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Chemical Antiquity in Metabolism

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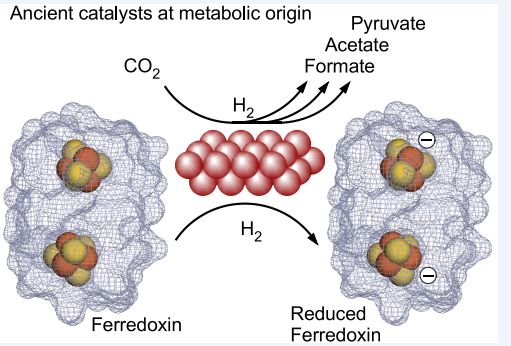
CONSPECTUS: Life is an exergonic chemical reaction. The same was true when the very first cells emerged at life's origin. In order to live, all cells need a source of carbon, energy, and electrons to drive their overall reaction network (metabolism). In most cells, these are separate pathways. There is only one biochemical pathway that serves all three needs simultaneously: the acetyl-CoA pathway of CO_2 fixation. In the acetyl-CoA pathway, electrons from H_2 reduce CO_2 to pyruvate for carbon supply, while methane or acetate synthesis are coupled to energy conservation as ATP. This simplicity and thermodynamic favorability prompted Georg Fuchs and Erhard Stupperich to propose in 1985 that the acetyl-CoA pathway might mark the origin of metabolism, at the same time that Steve Ragsdale and Harland Wood were uncovering catalytic roles for Fe, Co, and Ni in the enzymes of the pathway. Subsequent work has provided strong support for those proposals.



In the presence of Fe, Co, and Ni in their native metallic state as catalysts, aqueous H_2 and CO_2 react specifically to formate, acetate, methane, and pyruvate overnight at 100 °C. These metals (and their alloys) thus replace the function of over 120 enzymes required for the conversion of H_2 and CO_2 to pyruvate via the pathway and its cofactors, an unprecedented set of findings in the study of biochemical evolution. The reactions require alkaline conditions, which promote hydrogen oxidation by proton removal and are naturally generated in serpentinizing (H_2-producing) hydrothermal vents. Serpentinizing hydrothermal vents furthermore produce natural deposits of native Fe, Co, Ni, and their alloys. These are precisely the metals that reduce CO_2 with H_2 in the laboratory; they are also the metals found at the active sites of enzymes in the acetyl-CoA pathway. Iron, cobalt and nickel are relicts of the environments in which metabolism arose, environments that still harbor ancient methane- and acetate-producing autotrophs today. This convergence indicates bedrock-level antiquity for the acetyl-CoA pathway. In acetogens and methanogens growing on H_2 as reductant, the acetyl-CoA pathway requires flavin-based electron bifurcation as a source of reduced ferredoxin (a 4Fe4S cluster-containing protein) in order to function. Recent findings show that H_2 can reduce the 4Fe4S clusters of ferredoxin in the presence of native iron, uncovering an evolutionary precursor of flavin-based electron bifurcation and suggesting an origin of FeS-dependent electron transfer in proteins. Traditionally discussed as catalysts in early evolution, the most common function of FeS clusters in metabolism is one-electron transfer, also in radical SAM enzymes, a large and ancient enzyme family. The cofactors and active sites in enzymes of the acetyl-CoA pathway uncover chemical antiquity in metabolism involving metals, methyl groups, methyl transfer reactions, cobamides, pterins, GTP, *S*-adenosylmethionine, radical SAM enzymes, and carbon−metal bonds. The reaction sequence from H_2 and CO_2 to pyruvate on naturally deposited native metals is maximally simple. It requires neither nitrogen, sulfur, phosphorus, RNA, ion gradients, nor light. Solid-state metal catalysts tether the origin of metabolism to a H_2-producing, serpentinizing hydrothermal vent.

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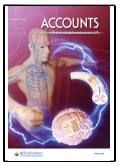

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INTRODUCTION

Life is an exergonic chemical reaction that branches out into roughly 1000 catalyzed and interconnected partial reactions (metabolism) and that, given sufficient reactants, produces a functional copy of its catalyst set (a daughter cell).[4] Understanding how life could have arisen involves forging links between the chemical reactions of living cells and chemical environments on the early Earth. The number of chemical environments on the early Earth where the origin of life was theoretically possible is vast. By contrast, the number of chemical environments on the early Earth that directly interface with the chemical reactions of living cells is limited. The physiology of modern cells can provide constraints that help to discriminate between the kinds of environmental catalysts that could have accelerated the primordial chemical reactions that gave rise to metabolism. Physiology can also help to identify ancient lifestyles, ancient reactions and ancient catalysts in metabolism.[5−7] Because no one was around to observe the origin of life 4 billion years ago, the physicochemical environment of origins is debated. But physiology adds needed constraints. For example, all cells, without exception, have to satisfy their basic requirements for carbon (CO_2 vs organics; autotrophy vs heterotrophy), electron donors (inorganic vs organic; lithotrophy vs organotrophy) and energy (photons vs chemical reactions; phototrophy vs chemotrophy) in order to grow. Given what we know about early Earth environments, which of these combinations is ancient?

Carbon

All ecosystems today start from CO_2 with autotrophs providing the reduced carbon compounds required by heterotrophs. Carbon from space is too reduced and structurally too heterogeneous to support fermentations,[8] and the Moon-forming impact transformed all accreted carbon on the early Earth into CO_2.[9,10] That means that CO_2 was the starting material for the first organic syntheses and the source of carbon for primary producers (autotrophs) that fueled the first ecosystems, the foundation of autotrophic theories for origins.[11−14] The Moon-forming impact generated a primordial atmosphere rich in CO_2 that dissolved in the ocean, generating a pH of roughly 6.5 from carbonic acid.[15] That localizes primordial CO_2 to the Earth's entire surface and oceans, but provides no direct clues about the specific environment where the CO_2 reduction process might have gotten started. The source of reductants narrows down the possibilities.

Electrons

The list of possible reductants for the first organic synthesis from CO_2 and the source of electrons for autotrophic pathways is short. Among the environmentally available reductants on the early Earth, only H_2 has a sufficiently negative midpoint potential to reduce CO_2 directly, but it requires alkaline pH and catalysts in order to do so. High pH (≥8) pulls the equilibrium in the reaction $H_2 \rightarrow 2H^+ + 2e^-$ to the right by removing protons, which shifts the midpoint potential of the H_2 oxidation reaction from −414 mV under standard physiological conditions (25 °C, 1 atm H_2, pH 7) to −533 mV (25 °C, 1 atm H_2) at pH 9, for example, using the Nernst equation. The midpoint potential of CO_2 reduction to formate is on the order of −430 mV.[16] In the absence of catalysts, H_2 in alkaline water will not react with dissolved CO_2 at significant rates. But if the solid-state transition metals Fe^0, Co^0 or Ni^0 or their alloys are included as catalysts for the reaction, H_2 readily chemisorbs onto the metal surface and quickly reduces CO_2 to a modest spectrum of specific organic compounds.[2,17−20] The reducing power of H_2 links the site of organic synthesis to the proximity of serpentinizing hydrothermal systems,[21,22] which naturally generate alkaline effluent (pH 9−11) with concentrations of H_2 reaching 10 mM or more.[23] The requirement for a solid-state catalyst (and high pH) furthermore ties organic synthesis rather specifically to the physical site of a serpentinizing vent, because the reducing conditions of serpentinizing systems actively deposit the native metals Fe^0, Co^0, Ni^0 and their alloys,[24] thereby providing the diffusible reductant (H_2, an inorganic electron donor), the alkaline pH needed to favor H_2 oxidation, and the native metal catalysts that allow chemisorbed H_2 and CO_2 to react.

Energy

All cells have a main exergonic chemical reaction that allows the cell to conserve energy. At origins, the first reactions to synthesize organics also had to be exergonic, otherwise the reactions would not have gone forward. Most autotrophs have separate and independent pathways of carbon and energy metabolism, with ATP from the latter energetically financing the former.[25] The search for ancient forms of energy metabolism is relatively simple because there are only two lineages of microbes that combine carbon and energy metabolism, obtaining their ATP from the reduction of CO_2 with H_2. They are strict anaerobes and they both employ the most ancient among CO_2 fixing pathways, the acetyl-CoA pathway:[12,14,26] acetogens and methanogens. In acetogen and methanogen metabolism, the main intermediates and products of the acetyl-CoA pathway are formate (an intermediate), pyruvate (the main source of carbon for biosynthesis), acetate and methane (the end products of acetogens and methanogens, respectively).[27,28] The acetyl-CoA pathway is linear, exergonic, the only CO_2-fixing pathway that occurs in bacteria and archaea[12,14] and can simultaneously support CO_2 fixation and ATP synthesis, and it is the only multistep biochemical pathway that can be replaced entirely by single metals as catalysts. The acetyl-CoA pathway requires a total of 127 enzymes—about 20 enzymes of the pathway itself (in acetogens and methanogens) plus >100 enzymes for the synthesis of required cofactors.[10] That massive enzymatic demand might not seem ancient at first sight. But solid-state

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Fe^0, Co^0 or Ni^0 or their alloys—as sole catalysts—convert H_2 and CO_2 to formate, acetate, pyruvate and methane overnight in alkaline water at 25−100 °C[2,17−20,29] (Figure 1). The

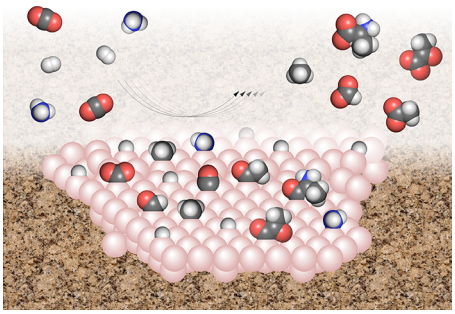

Figure 1. Schematic showing synthesis of methane, formate, acetate, pyruvate, and alanine on native metal catalysts deposited on mineral surfaces in hydrothermal pores at the onset of metabolism. The corresponding reactions (synthesis of formate, acetate and pyruvate from H_2 and CO_2, see text) including amino acid synthesis from 2-oxo acids, NH_3 and H_2,[30] symbolized here with alanine, take place in laboratory experiments, whereby the exact nature of the metal-bound intermediates is not known, although chemisorption of H_2 onto transition metal surfaces and the subsequent diffusion of hydrogen atoms on metal surfaces are well studied.[31] In the laboratory, Fe, Co, Ni and their alloys are catalytically active, the metal surface sketched represents generic transition metals. The scheme is based on the mechanisms proposed by Varma et al.[29] and Preiner et al.[2] and the findings of Kaur et al.[30]

reactions go forward because they are exergonic.[2,27,28] As a source of energy and physiologically central carbon compounds, the acetyl-CoA pathway is ancient. Its requirement for enzymes and cofactors in cells does not preclude its antiquity, because enzymes, once they arose in chemical evolution, merely accelerate reactions that tend to occur anyway: The exergonic chemical reactions catalyzed by ancient enzymes are older than the enzymes themselves.

Catalysis

In order to harness carbon, energy and electrons for synthesis, microbial metabolism requires enzymes as catalysts. The scheme in Figure 1 depicts recently observed[2,17−20,29,30] product formation in reactions of H_2 and CO_2 catalyzed by native metal surfaces on an inorganic support, which could represent a silicate support for a heterogeneous catalyst in the laboratory[17,20,30] or an environmental (host) rock in a serpentinizing environment[21−24] with the inorganic catalysts serving as precursors for similar reactions that later came to be catalyzed by enzymes.[26] In practice, solid-state catalysis is not as simple as sketched in Figure 1, as recent work by the team of Harun Tüysüz underscores. They have performed many such reactions, obtaining various amounts of formate, acetate, pyruvate under a variety of conditions with characterization of the surface of Fe, Co, Ni, and alloy catalysts post-reaction.[2,17−20] Across several independent studies, they found no significant surface alteration in most cases in particular when H_2 was used as reductant, but in the absence of H_2 evidence of oxide or oxyhydroxide formation in some cases, metal dissolution in some cases, and metal carbonate formation in others could be observed.[2,17−20] The degree to which the catalysts are altered can depend upon pH, temperature, time, pressure, the presence of H_2, the metals themselves, and whether different metals are alloyed or simply mixed.

In some combinations of catalysts and conditions, the CO_2-reducing reactions go forward without the addition of exogenous H_2 whereby the metal serves as reductant. For example, using Ni_3Fe as catalyst, CO_2 reduction to organic acids was observed[18] without exogenous H_2, though with yields that were lower by an order of magnitude or more compared to those obtained with the addition of H_2. In the absence of exogenous H_2, the reactions can be accompanied by dissolution of Fe as Fe^{2+} from the Ni_3Fe alloy, which is not observed when H_2 is supplied as reductant.[2,18] Varma et al.[29] reported efficient CO_2 reduction in the absence of exogenous H_2, where Fe^0 was serving as reductant, probably generating H_2 from water on the catalyst surface especially at high pH.[1,2] Reaction mechanisms on the surface of heterogeneous catalysts are notoriously difficult to study, but Henriques Pereira et al.[32] were able to show using ^2H_2O (D_2O) and $^1H_2$ gas with NAD^+ as an electron acceptor that Ni^0 acts as a true catalyst, generating NAD^1H, while Fe^0 can generate NAD^2H from D_2O in addition to NAD^1H from $^1H_2$. A complicating aspect is that the standard midpoint potentials at pH 7 for H_2 formation from water (−414 mV),[16] CO_2 reduction to formate (−430 mV),[16] and Fe^{2+} reduction to Fe^0 (−440 mV)[18] are not only very close to one another, but also vary with temperature, pressure and in particular pH so as to overlap in some conditions but not in others. A recent report[33] stated that several studies surveyed here provided no evidence for catalysis, because the turnover numbers they calculated were <1; however, they counted each atom of solid-state catalysts as "catalytically" active,[33] regardless of particle size. For H_2 activation, about 1% of the atoms on the surface of a typical heterogeneous metal catalyst such as iron are active,[34] and for 25 nm nanoparticles, only about 4% of the atoms are on the surface (H. Tüysüz, Personal communication); accordingly, the turnover numbers calculated[33] were roughly 1000-fold too low for the solid-state catalysts considered here when H_2 was used as the reductant. The implementation of heterogeneous catalysis for organic synthesis is still new in the origins field. It opens up many new avenues of pursuit, and it can be implemented using substrates, catalysts and products that align well with chemistry observed at H_2-producing hydrothermal vents.[23]

METALS INSTEAD OF FeS MINERALS

Conventional wisdom has it that early in biochemical evolution, inorganic surfaces served as catalysts that preceded cofactors and enzymes.[35] Much modern thinking on early biochemical evolution is centered around the idea that such inorganic surfaces were environmentally formed FeS minerals which served as ancient catalysts that preceded FeS clusters in evolution. This idea stems from the discovery of ferredoxin,[36] the first protein found to contain covalent Fe−S bonds[37] and FeS clusters. Ferredoxin is the strongest long-lived reductant in the cytosol and the main soluble one-electron carrier in anaerobes.[16] For decades, ferredoxin was implicated in the origin of metabolism as a link between the organic and inorganic worlds.[5,6,38] However, the surfaces that functionally predate the acetyl-CoA pathway (Figure 1) do not consist of iron sulfides, they consist of pure metals and the reactions do not require the presence of sulfur in any form.

The native transition metals that catalytically substitute for the entire acetyl-CoA pathway are exactly the same ones that

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Acc. Chem. Res. 2024, 57, 2267−2278

are coordinated in the active sites of the modern enzymes in this ancient pathway: Fe, Co and Ni (Figure 2). Nickel is

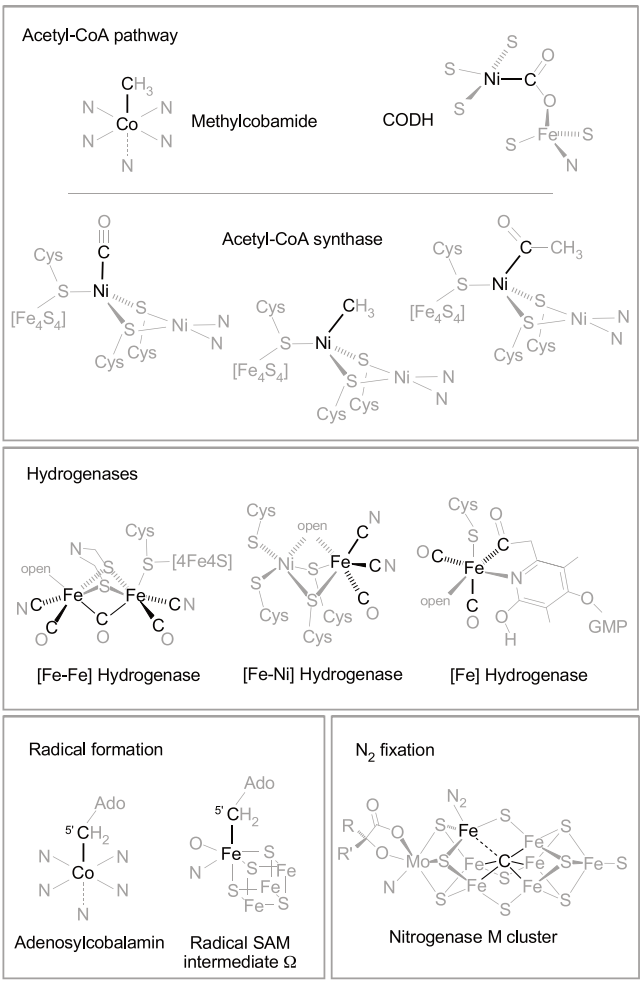

Figure 2. Carbon−metal bonds in active sites of ancient enzymes. The structures are from refs 39, 43, 44, 46−48, 51, and 52. The three intermediates in the acetyl-CoA synthase reaction that have been spectroscopically captured are from refs 41−43.

catalytically active in carbon monoxide dehydrogenase,[39] acetyl-CoA synthase,[40−43] [FeNi] hydrogenase,[44] and the Ni-containing tetrapyrrole F_{430} used in the final methane-synthesizing step of methanogenesis.[45] Cobalt is used in the methyl-transferring cobamide cofactor of the corrinoid iron−sulfur protein CoFeS[46] that donates methyl groups to acetyl-CoA synthase. Iron is used in [FeFe] hydrogenase[47] and [Fe] hydrogenase.[48] These ancient enzymes generate covalent bonds between carbon and the active site transition metal during the reaction mechanism, or harbor carbon−metal bonds in active site ligands (Figure 2). While iron sulfur clusters are common in proteins,[49] carbon−metal bonds are generally rare in biological reactions. Those involved in core carbon metabolism in acetogens and methanogens are ancient[50] (Figure 2), relicts from a time when the onset of metabolism (Figure 1) involved native metals as catalysts.

Of the structures shown in Figure 2, in addition to the metal cluster prosthetic groups shown, corrins and *S*-adenosylmethionine (SAM), acting through the radical SAM intermediate Ω,[52] are cofactors required by acetogens and methanogens. Several of the structures have an additional ancient property in common in that radical SAM enzymes are involved in their synthesis: the corrin backbone of cobalamin, the iron-guanylyl pyridinol cofactor of [Fe] hydrogenase, the active site of nitrogenase and of [Fe−Fe] hydrogenase all require radical SAM enzymes for the synthesis of the active cofactor.[53−56] Radical SAM enzymes harbor a 4Fe4S cluster that coordinates *S*-adenosylmethionine (SAM) via its amino and carboxyl groups. The 4Fe4S cluster in the enzyme initiates radical formation via the covalent intermediate Ω,[57] followed by homolytic cleavage of the carbon−iron bond to yield a 5′-deoxyadenosyl radical (5′dAdo•) that typically abstracts H• from the substrate to initiate the reaction mechanism.

All radical SAM enzymes contain a 4Fe4S cluster. They are reported as the largest enzyme family known[57] and in all cases, the function of the 4Fe4S cluster is one-electron transfer that forms the 5′dAdo• radical that initiates the radical reaction mechanism with the substrate. This kind of radical-initiating, one-electron transfer is a fundamentally different function from the myriad catalytic and electrostatic surface-binding functions initially envisioned for FeS minerals in the FeS world proposed by Wächtershäuser.[58] The metal-catalyzed acetyl-CoA pathway from H_2 and CO_2 to pyruvate (Figure 1) also departs sharply from Wächtershäuser's iron−sulfur world proposal in that (i) the reactions lack sulfur altogether, (ii) the primordial CO_2 fixation pathway is the acetyl-CoA pathway, not a reverse TCA cycle consisting of thioacids,[58] and (iii) the reductant is H_2, which was categorically excluded as the initial electron source "*since its reducing potential is not sufficient for reducing* CO_2".[59]

The midpoint potential of H_2 is a crucial parameter in biological H_2 utilization. How cells use H_2 as a reductant in the acetyl-CoA pathway was a puzzle for several decades because under standard conditions the midpoint potential of H_2 (−414 mV)[16] does indeed require electrons to flow energetically uphill to CO_2 (−430 mV),[16] even more so to reduce ferredoxin (−450 mV),[16] which is the physiological donor for CO_2 reduction in cells that use the acetyl-CoA pathway.[60] Yet modern cells readily use H_2 as a reductant for CO_2, whereby acetogens and methanogens even obtain energy from CO_2 reduction with H_2. Physiology always obeys the laws of thermodynamics. To reduce CO_2 with electrons from H_2, cells employ an elegant thermodynamic mechanism called flavin-based electron bifurcation, which splits the electron pair in H_2, sending one electron uphill to ferredoxin and the other downhill to a high potential acceptor (like NAD^+ or CoM−S−S−CoB) so that the overall reaction of H_2-dependent CO_2 reduction is exergonic.[61] Before the existence of enzymes, electron bifurcation was not required, because H_2 readily reduces CO_2 to formate, acetate and pyruvate in the presence of native Fe, Co and Ni catalysts at alkaline pH (Figure 1). But the acetyl-CoA pathway employs ferredoxin-dependent enzymes.[62] The electron donor for those enzymes is always reduced ferredoxin with a midpoint potential of near −500 mV.[16,61,63,64] How did metabolism generate reduced ferredoxin before the origin of the complex protein machinery involved in electron bifurcation? Again, native metals, which are naturally deposited in hydrothermal vents,[23] can replace enzymes for this ancient reaction. Brabender et al.[1] recently showed that H_2 can reduce *Clostridium pasteurianum* ferredoxin in the presence of Fe^0 as catalyst.

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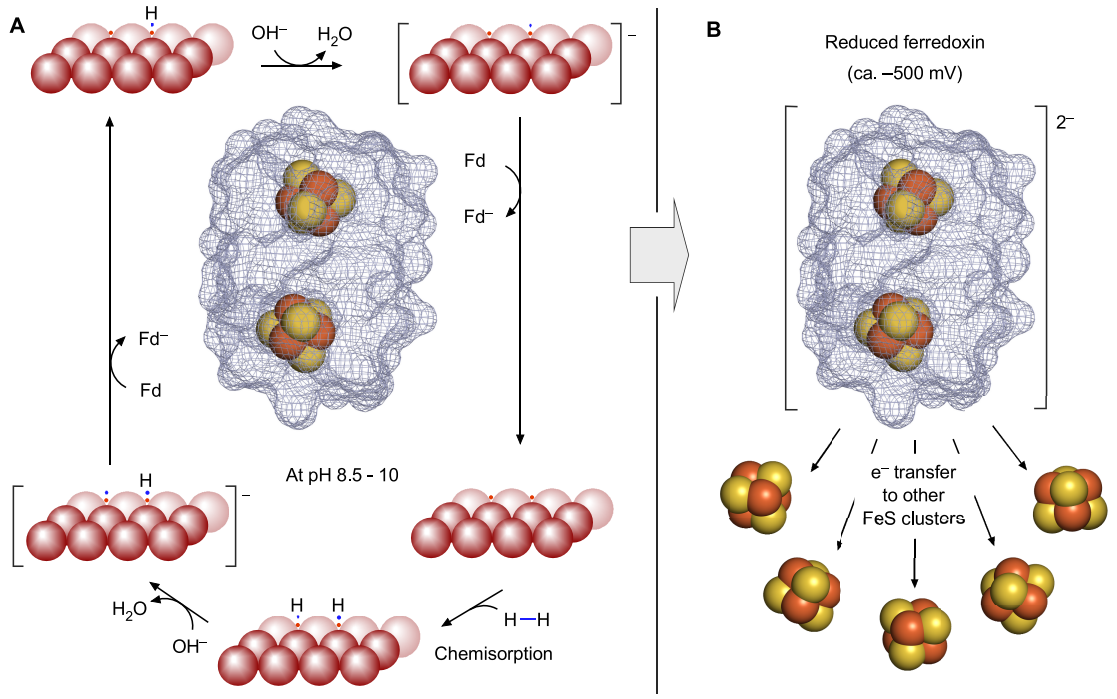

Figure 3. Proposal for the role of ferredoxin in early evolution. **A**. Proposed schematic mechanism for ferredoxin reduction by H_2 over Fe^0 in alkaline (pH 8.5 to 10) conditions.[1] Electrons indicated as small dots are solely for illustration purposes. Because iron conducts, the site of H_2 oxidation and ferredoxin (Fd) reduction need not be identical. The figure makes no statement about physical position of H atoms on the metal surface, distance between metal surface and Fd, or rates of electron transfer. **B**. A primordial role of ferredoxin could have been to transfer electrons from hydride-bearing metal surfaces (solid-state hydrogenases) to the aqueous phase as a soluble electron carrier for enzymes requiring a strong electron donor (see text). Though sometimes referred to as a catalyst in older literature,[6] ferredoxin is not a catalyst, it is an electron donor and acceptor for enzymatic reactions involving FeS clusters or cofactors[1] (in particular flavins, which transduce one-electron and two-electron transfers in metabolism).[16]

ELECTRON TRANSFER: THE PRIMORDIAL FUNCTION OF FeS CLUSTERS

Although cells can encounter extremely strong reductants in the environment, for example phosphite,[65] ferredoxin is the strongest reductant that is generated during metabolism of anaerobic chemotrophic cells and it requires electron bifurcation for synthesis.[16,64,66] From the 4Fe4S clusters of reduced low potential ferredoxin, electron transfer to all other FeS clusters in metabolism is energetically downhill. As with CO_2 reduction (Figure 1), the mechanism of ferredoxin reduction by H_2 and Fe^0 is not known, but one simple possibility is outlined in Figure 3A, involving chemisorption of H_2 onto the iron surface, H^+ removal by the alkaline aqueous phase, and electron transfer to ferredoxin.

The transfer of electrons from the solid-state metal surface to the 4Fe4S clusters of ferredoxin in solution[1] probably involves similar mechanisms as occur between FeS clusters in proteins: tunneling.[67] This is schematically drawn in Figure 3A. Though FeS clusters are traditionally discussed in the context of catalysis in early evolution, in modern metabolism their main function is electron transfer: in radical SAM enzymes and in redox enzymes generally. A striking example is the enzyme formylmethanofuran dehydrogenase, which contains 46 4Fe4S clusters in the structure of the active enzyme[68] that serve as electron conduits from reduced ferredoxin to the active site.

The ability of Fe^0 to catalyze the transfer of electrons from H_2 to ferredoxin suggests the existence of an intermediate state in early biochemical evolution in which ferredoxin became a soluble and diffusible single electron donor for early redox-dependent processes (Figure 3B), including CO_2 reduction via the acetyl-CoA pathway and N_2 reduction via nitrogenase, such that reactions that were once physically tied to the Earth's crust by virtue of a solid-state catalyst[1] became soluble, hence exportable from the site of life's origin to life as a free-living cell. Before the origin of free-living cells, other ancient proteins with FeS clusters in addition to ferredoxin, such as enzymes of the acetyl-CoA pathway,[1] could have interacted directly with hydride-laden metal surfaces, in a similar manner as sketched for ferredoxin in Figure 3A. If metabolism arose via redox reactions catalyzed on surfaces of Fe^0, Co^0 and Ni^0 (Figure 1), the first main function of FeS proteins would not have been catalysis, but the same as it is today: single electron transfer (Figure 3A and B).

THE BIOSYNTHETIC CORE OF 400 REACTIONS

In order for the first free-living cells to emerge, they had to be able to synthesize all of the essential building blocks of life in stoichiometrically useful amounts and more or less specific form, meaning that the rates of the reactions that generate the building blocks of life had to become similar by virtue of catalysts.[69] How many organic catalysts did that involve? Starting from H_2, CO_2, NH_3, H_2S, P_i, and water and salts, the synthesis of the 20 canonical amino acids, the eight nucleobases of DNA and RNA (excluding modifications), and the 18 main cofactors used by modern cells involves about 400 reactions that are thermodynamically favorable under the reducing conditions of serpentinizing hydrothermal vents.[3] One can collectively designate those reactions as the autotrophic biosynthetic core.[70] The biosynthetic core does

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not include synthesis of the ribosome,[71] tRNA modifications[72] or nucleic acid handling, it just comprises synthesis of the monomeric components.

The reverse citric acid cycle (rTCA) is a central hub of primordial metabolism as it provides the carbon backbones for amino acid synthesis, which in turn are the starting point for cofactor and nucleobase biosynthesis. Almost all of the reactions of the reverse citric acid cycle have been shown in recent work by Joseph Moran's group to operate in water using only metals and metal ions as catalysts.[30,73−75] Notably, the amino group of amino acids is almost always added as the last step in amino acid synthesis via reductive amination of the 2-oxo moiety of the corresponding 2-oxo acid. That can occur without enzymes using pyridoxal phosphate, by using metal catalysts via transamination reactions[76,77] or by using NH_3 and H_2 in the presence of Ni^0 as a solid-state catalyst in water at 25 °C, generating high yields and specific synthesis of 10 different amino acids from their 2-oxo precursors.[30]

We looked among the 400 reactions of the autotrophic core[3] for ancient traits in the form of FeS clusters and radical reactions. We identified 44 reactions catalyzed by FeS proteins (Supplementary Table 1), which is a conservative number due to database incompleteness and lack of characterization for all proteins underlying the reactions. Notwithstanding, FeS proteins were found to be involved in a wide range of core metabolic pathways, spanning from H_2 oxidation to central carbon metabolism including the acetyl-CoA pathway and the reverse tricarboxylic acid cycle, to cofactor biosynthesis (in the pathways for biotin, coenzyme B, cobalamin, coenzymes F_{420} and F_{430}, MoCo, NAD, thiamine diphosphate and tetrahydromethanopterin), amino acid and nucleobase biosynthesis. In *E. coli* about 2−3% of all proteins have been described as FeS proteins, the vast majority adopting a [4Fe4S] geometry,[49,78] the estimate increasing to roughly 5% when including candidate cases.[49] About 11% of reactions in the autotrophic core involve enzymes with FeS clusters. Nonetheless, the overrepresentation is substantial and not unexpected, as redox reactions are also overrepresented in the autotrophic core,[70] the reason being that carbon in CO_2 has to be reduced to the state of alcohols, carbonyls, methenyl, methylene, and methyl groups to make amino acids, cofactors and bases, while peripheral metabolism predominantly involves polymerization reactions of monomers, conjugations, acetal and hemiacetal formations, water eliminations and the like, which are typically redox neutral. Phosphorylations are also almost always redox neutral in metabolism, with one recently characterized and possibly very ancient exception involving ADP synthesis from AMP and phosphite (HPO_3^{2-}) using NAD^+ as the electron acceptor.[65]

Enzymes containing FeS clusters typically require FeS cluster assembly proteins—the Nif, Suf or Isc systems[79]—which incorporate iron as Fe^{2+} from the cytosol and S from cysteine into enzymes typically via cysteine sulfhydryl ligands. Enzymes of the Nif, Suf and Isc systems[79] typically contain FeS clusters, which are not counted here. For the synthesis of some FeS clusters, such as those in the active site of [FeFe] hydrogenase, additional FeS cluster-containing maturases are involved,[54] they are also not counted here. Among the 44 reactions involving FeS proteins, 10 of them were identified as SAM-dependent (~23%), with the FeS cluster initiating a radical reaction in almost all cases (Supplementary Table 1 and Supplementary Table 2).

RADICAL REACTIONS

A closer look into the autotrophic core reaction network reveals 14 reactions employing a radical mechanism (Supplementary Table 2), a conservative estimate, as databases are not complete and the properties of the enzymes are not known in all cases. It is generally held that radical enzymes are employed to catalyze demanding reactions to which there is no mechanistic alternative.[53,80] Radicals are highly reactive due to their unpaired electron, and can wreak havoc among molecules of the cell if not properly sequestered and controlled. In enzymes, radicals are generated by homolytic bond cleavage or one-electron transfer from metals and metal clusters or other one-electron carriers.

Radical SAM (rSAM) enzymes are the most common radical enzymes in the autotrophic core (Supplementary Table 2). The generation of the 5′−deoxyadenosyl radical (5′dAdo•) is initiated by one-electron transfer from a [4Fe4S] cluster to SAM, with the cytosolic electron donor being ferredoxin or flavodoxin.[80] The radical can act directly on the substrate, or induce a glycyl radical on the protein. Radical SAM enzymes are ancient, dating back to LUCA,[81] and possibly belonging to the first enzyme families.[53] It is therefore not surprising they accept an electron from ferredoxin, which is also ancient[5,6,38] and can acquire electrons directly from metal surfaces under H_2[1] (Figure 3). In modern *E. coli* cells, rSAM enzymes comprise over 10% of all iron−sulfur proteins,[49] and new ones are constantly being discovered. They are versatile and fulfill diverse cellular functions; they are known to take part in central pathways such as the biosynthesis of the [FeFe] hydrogenase H-cluster, the nitrogenase active site, the molybdopterin cofactor, thiamine diphosphate, biotin, modified tetrapyrroles, but also in RNA modification, DNA repair and others.[53] These pathways are essential to and had to exist before the first free living cells emerged.[70,81] They are ancient.

The energetic barrier for one-electron transfer to SAM is high. In enzymes this is compensated by coordination to the FeS cluster, active site polarity and other solutions.[53] In a prebiotic world it is possible that one-electron reductions of a small organic molecule (SAM) were easier to achieve, with iron-dependent mechanisms perhaps similar to that sketched in Figure 3. Although there are striking similarities between the B_{12} radical reactions and those catalyzed by radical SAM enzymes,[53,82] B_{12} is involved in only about 12 reaction classes in all of known metabolism,[53] while the rSAM family catalyzes over 100 different documented chemical reactions.[57] In adenosylcobalamin-dependent enzymes, the same 5′−deoxyadenosyl radical as the one derived from SAM is induced by homolytic bond cleavage of a cobalt−carbon bond.[80] Radical SAM reactions proceed through an organometallic intermediate called Ω, with a covalent bond being formed between iron of the [4Fe4S] cluster and SAM.[83] Homolytic cleavage of the Fe−C bond liberates the radical, in a similar fashion as for B_{12}, narrowing the gap between the mechanism employed by rSAM and B_{12} enzymes.[57]

One radical reaction that is not formally part of the biosynthetic core,[3] because it is not part of an amino acid, base or cofactor biosynthetic pathway, is included in this analysis nonetheless: methyl-CoM reductase (MCR). MCR catalyzes the terminal step in the methanogenic pathway[28] via a methyl radical intermediate[45] formed at the active site by a Ni-containing tetrapyrrole, F_{430}. By generating methane, MCR catalyzes a radical pulling reaction ($\Delta G^{\circ\prime} = -30$ kJ·mol^{-1})[28]

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Acc. Chem. Res. 2024, 57, 2267−2278

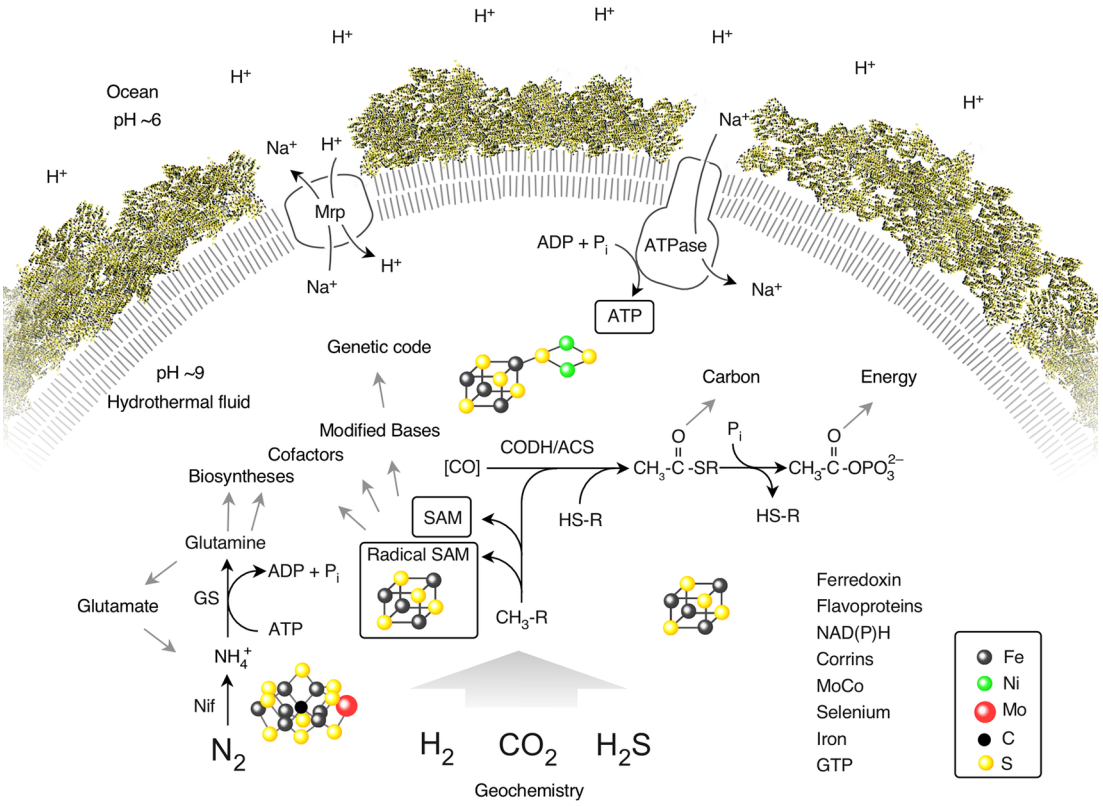

Figure 4. A schematic representation of a noncellular LUCA,[81] reproduced from ref 90, available under a Creative Commons CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0). Copyright 2018 Weiss et al.

for cobamide (a Co-tetrapyrrole)-dependent ion pumping at the MtrA-H methyltransferase reaction (also $\Delta G^{\circ\prime} = -30$ kJ·mol^{-1})[28] that synthesizes methyl-CoM and conserves energy as an ion gradient for ATP synthesis.[28] From that perspective, and looking at Figure 1, the involvement of metal-dependent reactions involving methyl groups at the core of methanogen energy metabolism makes sense: metabolic evolution has not found catalytic alternatives to these metal-catalyzed reactions, it has simply solubilized the metal-dependent methyl reactions with the help of tetrapyrroles and enzymes.

ANCIENT METHYL GROUPS PULL COFACTORS DEEP

The corrins required by acetogens and methanogens in the acetyl-CoA pathway are a set of diverse cobamides which differ in lower ligand[84,85] structure, whereby the corrin in the CoFeS protein initially isolated by Ragsdale and team[86] from *Clostridium thermoaceticum* had no lower ligand in the methyl-Co(III) or in the Co(II) or Co(I) forms, identifying 'base-off' corrins in proteins. Cobamides are required for methyl transfer reactions in which the methyl groups are bound by the free (upper) coordination site of the CoFeS Co atom[84,85] and transferred to a Ni atom in the active site of acetyl-CoA synthase, a rare metal-to-metal methyl transfer reaction.[46] The acetyl-CoA pathway, both in nonenzymatic (Figure 1) and enzymatic form,[14,62] is a pathway of methyl synthesis and methyl transfer: to a hydrogen atom in methanogenesis[45] and to CO in the acetyl-CoA pathway.[43] Methyl groups are ancient. There are eight SAM-dependent methylation steps involved in the synthesis of the corrin ring, suggesting that SAM is older than corrins. Methyl groups are also essential for the genetic code to work, with RNA methylations being essential for translation.[71,72,81]

GTP is ancient.[87] The genetic code requires the operation of the ribosome, whereby the ribosome is ancient, more ancient than any protein coding gene, as all protein coding genes are translated on ribosomes. GTP was the energy currency at the origin of translation[87] and it is the aromatic substrate for pterin synthesis (the GTP cyclohydrolase reaction). Pterins are ancient as they are the methyl carriers in the acetyl-CoA pathway of acetogens and methanogens, respectively, tetrahydromethanopterin differing from tetrahydrofolate by the C6 substituent and by additional methyl groups on the pterin ring.[88]

If we accept the robust inference that the acetyl-CoA pathway to pyruvate[14] of acetogens and methanogens is ancient, given its complete replacement by native forms of the metals (Figure 1) that serve in its enzymes and cofactors,[88] it follows that the following cofactors are also ancient: iron, nickel, cobalt, cobamides, SAM, MoCo, NAD(P)+ and flavins, coenzymes A, B and M, ATP and GTP, TPP, ferredoxin, methanofuran, tetrahydrofolate and tetrahydromethanopterin, F_{420} and F_{430}. They are required for the pathway to operate using proteins instead of solid-state metals as catalysts.

Otherwise extremely rare in biology, nickel is very common in ancient metabolism, not only in the acetyl-CoA pathway (Figure 2). The last step of methanogenesis entails the Ni-containing tetrapyrrole F_{430} in the MCR radical mechanism.[45] Yet Ni^0 alone catalyzes methane formation from H_2 and CO_2 in water[18] ($4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$, $\Delta G^{\circ\prime} = -131$ kJ·mol^{-1}), condensing the entire methanogenic pathway, which methanogens use in a stepwise manner to generate carbon backbones for biosynthesis and ion pumping for ATP

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Acc. Chem. Res. 2024, 57, 2267−2278

synthesis,[28] into a single reaction. Why use a multistep pathway when a single catalyst can perform the reaction? As metabolism evolved from solid−state catalysts to proteins with complex cofactors, highly exergonic single step reactions were split into series of linked reactions, each requiring more sophisticated catalysts that could introduce specificity and rate control.[89]

Perhaps unsurprisingly, the acetyl-CoA pathway pulls almost all universal cofactors into LUCA (Figure 4) except pyridoxal phosphate (transaminations) and biotin (carboxylations). The latter two are however essential for biosynthesis of amino acids[77] and bases. Heme and siroheme, which are essential for cytochrome-dependent electron transfers, are not present in the biosynthetic core and are not universal cofactors as they are not required either by hydrogenotrophic acetogens[27] or by hydrogenotrophic methanogens.[28]

CONCLUSION

The list of cofactors and catalysts that the acetyl-CoA pathway draws down into ancient biochemical evolution is almost identical to the list of cofactors identified in an earlier, phylogeny-based approach to the physiology of the last universal common ancestor LUCA.[81] A difference is that we now know that the overall reaction sequence of the acetyl-CoA pathway unfolds from H_2 and CO_2 overnight in the laboratory over transition metals in water under the alkaline conditions of serpentinizing hydrothermal vents,[2,17−20,29] and that several examples of acetogens and methanogens that grow in serpentinizing hydrothermal systems have been discovered.[91−94] These developments converge on one pathway, the acetyl-CoA pathway, and one kind of H_2-producing environment with native metal catalysts[23] at the origin of metabolism, helping to close the gaps between early Earth and early life.[89]

What do we not see in ancient metabolism? We do not see cyanide or nitrile-dependent[95] reactions in primary metabolism, ancient or otherwise. The CN and CO ligands of [FeFe] hydrogenase (Figure 2) are derived from the aliphatic moiety of tyrosine in a specific hydrogenase maturation process[54] although they can also be derived from glycine.[96] Cyanide is never observed in natural environments, not even in volcanic gases,[97] but abiotic glycine is produced by serpentinizing hydrothermal vents in amounts sufficient to support microbial growth.[94] Oró reported cyanide-dependent purine synthesis in 1960.[98] In the 60 years since, the gap between cyanide chemistry and life chemistry has widened, while the gap between the chemistry of hydrothermal vents and metabolism has narrowed. We also do not see RNA bases performing catalytic functions in core metabolism. The bases of RNA do not themselves catalyze any reactions in the acetyl-CoA pathway or in the biosynthetic core, although they do play an essential role in the peptidyl transferase reaction at the ribosome[99] and newer findings mechanistically implicate the modifications of tRNA in primordial peptide synthesis reactions.[100] GTP serves as a precursor for pterin synthesis (cyclohydrolase) and for DNA synthesis.[101] RNA bases are often attached to cofactors, and even though every radical SAM reaction requires bound adenosyl, the base plays no role, the active moiety of the reaction is the 5′ − CH_2• of ribose.[57] FeS clusters are mainly cofactors of electron transfer (Figure 3), but also serve to coordinate Ni, which exerts catalytic function in reactions central to the acetyl-CoA pathway.[43]

Native Fe, Co, or Ni can replace the entire acetyl-CoA pathway without the help of nitrogen, sulfur or phosphorus. For the bedrock-level origin of metabolism, reactions of H_2 and CO_2 in water on metal surfaces at alkaline pH (conditions of serpentinizing systems) are sufficient.[2,17−20,29] While meteorites can also deliver native metals as catalysts,[102] serpentinizing hydrothermal vents also provide a continuous stream of H_2 as reductant[23] in addition to microcompartments[25] and temperature gradients that can concentrate products of synthesis for further reaction.[103] Though the ion gradients of serpentinizing systems could have powered primitive ATP synthases prior to the origin of biological ion pumping,[23,25,81,89] we see no requirement for ion gradients[104] at the origin of the biosynthetic core, with metabolism emerging from reactions of aqueous H_2 and CO_2 interfacing with solid-state catalysts (Figure 1). The first organic compounds in this view were organic acids, including 2-oxoacids,[74] with the incorporation of N taking place exactly as in metabolism, but with H_2 as reductant and Ni as catalyst.[30] The incorporation of S is facile.[79] The incorporation of phosphate, however, is still not resolved. Using metabolism as a guide, it might have entailed reactions of phosphate with reactive carbonyl moieties[26] or it might have involved reactions of phosphite, as suggested by phosphite-dependent substrate phosphorylations recently discovered in organisms that use the acetyl-CoA pathway.[65] That Ni, Co, and Fe served as catalysts in the enzymatic reactions of the acetyl-CoA pathway,[40] and that these reactions are ancient,[12] was evident 40 years ago. Since that time, serpentinizing hydrothermal vents[105] have conjoined the metals in their native catalytic form (i) with the source of reductant (H_2) that acetogens and methanogens still use today and (ii) with specific products of the acetyl-CoA pathway, marking chemical antiquity in metabolism that connects life to Earth.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.accounts.4c00226.

Supplementary Table 1 with a list of reactions of the autotrophic core[3] catalyzed by FeS proteins, Supplementary Table 2 with a list of reactions of the autotrophic core[3] catalyzed by radical enzymes, and supplementary references (PDF)

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Notes

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