



CO₂-Fixation Strategies in Energy Extremophiles: What Can We Learn From Acetogens?

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Domestication of CO₂-fixation became a worldwide priority enhanced by the will to convert this greenhouse gas into fuels and valuable chemicals. Because of its high stability, CO₂-activation/fixation represents a true challenge for chemists. Autotrophic microbial communities, however, perform these reactions under standard temperature and pressure. Recent discoveries shine light on autotrophic acetogenic bacteria and hydrogenotrophic methanogens, as these anaerobes use a particularly efficient CO₂-capture system to fulfill their carbon and energy needs. While other autotrophs assimilate CO₂ via carboxylation followed by a reduction, acetogens and methanogens do the opposite. They first generate formate and CO by CO₂-reduction, which are subsequently fixed to funnel the carbon toward their central metabolism. Yet their CO₂-reduction pathways, with acetate or methane as end-products, constrain them to thrive at the “thermodynamic limits of Life”. Despite this energy restriction acetogens and methanogens are growing at unexpected fast rates. To overcome the thermodynamic barrier of CO₂-reduction they apply different ingenious chemical tricks such as the use of flavin-based electron-bifurcation or coupled reactions. This mini-review summarizes the current knowledge gathered on the CO₂-fixation strategies among acetogens. While extensive biochemical characterization of the acetogenic formate-generating machineries has been done, there is no structural data available. Based on their shared mechanistic similarities, we apply the structural information obtained from hydrogenotrophic methanogens to highlight common features, as well as the specific differences of their CO₂-fixation systems. We discuss the consequences of their CO₂-reduction strategies on the evolution of Life, their wide distribution and their impact in biotechnological applications.

Keywords: acetogenic bacteria, hydrogenotrophic methanogens, CO₂-fixation, formate dehydrogenase, evolution, biotechnology, coupled reaction

INTRODUCTION

CO₂, the most oxidized state of carbon, has become a major concern to society due to its greenhouse gas properties and its increasing accumulation in our atmosphere since the 20th-century. Efficient CO₂-sequestration techniques, as well as concomitant applications in biochemical synthesis and alternative energy source storage, being developed to reduce its impact on global warming (Schuchmann and Müller, 2013). Yet CO₂ is a stable, inert molecule. The few applicable

chemical processes allowing its unfavorable fixation (like the Monsanto and Cativa processes) require high temperatures and pressures as well as expensive and polluting catalysts while only exhibiting moderate catalytic rates (Appel et al., 2013; Fujita et al., 2013; Schuchmann and Müller, 2013). New alternative chemistry based on metal-organic framework (Hou et al., 2019) or transition metal-free catalysis (Cherubini-Celli et al., 2018) are upcoming and might be applied in the near future. Nevertheless, none of these artificial processes matches the efficiency of their biological counterpart.

At least six different autotrophic carbon fixation pathways exist among the domains of Life (Berg et al., 2010; Fuchs, 2011; Appel et al., 2013). The most common scenario is a two-step process where CO₂ is branched on a reactive group (carboxylation) and then reduced (e.g., Calvin–Benson–Bassham or the 3-hydroxypropionate 4-hydroxybutyrate cycle). To date, there is only one exception that uses the reverse way, CO₂-reduction before carboxylation: the reductive acetyl-CoA pathway. This pathway constitutes the “cheapest” option to fix CO₂ in term of energy-consumption and is thought to be the most ancient one (Berg et al., 2010; Martin and Thauer, 2017).

The reductive acetyl-CoA pathway has two CO₂ entry points: the methyl-branch, where a reductive cascade turns CO₂ in a methyl-group (Figure 1A, reaction from the CO₂-activation to the methyl-H₄F for acetogens or methyl-H₄MPT for methanogens) and the carbonyl-branch. In the latter, CO₂ is converted into carbon monoxide (CO; Figure 1A), further combined with the methyl group and Coenzyme A (CoA) to ultimately produce acetyl-CoA, the “turntable” of the central carbon metabolism (Ragsdale and Pierce, 2008; Thauer et al., 2008; Berg et al., 2010; Sousa et al., 2013; Fuchs and Berg, 2014).

Hydrogenotrophic methanogens (Euryarchaea, simplified as methanogens below) and autotrophic acetogens (Bacteria, simplified as acetogens below) use the reductive acetyl-CoA pathway to derive their cellular carbon and energy by growing on H₂ plus CO₂. The final product for methanogens and acetogens are methane and acetate, respectively. Under physiological conditions, such metabolism provides less than half a molecule of ATP per acetate/methane, constraining these organisms to live at the “thermodynamic limits of Life” (Buckel and Thauer, 2013; Schuchmann and Müller, 2014). Nevertheless, methanogens and acetogens are found in various ecological niches, ranging from rumen to deep-sea volcanoes and they are crucial actors in organic matter conversion and element cycling (e.g., carbon assimilation and nitrogen fixation). Despite drastically low energy yields, their doubling time is surprisingly short: ranging from only one to a few hours under laboratory conditions (Thauer et al., 2008; Basen et al., 2018).

The energy metabolism of acetogens and methanogens was puzzling for a long time until the discovery of energy conserving enzymes (i.e., Rnf and Ech membrane complexes), which use low-potential electrons from ferredoxins, reduced by H₂ oxidation via flavin-based electron bifurcation (Figure 1D). The use of low-potential electrons provided a rational explanation as to how these organisms derive enough energy to survive and grow under such stringent metabolic conditions (Buckel and Thauer, 2013, 2018; Schuchmann and Müller, 2014;

Peters et al., 2018). Considered to be among the first metabolic processes, methanogenesis and acetogenesis might have been crucial for shaping ecosystems since the first Lifeforms arose.

This review summarizes our current understanding of the CO₂-activation steps orchestrated by these fantastic machineries, which evolved to fulfill the physiological needs for carbon-assimilation and energy-conservation. The structural knowledge gathered from hydrogenotrophic methanogens provides insights in the shared and distinct features between the acetogenic and methanogenic CO₂-conversion systems, due to both metabolic adaptation and ecological specialization.

THE CO₂-REDUCTION/FIXATION COMPLEX IN METHANOGENS

The entire energy metabolism of methanogens relies on highly efficient CO₂-capture. This challenging task is overcome by the formyl-methanofuran dehydrogenase (Fwd) complex catalyzing both the reduction of CO₂ and the conversion of formate (HCOO⁻) into a formyl group (Figures 1A, 2). So far, two isoforms of this enzyme are described, containing either a molybdenum- or tungsten-dependent formate dehydrogenase (Fdh) subunit (Bertram et al., 1994; Thauer et al., 2008; Leimkühler and Iobbi-Nivol, 2016; Wagner et al., 2018). Depending on the organism, the molybdo/tungstopterin cofactor can be coordinated by a cysteine or seleno-cysteine. Unlike nearly all described Fdh that perform formate oxidation releasing CO₂, methanogenic and acetogenic Fdh physiologically run toward CO₂-fixation. Until now, only a few enzymes found in the *Synthrophobacter* genus share this feature (de Bok et al., 2003).

The reaction remained a mystery for a long time: how can the enzyme couple formate to the C1-carrier methanofuran (MFR) without any ATP investment (Bertram et al., 1994)? The secret was eventually unraveled by its crystal structure (Wagner et al., 2016). The overall complex is constituted of an unprecedented electron transfer apparatus containing a total of 46-[Fe₄S₄] clusters flanked by two catalytic modules, a tungstopterin-dependent Fdh and a binuclear metallo-hydrolase. Based on the molecular details, a scenario of the reaction has been proposed where; (1) CO₂ is funneled to the active site of Fdh by a selective, hydrophobic channel; (2) CO₂ is reduced to formate (Figures 1A,B, 2A,B; Wagner et al., 2016); (3) a second hydrophilic tunnel channels and accumulates formate at the active site of the metallo-hydrolase; and (4) formate is condensed on the amino-group of MFR as a formyl-group. The accumulation of formate is predicted to thrive the conversion of formate to a formyl group on MFR without the investment of ATP, an endergonic reaction under standard conditions. However, since Fdh are reversible, the driver of the overall reaction is the electron donor.

From MFR the formyl group is transferred to the second C1-carrier tetrahydromethanopterin (H₄MPT), successively dehydrated and fully reduced (i.e., by the F₄₂₀ cofactor or alternatively with H₂ by the [Fe]-hydrogenase) to a methyl group (dashed line, Figure 1A). The methyl-H₄MPT represents the crossroad between carbon-assimilation and energy conservation

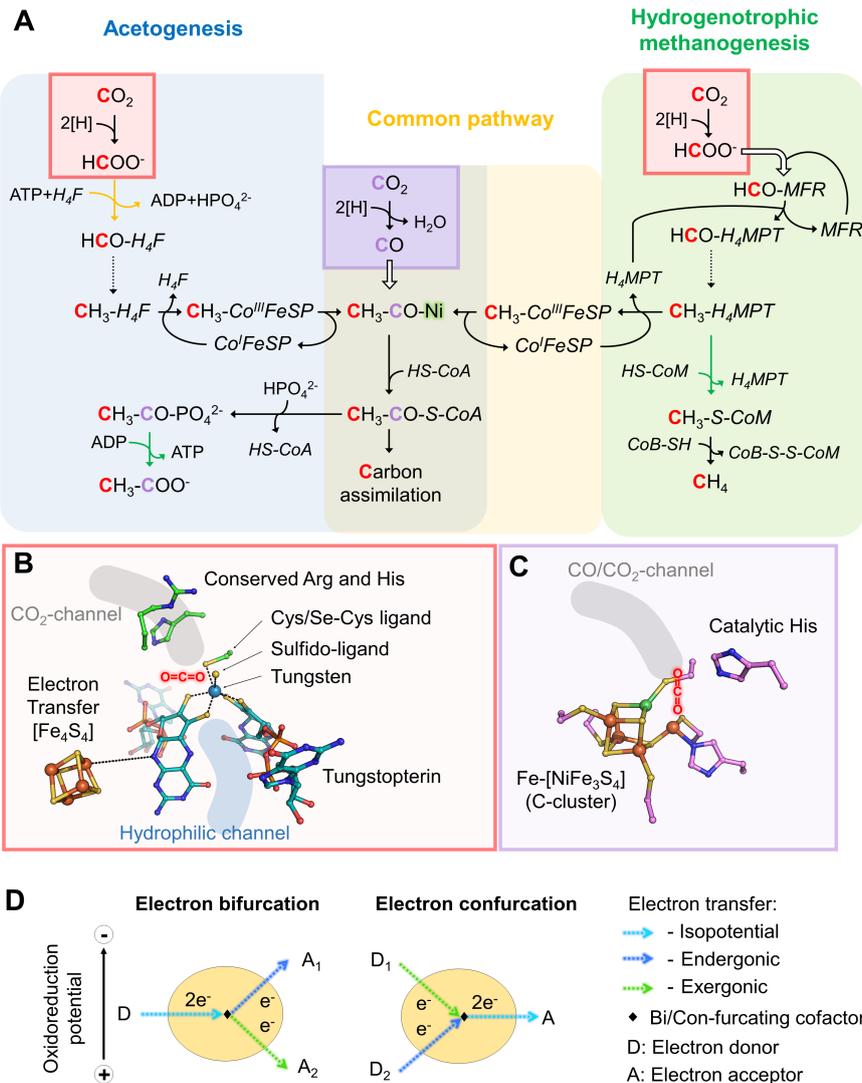


FIGURE 1 | Variations in the reductive acetyl-CoA pathway between acetogenic bacteria and hydrogenotrophic methanogens, implicated active sites and mechanisms. **(A)** Differences in the reductive acetyl-CoA pathway between acetogenic bacteria (left and middle) and methanogenic archaea (right and middle). Acetogens and methanogens share a conserved “carbonyl” branch (common pathway) used to build biomass for both and to conserve energy for acetogens. The green arrows correspond to reactions coupled to energy-conservation (ATP or electrochemical ion gradient generation across the membrane) and the orange one to ATP hydrolysis-coupled reaction. Dashed arrows correspond to three successive reactions: dehydration and two reduction steps. White arrows indicate the usage of an internal channeling system between two active sites. Red and purple squares highlight CO₂-reduction events, in red Fdh reaction and in purple the CODH reaction. The ACS contains the A-cluster harboring the binuclear Nickel center highlighted by a green glow. The cofactors involved in these processes are: tetrahydrofolate (H₄F), tetrahydromethanopterin (H₄MPT), coenzyme A (CoA-SH), methanofuran (MFR), reduced/oxidized corrinoid FeS containing protein (Co/CH₃-Co^{III}-FeSP), coenzyme B (CoB-SH), coenzyme M (CoM-SH). **(B)** Close up of a Fdh catalytic site (PDB code 5T5I) containing the tungstopterin, which could be replaced by molybdopterin for other Fdh. Carbons are colored in green for the residues involved in the catalysis and dark cyan for the tungstopterin. Dashed line between the [Fe₄S₄]-cluster and the pterin represents the hypothetical electron transfer from the cluster to the tungstopterin. **(C)** Close up of the catalytic site of CODH from *Moorella thermoacetica* (PDB code 1MJG) containing the C-cluster. Carbons from protein residues are colored in light pink. For both panels, **B** and **C**, nitrogen, oxygen, phosphorous, sulfur, iron, tungsten, and nickel are colored as dark blue, red, light orange, yellow, orange, metallic blue, and green, respectively. A molecule highlights the putative CO₂ position in both panels. **(D)** Scheme of electron bifurcation/confurcation mechanism. During electron bifurcation, a two-electron transfer from an electron donor (D) is bifurcated by a specific cofactor to both endergonic and exergonic one-electron transfers to two different acceptors (A₁ and A₂). The overall reaction is slightly exergonic. The opposite reaction occurs during electron confurcation.

(Thauer, 2012). The latter is formed during the methyl group transfer from nitrogen-bound methyl-H₄MPT to the thiol group of the coenzyme-M acceptor. The methyl-transfer is coupled to a sodium translocation across the membrane, used to feed the ATP-synthase, which is generating only half an

ATP per processed C1-unit (Schäfer et al., 1999). Finally, methylated coenzyme-M becomes oxidized to a heterodisulfide with coenzyme-B (CoB-S-S-CoM), releasing methane by using the F₄₃₀-cofactor (Thauer et al., 2008; Thauer, 2012; Sousa et al., 2013).

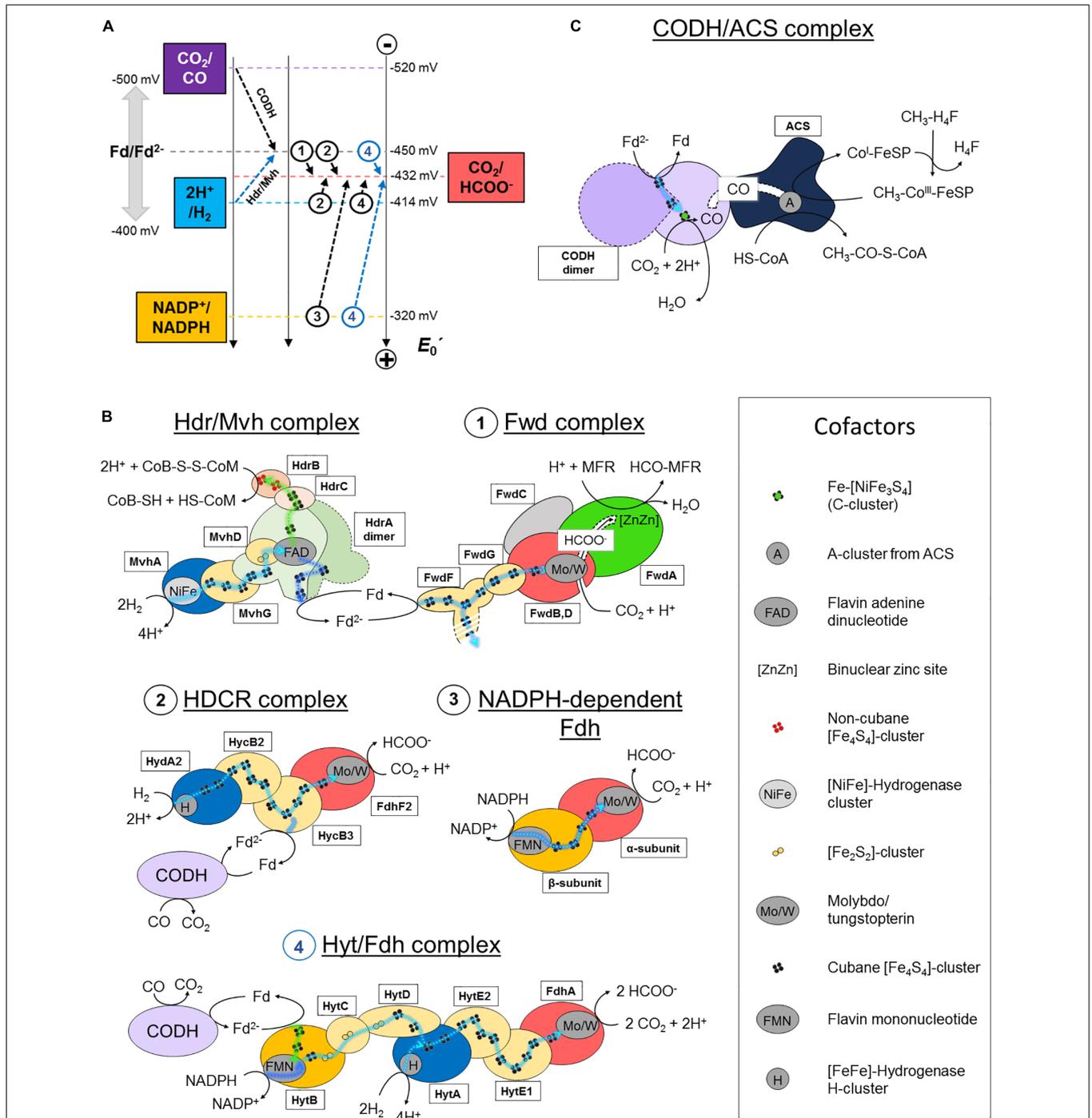


FIGURE 2 | CO₂-activation strategies in hydrogenotrophic methanogens and acetogens. **(A)** Standard redox potential (E_0') of redox couples implicated in CO₂-activation in methanogenic and acetogenic processes. Dashed arrows schematize the reactions performed by the enzymes listed in panel **B**. A name or circled number indicates which complex is implicated. The blue arrows correspond to a coupled electron confurcating reaction. To simplify the scheme, the endergonic reaction of the Hdr/Mvh complex (CoB-S-S-CoM/CoB-SH + CoM-SH = -140 mV) has been omitted. Standard redox potentials were taken from Schuchmann and Müller (2014). Ferredoxin can exhibit potentials ranging from -400 to -500 mV, depending on the organism. An averaged potential of -450 mV is thus used in the figure. **(B,C)** Schemes of the characterized and putative organizations of the enzymes involved in CO₂-reduction in hydrogenotrophic methanogens and acetogens. Catalytic subunits are colored according to their substrate as in panel **A**. All electron transfers except for con/bifurcation events are shown as cyan dashed lines. For con/bifurcation events, the endergonic reactions are colored in dark blue and exergonic in light green, as illustrated in **Figure 1D**. Due to the absence of acetogenic structural data, hypothetical architectures are represented based on their original publications, biochemical data and homologies (Yamamoto et al., 1983; Schuchmann and Müller, 2013; Wang et al., 2013). Monomeric forms are schematized. The localization of the electron confurcation event in the Hyt complex is purely hypothetical. The [NiFe]-hydrogenase module from the Hdr/Mvh complex can be replaced by a Fdh. All known cofactors involved in the different reactions of panels **B** and **C** are listed. Fd stands for oxidized ferredoxin and Fd²⁻ for reduced ferredoxin.

ELECTRON-BIFURCATION FUELS METHANOGENIC CO₂ FIXATION

The electron donor for the Fwd complex is predicted to be reduced ferredoxin or a direct electron transfer by the heterodisulfide reductase (Costa et al., 2010; Kaster et al., 2011; Milton et al., 2018). Two versions of this enzyme have been described in hydrogenotrophic methanogens depending on the coupled electron donor: a [NiFe]-hydrogenase or a Fdh (the structurally characterized hydrogenase-dependent one called the Hdr/Mvh complex is shown in **Figures 1, 2B**).

The overall process starts with the transfer of two electrons from the donor (H₂ or formate) to a flavin. Flavin-based electron bifurcation then splits the two electrons at different potentials (**Figures 1D, 2B**). The high-potential electron is used for the exergonic reduction of the heterodisulfide. The low-potential electron reduces ferredoxin or might even be directly delivered to the 46-[Fe₄S₄] relay of the formyl-methanofuran dehydrogenase to allow CO₂-capture. The whole reaction is performed in two rounds (Wagner et al., 2017).

DIVERSITY OF CO₂-ACTIVATION SYSTEMS IN ACETOGENS

Like methanogens, acetogenic bacteria perform initial CO₂-reduction via molybdo/tungstopterin-dependent Fdh. However, in contrast to methanogens, all described acetogenic CO₂-reducing systems produce detectable formate, indicating that CO₂-reduction and formate condensation are uncoupled (Yamamoto et al., 1983; Schuchmann and Müller, 2013, 2014; Wang et al., 2013). Formate conversion into a formyl group on a C1-carrier (i.e., tetrahydrofolate, H₄F) is thermodynamically unfavorable and acetogens must therefore invest one ATP. Then, the successive dehydration and reduction into methenyl, methylene and finally methyl group can occur, similar to the methanogenic process but involving different systems, reductants and cofactors (Mock et al., 2014; Schuchmann and Müller, 2014). The methyl group is further fused to CO and CoA by the CO dehydrogenase/acetyl-CoA synthase complex (CODH/ACS) to form acetyl-CoA (see below, **Figures 1, 2C**; Ragsdale, 2008; Can et al., 2014).

Despite a common Fdh module, acetogens evolved their CO₂-fixation system in many variations, a real example of “mix and match” from the redox module toolbox, as already shown for sulfate-reducing organisms (Grein et al., 2013). Some of them have been reported to be molybdopterin dependent (e.g., *Acetobacterium woodii*) while others require tungstopterin (e.g., *Moorella thermoacetica*, *Clostridium autoethanogenum*, etc.). There are selenium-dependent or selenium-free formate dehydrogenases and some organisms encode both (Yamamoto et al., 1983; Schuchmann and Müller, 2013; Wang et al., 2013). This diversity allows the use of different electron donors (Schuchmann and Müller, 2014).

Acetobacterium woodii uses a hydrogen-dependent CO₂-reductase (HDCR) which couples the oxidation of H₂ to the reduction of CO₂ (Schuchmann and Müller, 2013). The enzyme

is composed of a selenocysteine–molybdopterin dependent Fdh linked to a [FeFe]-hydrogenase via an electron bridge (**Figure 2B, 2**). The genome also encodes a cysteine-dependent Fdh isoform, supposedly expressed under selenium deprivation. The reduction of CO₂ to formate with H₂ is slightly endergonic at standard conditions (**Figure 2A**). However, at the relatively important threshold concentration of H₂, necessary for acetogenesis in *A. woodii* (measured at 0.0025 Bar, around thirty times superior to the threshold of hydrogenotrophic methanogens; Thauer et al., 2008), the equilibrium concentration of generated formate becomes sufficient to fuel the methyl branch of the pathway (Schuchmann and Müller, 2014). The enzyme can also perform carbon fixation by ferredoxin oxidation, albeit exhibiting a 1000-times lower reaction rate (**Figure 2B, 2**). This ability is thought to be crucial in presence of CO, a strong inhibitor of hydrogenases. The coupling of HDCR with CODH is an efficient way to regenerate reduced ferredoxin.

Moorella thermoacetica contains a two subunit NADPH-dependent Fdh containing selenocysteine and tungstopterin cofactor, which catalyzes the reversible formate generation through NADPH oxidation (**Figure 2B, 3**; Thauer, 1972; Yamamoto et al., 1983). Despite being thermodynamically highly unfavorable under standard conditions, it appears that the NADPH-dependent Fdh is the only formate generating enzyme in *M. thermoacetica*. High NADPH/NADP⁺ and CO₂/formate ratios are necessary to push the reaction toward carbon reduction.

Clostridium autoethanogenum exploits a seven subunit complex (Hyt/Fdh), the so far most complicated formate-generating system in acetogens (**Figure 2B, 4**; Wang et al., 2013; Schuchmann and Müller, 2014). The selenium-dependent tungstopterin-containing Fdh module performs CO₂-reduction by receiving electrons from H₂-oxidation via a [FeFe]-hydrogenase subunit (similar to HDCR) or by concomitant oxidation of NADPH and ferredoxin through an internal confurcation event (**Figures 1D, 2B, 4**). Like in HDCR, the reduced ferredoxin could directly come from CO-oxidation by the CODH. Where and how the electron confurcation is carried out is still unresolved, as the only known flavin cofactor present in the complex (in HytB) is thought to be not involved (Wang et al., 2013). A novel type of electron bifurcation is thus suspected, one that is similar to the related electron-bifurcating hydrogenase. The structural features of this bifurcation mechanism have to be deciphered and as said by Buckel and Thauer (2018): “A crystal structure is urgently needed to solve this problem.”

A COMMON CO₂-FIXATION SYSTEM: THE CO-DEHYDROGENASE/ACETYL-COA SYNTHASE

While methanogens and acetogens employ different strategies to reduce CO₂ for the methyl-branch, the activation step of the carbonyl-branch, catalyzed by Ni,Fe-containing CODH, is remarkably conserved (Lindahl, 2002; Jeoung et al., 2019).

The initial CO₂-reduction to CO, powered by low-potential electrons from ferredoxins or flavodoxins (Ragsdale et al., 1983;

Can et al., 2014; Schuchmann and Müller, 2014), occurs at the C-cluster composed of a Fe-[NiFe₃S₄] (**Figure 1C**). CO is transferred to the ACS by a long internal hydrophobic channel (**Figure 2C**; Doukov et al., 2002; Can et al., 2014). Here, it is fixed on the A-cluster, which is composed of a Ni-[Fe₄S₄] cluster bridged to another Ni atom (Ragsdale and Pierce, 2008). Ultimately, the ACS forms acetyl-CoA by associating the CO-ligand, CoA and the methyl-ligand from the methyl-branch. A cobalamin-containing FeS protein (CoFeSP) serves as a shuttle for the methyl group between the Methyl-H₄F and the ACS. The transfer mechanism from the CoFeSP to the ACS is so far unknown. The enzyme thus performs the biological equivalent of the Monsanto and Cativa processes, where CO and methanol are converted to acetate by metal-based catalysts (Appel et al., 2013).

Even if the overall reaction is the same between methanogens and acetogens some subtleties concerning the CODH/ACS composition exist. According to the classification of Lindahl (2002), archaea and predominantly methanogens use preferentially Ni,Fe-CODH of Class I and II (also called acetyl-CoA decarbonylases/synthases), which consist of five different subunits that form oligomeric complexes of approximately 2-MDa. This super-complex contains the CODH/ACS (Doukov et al., 2002; Can et al., 2014), the CoFeSP and the enzyme responsible for methyl-transfer from methyl-H₄F to cobalamin. These three sub-complexes are separated in acetogenic systems. The CODH subunit in methanogens contains two extra [Fe₄S₄]-clusters, putatively implicated in the rerouting of electrons. Acetogenic complexes have been extensively studied (Ragsdale and Kumar, 1996; Ragsdale and Pierce, 2008) thanks to a few available crystal structures of the whole CODH/ACS complex from *M. thermoacetica* (Doukov et al., 2002; Darnault et al., 2003; Kung et al., 2009) and the knowledge gathered on this enzyme has already been reviewed (Can et al., 2014).

Beside these slight differences, all classes of the CODH/ACS complex are thought to be homologous and thus may have been acquired from a common ancestor (Sousa et al., 2013).

CONCLUSION AND PERSPECTIVES

As previously depicted, the Fdh subunit and CODH/ACS complex are conserved in methanogens and acetogens. These elementary modules are therefore thought to have evolved before the divergence of acetogens and methanogens, thus in the Last Universal Common Ancestor (LUCA) (Sousa et al., 2013; Martin and Thauer, 2017). Because they harbor “ancestral” cofactors like Fe-S clusters or tungstopterin and since the substrates H₂ and CO₂ should have been abundant in Early Earth, these pathways are considered to be among the first, if not the first, biological energetic processes (Fuchs, 2011; Sousa et al., 2013; Martin and Thauer, 2017). Understanding the mechanisms and limitations of methanogenesis and acetogenesis will help to unravel the fundamental questions of how Life arose from the pre-existing inorganic world and could provide information about its first evolutionary steps in the new organic one.

While the carbonyl-branch of the reductive acetyl-CoA pathway might be an early and highly conserved invention

in LUCA, the methyl-branch is not. Here, methanogens and acetogens use non-homologous enzymes to perform similar reactions. This parallel evolution gave birth to a variety of formate-generating, CO₂-reducing enzymes, albeit using similar modules (Sousa et al., 2013) and invented different strategies for C1-reduction and formate condensation by the use of different C1-carriers. The evolutionary plasticity of the methyl-branch compared to the strict conservation of the carbonyl-branch might derive from its requirement for low-potential electrons. Because of the low-potential of the CO₂/CO couple, the CODH could not adapt to a partner other than ferredoxin for CO generation, while the CO₂-reduction to formate can accommodate different electron donors, allowing variability of enzymes according to the metabolic needs for physiological requirements.

Furthermore, the functional modules coupled to Fdh systems might be the foundation for other “modern” enzymes, from the formate-hydrogen lyase complex to the respiratory complex I (Marreiros et al., 2016). Elucidating methanogenic and acetogenic enzymes has therefore the potential to provide hints to how the ancestral energetic pathways diversified, thereby creating new processes and gradually giving birth to the plethora of bio-energetically important complexes.

A striking difference between formate generating enzymes from acetogens and methanogens is the energy investment. While methanogens bypass the latter (**Figure 1A**), acetogens need to sacrifice one ATP to allow formate fixation. They counterbalance this energy loss via substrate level phosphorylation of acetyl-phosphate in the last step of acetogenesis. In comparison, no ATP is generated through methanogenesis (**Figure 1A**). Nevertheless, ATP sparing is critical for energy-limited extremophiles and one could ask why acetogens did not develop an equivalent of the Hdr/Mvh/Fwd coupling system. The explanation could come from the use of low-potential ferredoxins. The last step of methanogenesis releases CoB-S-S-CoM, which is recycled by the heterodisulfide reductase (downhill reaction) with the concomitant generation of low-potential electrons (uphill reaction). Most of these low-potential electrons generated in the cell are assumed to be dedicated for the CO₂-fixation (**Figure 2B**, 1; Thauer, 2012).

Acetogenic bacteria are restricted to ecological niches with higher H₂ pressure than methanogens. The main reason is that electron bifurcating [FeFe]-hydrogenases are necessary for ferredoxin reduction (Schuchmann and Müller, 2012, 2014), the electron acceptor for the downhill reaction being NAD(P)⁺. According to the current knowledge, the uphill electron generated during the flavin-based electron bifurcation could have a lower potential if the electron downhill is the heterodisulfide ($E_0' \approx -140$ mV) compared to NAD(P)⁺ ($E_0' = -320$ mV). Therefore, the ferredoxins reduced via electron bifurcation in methanogens are expected to have higher reducing power compared to acetogens. Thus, in the latter the potential could not be low enough to allow both, formate generation and conversion to formyl group, unlike in methanogens. Thus, despite sparing one ATP, coupling formate generation and fixation may be not favorable for acetogenic bacteria and will not sustain a metabolic high-flow toward acetyl-CoA synthesis.

A way to bypass H₂ is CO-oxidation. To handle CO, acetogens use different strategies. *A. woodii* thrives episodically on weak CO concentrations, possible due to the reversibility of the CO inhibition of the HDCR system and the ability to oxidize ferredoxin, albeit with a weak turnover (Figure 2B, 2; Schuchmann and Müller, 2013; Bertsch and Müller, 2015; Ceccaldi et al., 2017). Acetogenic bacteria, which use CO as substrate, like *M. thermoacetica* and *C. autoethanogenum*, exhibit metabolic adaptations. For instance, albeit it has not been tested so far, the NADPH-dependent Fdh system from *M. thermoacetica* should be insensitive to CO as it is not directly using the CO-sensitive hydrogenase, like the HDCR. However, the enzyme depends on a high NADPH/NADP⁺ ratio or high pressure of CO₂. The Hyt/Fdh system from *C. autoethanogenum*, a chimera between HDCR and the electron bifurcating/confurcating hydrogenase (Figure 2B, 4), shows a reactional plasticity by switching from the CO-sensitive hydrogenase to NADPH plus ferredoxin oxidation to drive CO₂-reduction despite inhibition (Wang et al., 2013). Interestingly, to date, a formyl group generation directly driven by CO-oxidation has never been found in any CO fermenting acetogen. Still, the low redox potential of the CO₂/CO couple could allow an Fwd-like coupled mechanism, sparing a molecule of ATP, crucial for such energetic extremophiles.

The diversity of the electron-donating Fdh systems reflects and allowed the widespread distribution of these microbes, from H₂ rich to CO saturated niches. However, their dependence on oxygen-sensitive cofactors constrains them to strictly anaerobic but also metal-rich environments, since such carbon fixation pathways require more metallic cofactors than the others. Studying the diversity of these systems provides modern snapshots of the evolution of such “ancestral” organisms to accommodate various ecological niches.

Because syngas (H₂/CO₂/CO) is the main source of carbon and energy for hydrogenotrophic methanogens and acetogens, they are excellent “bio-converters.” For instance, acetogens turn

industrial waste gases, rich in H₂, CO and CO₂, to butanediol, ethanol or acetate, potential biofuels or starting points for new chemical synthesis (Wang et al., 2013; Mock et al., 2015; Liew et al., 2016). With the discovery of genetically tractable acetogens (Liew et al., 2016; Basen et al., 2018) the possibilities for bio-compound synthesis, and bioremediation are expanding.

Moreover, acetogenic CO₂-activation systems as HDCR and Hyt/Fdh are a treasure trove to realize the Holy Grail reaction of our century: the reversible hydrogenation of CO₂ to formate, offering a stable way to store energy with the concomitant advantage of trapping the greenhouse gas (Schuchmann and Müller, 2013; Müller, 2019).

Studies of acetogenic physiology and carbon fixation pathways are still an ongoing growing field. More work has to be conducted to truly understand their enzymes, metabolic fluxes, the molecular juggling of their reactions and their limitations. It is crucial to ensure the success of biotechnological applications, including synthetic biology, that will – let’s hope – bring a brighter future.

AUTHOR CONTRIBUTIONS

All authors participated to the manuscript writing.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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