.* performed the reactions, the reaction products remained bound to the surface of the metals, such that they had to be cleaved by alkaline lysis to be measured; however, it was not determined whether the products were bound to the metals by carbon–metal or by oxygen–metal bonds [113]. Additionally, the natural iron nickel alloy **awaruite** (Ni_3Fe) has been shown to catalyze the synthesis of methane from CO_2 and H_2 at temperatures between 200°C and 400°C [114]. Native metals have interesting properties in the context of early metabolic evolution [114]. Furthermore, native iron can serve as the electron donor for the growth of both methanogens [116,117] and acetogens [118,119], although the exact mechanism of electron flow from the metal to metabolism remains unresolved [119].

It is possible that native metals are catalyzing the organic syntheses reported in modern hydrothermal vents [36]. Awaruite is common in rocks that host serpentinizing hydrothermal systems, because it is formed during serpentinization in phases where high H_2 partial pressures occur [121]. The reason why native nickel and native iron catalyze the synthesis of intermediates and end-products of the acetyl-CoA pathway [113] more efficiently than Fe(II) minerals, such as FeS [122], is mechanistic: CO_2 reduction in biology occurs via two-electron reactions [43]. Under anaerobic conditions, native iron and nickel readily undergo two-electron reactions, but FeS minerals can only undergo one-electron reactions, Fe(II) to Fe(III) valence changes [22,123], unless external electrical current is applied [124]. Native iron even catalyzes amino acid synthesis [125]. In the presence of native nickel and iron, the acetyl-CoA pathway emerges from H_2O and CO_2 [113], setting it apart from other segments of physiology as a simple and natural starting point for biochemical origins.

are traces of biochemical reactions that might have existed before ATP became the universal energy currency. For that, they looked at metabolic networks to see whether an ancient core remains if all the ATP-dependent reactions are removed. They found such a core comprising 260 metabolites connected by 315 reactions, some of which were still energetically uphill. Those energetically uphill reactions did not involve ATP but did involve two currencies of ancient metabolic energy instead: thioesters and FeS-dependent reactions.

The involvement in ancient core metabolism of thioesters, which have a high free energy of hydrolysis and are an energy currency similar to ATP [16], meshes well with the findings of Semenov *et al.* [18], who constructed thioester-driven systems of oscillating chemical reactions in the laboratory. Furthermore, the involvement of FeS-dependent reactions in the ancient core network [16] fits well with the biochemical axiom that metals and FeS clusters are relics of ancient metabolism [21,22,64–66]. In an origin of life context, FeS clusters are usually discussed as ancient catalysts [22,66–68]. However, their main role in metabolism is not in catalyzing reactions, but rather in enabling electron flow via one-electron-transfer reactions [69,70]. In cells that harness H_2 as a source of electrons for carbon and energy metabolism, electrons enter metabolism via hydrogenases and a soluble FeS protein called ferredoxin, 90% of which is present in the reduced form in growing anaerobes [61] at concentrations of ~80–400 μM [39,71]. The generation of reduced ferredoxin from H_2 is an energetic challenge in its own right and involves the process of flavin-based **electron bifurcation**, which is the most recently recognized mechanism of biological energy conservation [23,24,61–63]. The ancient core metabolic network uncovered by Goldford *et al.* [16] underscores the antiquity of the acetyl-CoA pathway, thioesters, and FeS-dependent reactions at the onset of metabolic evolution.

Tracing Ancient Metabolic Pathways through Genomic Analysis

Genomes harbor another resource that can be tapped to probe ancient evolution: gene sequences that can be used to generate phylogenetic trees. The standard approach to

investigate early evolution with phylogeny has been to make trees of some conserved component of the cell, such as the ribosome, and to map biochemical pathways or physiological traits onto the tree to infer what might be ancient by scoring the properties of the lineages that branch deep. However, LGT often underlies gene distributions among bacteria and archaea [72] and the evolution of genes that define physiological traits [39].

The LGT problem is quantitatively severe [72,73]. A recent study investigated trees for all 250 000 prokaryotic genes families (clusters) from 2000 genomes that are sufficiently conserved to make trees [25]. Approximately 11 000 of the clusters had homologs in bacteria and Archaea, but 97% of those trees uncovered evidence for LGT between bacteria and archaea [25]. The remaining 3% (355 of the 11 000 present in archaea and bacteria) recovered monophyly for archaea and bacteria [25]. That does not mean that those 355 genes were inherited vertically, but it does mean that they were likely present in the common ancestor of bacteria and Archaea, which in current views was the LUCA.

Those 355 genes shed light on the physiology and habitat of LUCA, depicting it as an anaerobic thermophile that was rich in transition metal clusters, one-electron reactions, radical *S*-adenosyl methionine (SAM) enzymes, and methyl transferase reactions [25,26]. Regarding ancient metabolism, genomes point in the same direction as the early Earth environments, energetics, and networks described earlier: LUCA used the acetyl-CoA pathway and lived off gases including H_2, CO_2, CO, and N_2 [25,26]. Moreover, the known organometallic bonds of modern metabolism clearly tend to cluster in LUCA (Figure 1) [25,26].

Carbon–Metal Bonds in Nitrogen Fixation

Cell mass is 50% protein and 20% RNA [74], corresponding to 50% carbon and 10% nitrogen by dry weight. Given that proteins and RNA are indispensable for life, the entry of nitrogen into metabolism is crucial to metabolism, both modern and ancient. While H_2 and CO_2 readily provide carbon energy and electrons for acetogens and methanogens, nitrogen must also be provided for cells to grow. In modern ecosystems, the source of nitrogen is N_2, and there is only one enzyme known that can reduce N_2: nitrogenase. Nitrogenase is an ancient enzyme [75] the subunits of which trace to LUCA [25,76]. There is only one basic type of nitrogenase active site, which can contain molybdenum (Mo), vanadium (V), or Fe [76,77]. The active site of nitrogenase points to a unique biological solution for a hard chemical problem. The center of the nitrogenase active site harbors a carbide carbon atom that is complexed by iron [78,79]. It is the only biological carbide described to date. Its insertion starts from the methyl group of SAM [80,81], an ancient cofactor that also had a central role in the physiology of LUCA [25]. The nitrogenase active site (Figure 1) has a phenomenal spectrum of enzymatic activities. Not only is it the biological model for the Haber–Bosch process of ammonium synthesis from N_2 [36,77], but it also catalyzes industrially relevant reactions involving carbon. These include Fischer–Tropsch reactions (hydrocarbon synthesis from CO_2), although sometimes at low rates, as well as reactions of various organic and inorganic nitrogen compounds [82–84]. Nitrogenase accepts electrons from low potential ferredoxin, which is generated in H_2-dependent diazotrophs via hydrogenase with the help of electron bifurcation [61–63]. According to current structural models [76,77,85,86], the atom that binds N_2 is Fe, not Mo or V (or the Mo-corresponding Fe) in the three nitrogenase isoforms. The carbide, which has not yet been identified in the Fe enzyme [76], is encased in carbon–metal bonds (Figure 1).

Carbon–Metal Bonds in Hydrogen Oxidation

Before the origin of photosynthesis, H_2 was the source of electrons for primary production [39]. These electrons come into metabolism via the action of the enzyme hydrogenase. Three types of hydrogenase active site are known: [Fe–Ni] hydrogenase [87,88], [Fe] hydrogenase [88,89],

and [Fe–Fe] hydrogenase [88,90,91] (Figure 1). The three proteins are structurally unrelated [88] and their active sites are also different and require different maturases [64,91–93]. Specifically, the [Fe–Ni] active site is found in bacteria and archaea, occurring as a functional module in many enzymes, including soluble hydrogenases [87], membrane-bound hydrogenases related to complex I of the respiratory chain [94], H_2-dependent CO_2 reductase [95], and many others [96]. [Fe–Ni] hydrogenase subunits trace to LUCA [25]. [Fe–Fe] hydrogenase has only been found among bacteria [88] and eukaryotes [97]. It has an essential role in electron bifurcation [61], both in the H_2-oxidizing direction in acetogens [10] and in the H_2-producing direction in fermenters [92,98]. [Fe] hydrogenase has only been found among Archaea so far, where it has an important role in methanogenesis [11]. Given that it relates to LUCA, the distributions of [Fe–Fe] hydrogenase (bacteria) and [Fe] hydrogenase (archaea), in light of their crucial role in H_2-dependent acetogenesis and methanogenesis, suggest an origin of these two hydrogenases at the base of the respective domains, subsequent to divergence from LUCA, but before the origin of free-living H_2-dependent cells (Figure 2). Although they have structurally distinct active sites and unrelated polypeptide sequences, all three hydrogenases harbor carbon–metal bonds as ligands (Figure 1), reflecting evolutionary convergence [92] driven by constraints imposed by reaction mechanisms.

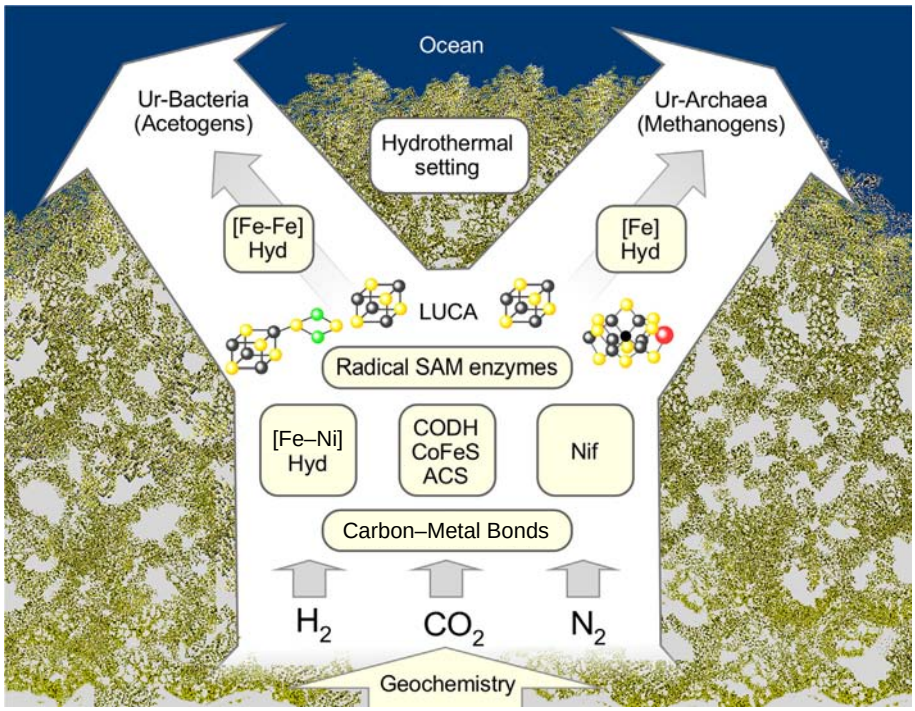

Figure 2. Schematic Depiction of Early Physiological Evolution in a Hydrothermal Setting. Carbon–metal bonds are involved in the entry of hydrogen (H_2) and dinitrogen (N_2) into metabolism, and in the exergonic pathway of biological carbon dioxide (CO_2) reduction. According to genomic studies, the last universal common ancestor (LUCA) may have inhabited a hydrothermal setting and lived off gases [25,26], because several enzymes and cofactors with carbon-metal bonds trace to LUCA. The [Fe] and [Fe–Fe] hydrogenases do not trace to LUCA but are specific to Archaea and bacteria, respectively, where they have essential roles in the carbon and energy metabolism of hydrogenotrophic methanogens and acetogens (see main text). Abbreviations: ACS, acetyl-CoA synthase; CODH, carbon monoxide dehydrogenase; Hyd, hydrogenase; Nif, nitrogenase; CoFeS, corrinoid iron sulfur protein; SAM, *S*-adenosyl methionine; Ur-Archaea, archaeal ancestor; Ur-bacteria, bacterial ancestor ('Ur-' is a German prefix meaning 'primordial').

Carbon–Metal Bonds in Ancient Metabolic Pathways

As described earlier, carbon–metal bonds are found in ancient metabolic pathways, linking H_2, CO_2, and N_2 to life in organisms that synthesize their cell mass from these gases. Although it may appear that carbon–metal bonds are common in prokaryotes, few others have been characterized in primary metabolism and both involve cofactors. One is the first organometallic bond characterized, the classical cobalt–carbon bond in adenosylcobalamin, reported in 1961 by Dorothy Hodgkin [99], and the other is the most recent organometallic bond characterized, an iron–carbon bond in the radical SAM reaction intermediate Ω reported by the team of William and Joan Broderick in 2018 [93,100] (Figure 1). Both of these cofactors are involved in generating radicals for enzymes that catalyze reactions with a radical-dependent mechanism, reactions that are particularly common in cofactor biosynthesis [101,102] and particularly prevalent in LUCA [25].

The collection of carbon–metal bonds in Figure 1 is surely incomplete and others will likely come to light. Since 1985, biologically generated carbon–metal bonds were suggested for the acetyl-CoA pathway [47] which is now known to be replete with carbon–nickel bonds [50]. Carbon–nickel bonds had also been discussed as possible reaction intermediates in the mechanism of methyl coenzyme M reductase [103,104], but recent findings reveal a methyl radical intermediate [104,105]. Carbon–metal bonds have also been proposed in O_2-tolerant, Cu- and Mo-dependent CO-oxidizing enzymes [106], which are unrelated to the anaerobic CODH depicted in Figure 1, but the case is unresolved.

It appears that, in 4 billion years of evolution, biology has not evolved an alternative to the CODH/ACS-dependent acetyl-CoA pathway for exergonic CO_2 fixation that lacks carbon–metal bonds; neither has nature found an alternative to the carbon–metal bond in the active site of nitrogenase to funnel N_2 into metabolism. Nature has also so far not revealed an enzymatic reaction that will extract electrons from H_2 without the participation of carbon–metal bonds. Only three metals, Fe, Co, and Ni, have been observed in organometallic bonds so far and include Ni-carboxyl, Ni-carbonyl, Ni-methyl, and Ni-acetyl in CODH/ACS [49,50,55–59], Co-methyl in CoFeS [51–54], Fe-carbide in nitrogenase [77–79,87], Fe-carbonyl, Fe-acyl, and Fe-nitrile in hydrogenases [87–91], Co-methylene in adenosylcobalamin [99], and Fe-methylene in the SAM intermediate Ω [93,100]. In enzymes and in industry, the catalytic activity of Fe, Co, and Ni resides in the ability of the unpaired electrons in their 3*d* orbitals to undergo back bonding and to forge metastable bonds [48,107].

As microbiologists probe new environments, more carbon–metal bonds might be found. As one possible example, newly characterized acetogens from a serpentinizing system called the Cedars inhabit effluent that is saturated with H_2 [32]. The effluent has a strongly reducing midpoint potential that can reach values of up to –900 mV, easily sufficient to reduce ferredoxin if the right catalysts are available [32]. Although effectively drowning in H_2, the bacteria appear to lack known hydrogenases [32], raising interesting questions, such as whether they access electrons from H_2 by mechanisms that do not require hydrogenases, or whether known hydrogenase side activities of ferredoxin-dependent organometallic enzymes, such as nitrogenase [83] or CODH [108], fill that catalytic void. Alternatively, it is possible that organisms living in environments such as the Cedars have novel hydrogenases that, based on hydrogenases known so far, might be expected to harbor carbon–metal bonds.

Although rare and ancient in biology, carbon–metal bonds are common in chemical industry [36,77,107], they are always around us and we encounter them every day: imagine a life without steel. Steel is iron containing ~0.1–5% carbon atoms (Figure 3) [109,110]. Carbon–metal bonds are what gives steel greater hardness and tensile strength over iron alone; the more carbon steel

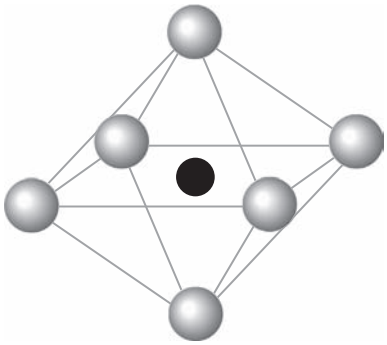

Figure 3. Carbon–Metal Interactions in Steel. The diagram depicts the position of a carbon atom (black) in a lattice of iron (Fe) atoms (gray) in a subsection of a crystal structure for a sample of steel containing a molar ratio of Fe to C atoms of ~24:1. Redrawn after [109,120]. In contrast to Figure 1 (in the main text), the lines in this figure do not indicate bonds, but instead highlight the geometry of elemental iron atoms surrounding an elemental carbon atom in martensite steel [109,120].

contains, the harder and more brittle it becomes [109]. Many experiments investigating CO_2 reduction under hydrothermal conditions have been reported in which the catalysts investigated had small effects, but the steel-walled reactors themselves appeared to be catalytic in the synthesis of a small amount of reduced carbon compounds (reviewed in [110]). In light of the activities associated with carbon–metal bonds and the biological activities of native metals [111–119] (Box 1), the catalytic properties of steel would appear to have more in common with biology than one might think.

Concluding Remarks and Future Perspectives

Carbon–metal bonds in enzymes and cofactors are ancient, few in number, and crucially positioned at the interface of metabolism and the environment; they have been and still are required for the entrance of carbon, nitrogen, and hydrogen gases into metabolism. However, unlike organic cofactors, thioesters, and FeS clusters found in ancient metabolism, organometallic bonds are not universal among microbes, which leads to a variety of questions regarding their mechanisms and evolution (see Outstanding Questions). Given their insuperably primordial position in metabolism, bringing H_2, CO_2, and N_2, to life, carbon–metal bonds in physiology might well be as old as it gets, a kind of biochemical bedrock.

Outstanding Questions

What are the molecular mechanisms that microbes use to access native metals as electron donors for growth? Do the mechanisms involve the formation of carbon–metal bonds?

What is it about carbon–metal bonds in the reactions that channel H_2 and N_2 into metabolism that makes them catalytically indispensable?

Besides Fe, Ni, and Co, are there other metals that forge bonds with carbon during catalysis or promote biogenic catalysis, either within cells or in the environment?

Newly characterized bacteria that inhabit the Cedars, a serpentinizing system saturated with H_2, appear to lack known hydrogenases. How do they generate reduced ferredoxin?

Why is it that [Fe] hydrogenase is specific to Archaea while [Fe–Fe] hydrogenase is specific to bacteria?

Cells that access native metals or that live in saturated H_2 environments might be able to circumvent electron bifurcation as a means of generating low potential ferredoxins. Do such routes exist and, if so, what mechanisms and catalysts are involved?

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