Review

Acetate Metabolism in Anaerobes from the Domain *Archaea*

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Abstract: Acetate and acetyl-CoA play fundamental roles in all of biology, including anaerobic prokaryotes from the domains *Bacteria* and *Archaea*, which compose an estimated quarter of all living protoplasm in Earth's biosphere. Anaerobes from the domain *Archaea* contribute to the global carbon cycle by metabolizing acetate as a growth substrate or product. They are components of anaerobic microbial food chains converting complex organic matter to methane, and many fix CO_2 into cell material via synthesis of acetyl-CoA. They are found in a diversity of ecological habitats ranging from the digestive tracts of insects to deep-sea hydrothermal vents, and synthesize a plethora of novel enzymes with biotechnological potential. Ecological investigations suggest that still more acetate-metabolizing species with novel properties await discovery.

1. Introduction

Acetate and acetyl-CoA play a prominent role in the metabolism of all three phylogenetic domains of life, including anaerobic prokaryotes from the domains *Bacteria* and *Archaea*, which contribute to an estimated quarter of all living protoplasm in Earth's biosphere [1]. Anaerobes from the domain *Archaea* play significant roles in the global carbon cycle by metabolizing acetate as a growth substrate or product. Many anaerobes also fix CO_2 into cell material via synthesis of acetyl-CoA [2].

In anaerobic environments where terminal electron acceptors (Fe(III), Mn(IV), SO_4^{2-}, S^0, NO_3^-) are absent, anaerobes from both prokaryotic domains convert complex organic matter to CH_4 and CO_2, providing an essential link in the global carbon cycle (Figure 1). In Step 1, CO_2 is incorporated into

biomass driven primarily by photosynthesis. In environments where O_2 is abundant, microbes oxidize the biomass, producing CO_2 that re-enters the carbon cycle (Step 2). A significant portion of the biomass is diverted to an assortment of anaerobic habitats devoid of terminal electron acceptors (Step 3), where anaerobic microbial food chains, comprised of at least three distinct metabolic groups, digest the biomass to CO_2 and CH_4 (Steps 4–7). The fermentative group converts the complex biomass primarily into, acetate along with lesser amounts of volatile fatty acids, H_2, and CO_2 (Steps 4 and 5). The fatty acids are converted chiefly into acetate plus either formate or H_2 by the acetogenic group (Step 6). Thus, acetate emerges as the principal product of the fermentative and acetogenic groups. The CH_4-producing (methanogen) group is sub-divided into acetate-utilizing (acetoclastic) and CO_2-reducing species. Acetoclastic species convert the methyl group of acetate to CH_4 and the carbonyl group to CO_2. The CO_2-reducing species reduce CO_2 to CH_4 with electrons derived from H_2 or formate. At least two-thirds of the CH_4 produced derives from acetate, the central intermediary in anaerobic microbial food chains. A portion of the CH_4 is oxidized to CO_2 (Step 8) by associations of anaerobes that utilize sulfate, nitrate, manganese, or iron as terminal electron acceptors [3]. The CO_2 and remaining CH_4 diffuse into aerobic zones (Steps 9 and 10), where O_2-requiring methanotrophic microbes oxidize the CH_4 to CO_2 (Step 11), completing the carbon cycle. Anaerobes also participate in chemoautotrophic habitats, where they fix carbon dioxide in catabolic and anabolic pathways. Although the fermentative and acetogenic groups are largely populated with characterized isolates from the domain *Bacteria*, all characterized methanogens are classified in the domain *Archaea*. Acetate-utilizing anaerobes classified in the domain *Archaea* also proliferate in environments where terminal electron acceptors are abundant and obtain energy through anaerobic respiration, converting acetate to CO_2.
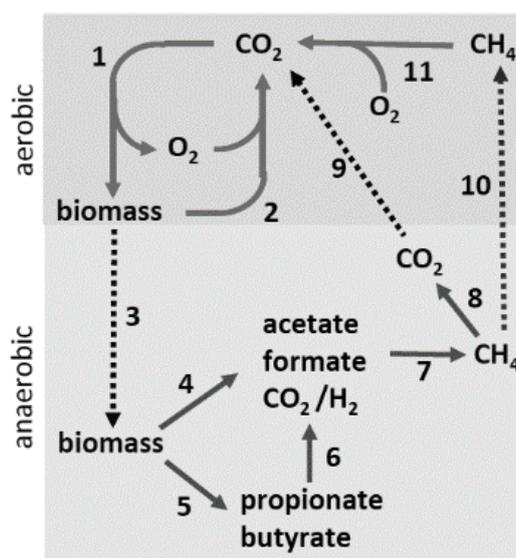
Figure 1. The global carbon cycle. Solid lines indicate steps in the cycle (see text) and dotted lines indicate transfer of material between aerobic and anaerobic environments.

This review encompasses the role of acetate, which has the greatest influence on the ecology of environments, in the energy conversion pathways of anaerobes from the domain *Archaea*; however, acetate and acetyl-CoA also play a prominent role in the biosynthetic pathways of anaerobes from the domain *Archaea* [2].

2. Acetate Production

2.1. Heterotrophic Energy-Converting Pathways Producing Acetate

Investigations of heterotrophic hyperthermophilic species from the domain *Archaea* have revealed pathways that deviate substantially from pathways in heterotrophic organisms from the domain *Bacteria*. *Pyrococcus furiosus*, for example, grows at 100 °C and ferments carbohydrates to acetate, CO_2, and H_2 by an unusual Emden–Meyerhof pathway involving the novel enzymes ADP-dependent glucokinase, ADP-dependent phosphofruktokinase, glyceraldehyde-3-phosphate ferredoxin oxidoreductase (GAPOR), phosphoenolpyruvate synthase, pyruvate: Ferredoxin oxidoreductase (POR), and ADP-forming acetyl-CoA synthetase (Figure 2) [4]. Other heterotrophic acetate-producing hyperthermophiles that utilize one or more of these enzymes include species from the genera *Thermococcus*, *Desulfurococcus*, *Staphylothermus*, and *Archaeoglobus* [5–8]. In the glycolytic pathway exemplified by *P. furiosus* (Figure 2), ferredoxin reduced by GAPOR and POR is re-oxidized by a membrane-bound hydrogenase that generates an ion gradient driving ATP synthesis [4]. GAPOR is an oxygen-sensitive homomonomer with a molecular mass of 63 kDa, and contains a pterin cofactor, one tungsten, and six iron atoms per monomer [9]. Pyruvate is oxidized to acetyl-CoA, catalyzed by pyruvate: Ferredoxin oxidoreductase (POR). The enzyme has a molecular mass of 100 kDa and is comprised of three subunits (45, 31, and 24 kDa), and contains thiamine pyrophosphate (TPP) and two ferredoxin-type [4Fe-4S] clusters [10]. The enzyme requires CoASH but not TPP for pyruvate oxidation activity. The POR also catalyzes the formation of acetaldehyde from pyruvate in a CoA-dependent reaction, although the cofactor plays a structural rather than catalytic role [11]. Acetyl-CoA is converted to acetate by an ADP-forming acetyl-CoA synthetase, which generates ATP by substrate level phosphorylation [12,13]. Conversion of acetyl-CoA to acetate by acetyl-CoA synthetases is characteristic of the domain *Archaea*, in contrast to the domain *Bacteria*, in which phosphotransacetylase and acetate kinase predominate [14]. A reaction mechanism has been proposed for the heterotetrameric ($\alpha_2\beta_2$) enzyme from *P. furiosus* that follows a four-step mechanism including transient phosphorylation of two active site histidine residues (Equations (1)–(4)) [15].

$$E + \text{acetyl-CoA} + \text{Pi} = E\cdot\text{acetyl}\sim\text{P} + \text{CoASH} \tag{1}$$

$$E\cdot\text{acetyl}\sim\text{P} = \text{acetate} + E\text{-His}^{257}\alpha\sim\text{P} \tag{2}$$

$$E\text{-His}^{257}\alpha\sim\text{P} = E\text{-His}^{71}\beta\sim\text{P} \tag{3}$$

$$E\text{-His}^{71}\beta\sim\text{P} + \text{ADP} = \text{ATP} + E \tag{4}$$
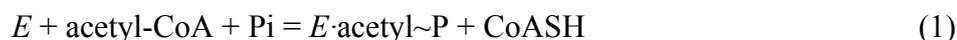
Archaeoglobus fulgidus is an example of a heterotrophic hyperthermophile from the domain *Archaea* that utilizes terminal electron acceptors in place of reducing protons and producing H_2. A strain of *A. fulgidus* grows with starch as the sole source of carbon and energy by an incomplete oxidation of glucose to acetate and CO_2, utilizing a modified Embden–Meyerhof pathway resembling that of *P. furiosus* [8]. However, in contrast to H_2-producing *P. furiosus*, electrons derived from the oxidation are transferred to sulfate, producing sulfide (Equation (5)):

$$C_6H_{12}O_6 + H_2SO_4 = 2CO_2 + 2C_2H_4O_2 + H_2S + 2H_2O. \tag{5}$$
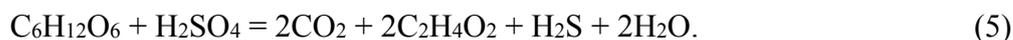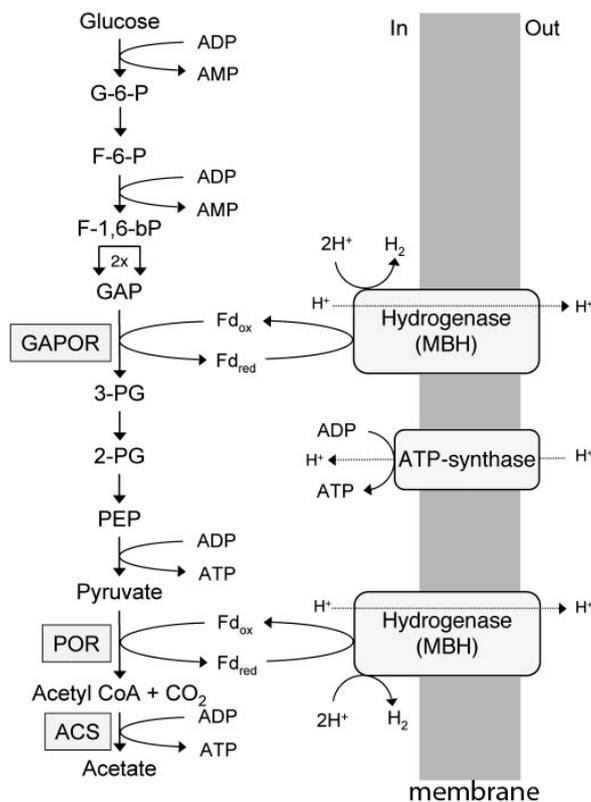
Figure 2. Electron transport and energy conversion during glucose catabolism by *P. furiosus*. G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate; F-1,6-bP, fructose 1,6-bisphosphate; GAP, glyceraldehyde phosphate, GAPOR, GAP: Ferredoxin oxidoreductase; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; POR, pyruvate: Ferredoxin oxidoreductase; ACS, acetyl-CoA synthase. Reproduced with permission [4]. Copyright (2006) National Academy of Sciences, USA.

2.2. Chemolithotrophic Energy-Converting Pathways Producing Acetate

Biochemical and quantitative proteomic analyses of *M. acetivorans* revealed a pathway for CO-dependent growth of *M. acetivorans* in which both acetate and CH_4 are products [16,17] (Figure 3). In the pathway, electrons derived from the oxidation of CO (Reaction 1a–c) are used to reduce CO_2 to a methyl group attached to THSPt (Reactions 2–6), similar to the pathway of obligate H_2-utilizing CO_2-reducing methanogens, which are the subject of several reviews recommended to the reader [18–21]. The CO dehydrogenase/acetyl-CoA synthetase CODH/ACS is proposed to oxidize CO-reducing ferredoxin, which donates electrons in the first reductive step (Reaction 2). It is possible that ferredoxin also donates electrons to FpoF, catalyzing the reduction of coenzyme F_{420} to $F_{420}H_2$ (Reaction 1c) that donates electrons to complete the reduction of CO_2 to the methyl level (Reactions 5 and 6). Indeed, it was recently shown that the FpoF of *Methanosarcina mazei* catalyzes reaction 1c [22] and a homolog in *M. acetivorans* (encoded by MA3732) is upregulated 11-fold in CO- *vs.* acetate-grown cells [16]. The methyl group of methyl-THSPt is reduced to CH_4 by reactions common to all methanogenic pathways (Reactions 7–9), albeit with two exceptions. First, a membrane-bound complex (FpoA-O) oxidizes $F_{420}H_2$ and transfers electrons to HdrDE mediated by MP, which generates a proton gradient (Reactions

9 and 10) driving ATP synthesis (Reaction 14). Second, transfer of the methyl group of methyl-THSPt to HS-CoM (Reaction 7) is thought to be catalyzed by two enzymes, the membrane-bound Mtr complex coupled to Na^+ translocation, common to all methanogenic pathways; and a soluble enzyme (CmtA) unique to the CO-dependent CO_2 reduction pathway [23]. Acetate is a prominent product in addition to CH_4. Levels of CODH/ACS, phosphotransacetylase, and acetate kinase are prominent in CO-grown cells [16], supporting a route to acetate wherein CODH/ACS synthesizes acetyl-CoA with the methyl group of methyl-THSPt, CO, and CoA-SH (Reaction 11), which are further converted to acetate by phosphotransacetylase and acetate kinase (Reactions 12 and 13) with the production of ATP. Thus, ATP is synthesized via both substrate level and chemiosmotic mechanisms.

A. fulgidus uses essentially the same pathway during CO-dependent growth, employing similar enzymes and electron carriers [24–28], except for conversion of acetyl-CoA to acetate, which is catalyzed by either an AMP- or ADP-forming acetyl-CoA synthetase [29–31]. *A. fulgidus* does not produce methane, and acetate is the sole product in the absence of sulfate.
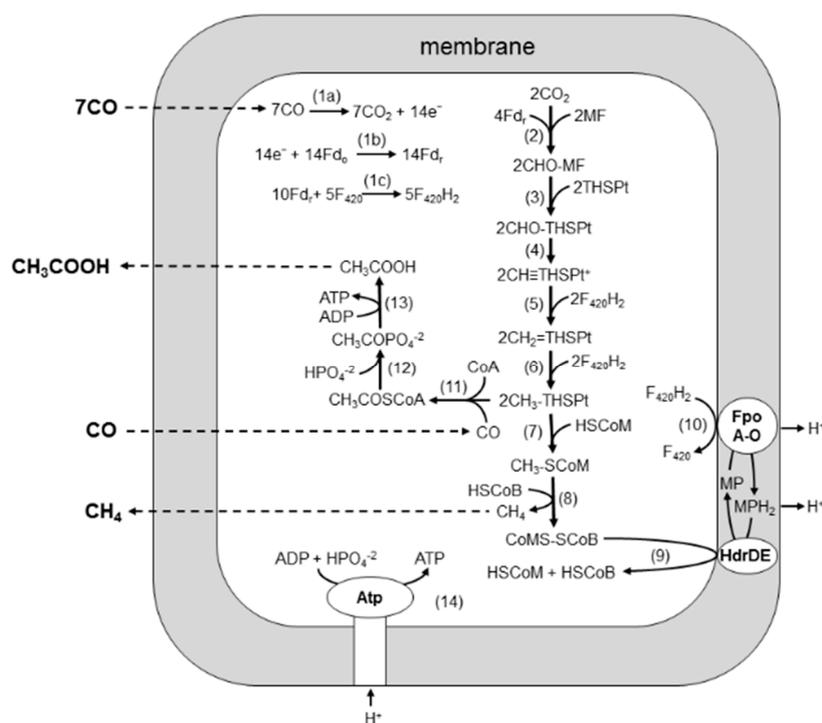
Figure 3. Pathway for conversion of CO to acetate and CH_4 by *M. acetivorans*. See text. Fd_o, oxidized ferredoxin; Fd_r, reduced ferredoxin; F_{420}, coenzyme F_{420}; MF, methanofuran; THSPt, tetrahydrosarcinapterin; HSCoM, coenzyme M; HSCoB, coenzyme B; Fpo, $F_{420}H_2$ dehydrogenase complex; MP, methanophenazine; Hdr, heterodisulfide reductase. Reproduced from [16] with permission. Copyright (2006) National Academy of Sciences, USA.

3. Acetate Utilization

3.1. Acetotrophic Energy-Converting Pathways Producing Methane

Methane-producing species from the genera *Methanosarcina* and *Methanosaeta* are the only known acetoclastic genera in the domain *Archaea*. The majority of studies on the acetoclastic pathway have

involved *Methanosarcina* species, for which *M. acetivorans* is a model (Figure 4). Carbon transfer reactions in the pathway can be divided into two parts: (1) Reactions 1–3: activation to acetyl-CoA and cleaving the C-C bond of the acetyl group, yielding CH_3-THSPt and CO_2, which are unique to the pathway; and (2) Reactions 4–6: reducing the methyl group of CH_3-THSPt to CH_4, which is common to all methanogenic pathways. Methanogenesis, and by inference reactions common to all methanogenic pathways, is thought to have evolved soon after the origin of life, approximately 3.75 billion years ago [32,33]. However, evolution of the acetoclastic pathway is proposed to have evolved approximately 250–300 million years ago during the end-Permian carbon cycle that contributed to the mass extinction of that period [34]. Reactions 2 and 3, catalyzed by acetate kinase and CO dehydrogenase/acetyl-CoA synthase CODH/ACS, are thought to be ancient enzymes evolving soon after the origin of life [35,36]. Homologs of acetate kinase and phosphotransacetylase are key enzymes in energy-yielding pathways of fermentative and acetogenic species from the domain *Bacteria*, converting acetyl-CoA to ATP and acetate. These observations are consistent with the evolution of the aceticlastic pathway by horizontal gene transfer of enzymes catalyzing Reactions 1–3 from the domain *Bacteria* and grafting on to Reactions 4–6, common to more ancient methanogenic pathways. Reactions 4 and 5, and the enzymes catalyzing them, are the subject of recent reviews that are recommended to the reader [37,38].
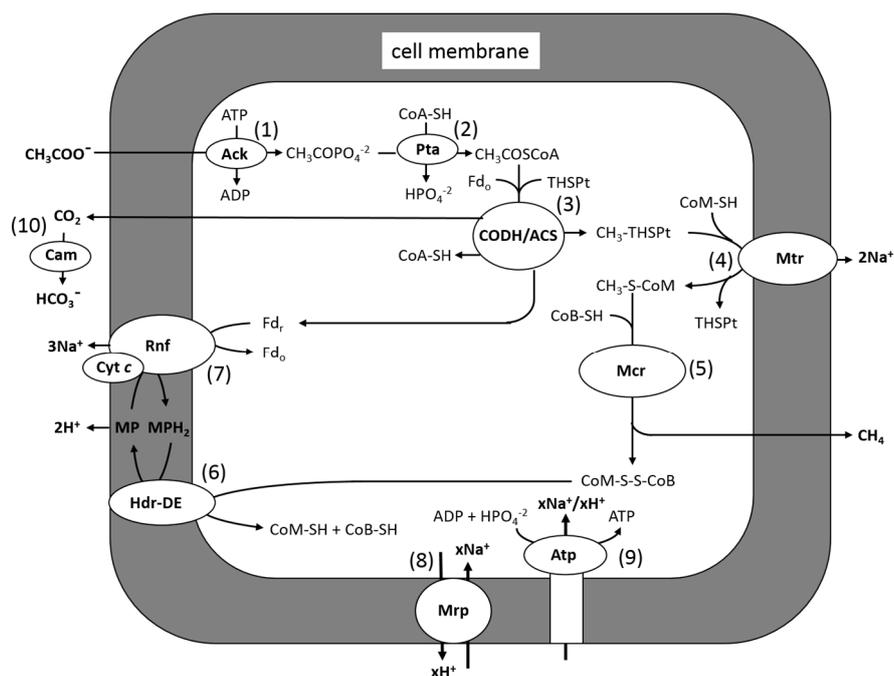
Figure 4. Pathway of aceticlastic methanogenesis in *M. acetivorans*. Ack, acetate kinase; Pta, phosphotransacetylase; CoA-SH, coenzyme A; THSPt, tetrahydrosarcinapterin; Fdr, reduced ferredoxin; Fdo, oxidized ferredoxin; Cdh, CO dehydrogenase/acetyl-CoA synthase; CoM-SH, coenzyme M; Mtr, methyl-THSPt:CoM-SH methyltransferase; CoB-SH, coenzyme B; MP, methanophenazine; Hdr-DE, heterodisulfide reductase; Rnf, Rnf complex; Mrp, Mrp complex; Atp, ATP synthase. Modified from [39].

The first crystal structure for any acetate kinase was from the acetoclastic methanogen *Methanosarcina thermophila*, which revealed properties suggesting that the enzyme is the founding

member of the Acetate and Sugar Kinase/Hsc70/Actin (ASKHA) superfamily of phosphotransferases [35]. Structural and biochemical investigations established a direct in-line mechanism in which the carboxylate anion attacks the γ-phosphate of ATP enabling transfer of the phosphate group [40–48]. The first crystal structure of any phosphotransacetylase was also from *M. thermophila*, the structural and biochemical analyses of which indicate a mechanism that proceeds through base-catalyzed abstraction of the HS-CoA thiol proton and subsequent nucleophilic attack of $^-$S-CoA on the carbonyl carbon of acetyl phosphate [49].

The CODH/ACS complex [50–59] cleaves the C-C and C-S bonds of acetyl-CoA, transferring the methyl group to THSPt and oxidizing the carbonyl group to CO_2 with transfer of electrons to ferredoxin [60–62] (Reaction 3). The same enzyme complex functions in reverse to synthesize acetyl-CoA for cell carbon in CO_2-reducing methanogens and oxidizes exogenous CO in the pathway of CO conversion to CH_4, CO_2, and acetate in *M. acetivorans*. Of ancient origin, primitive ancestors of CODH/ACS likely played a central role in the early evolution of life [36,63,64]. Although a two-subunit enzyme has been purified and characterized from an acetate-utilizing species of the genus *Methanosaeta* [54,57–59], the majority of mechanistic studies have been with the five-subunit ($\alpha\beta\gamma\delta\epsilon$) complexes from the acetate-utilizing species *M. thermophila* and *Methanosarcina barkeri*. The complexes are resolvable into three components containing the $\alpha\epsilon$, $\gamma\delta$, or β subunit(s) [65]. The $\alpha\epsilon$ component catalyzes the oxidation of CO and reduction of ferredoxin [60,66]. The crystal structure from *M. barkeri* shows a $\alpha_2\epsilon_2$ arrangement with the α subunit containing four 4Fe-4S clusters and a $NiFe_3S_4$ cluster bridged to an exogenous iron atom called the "C" cluster [67]. Two of the 4Fe-4S clusters are postulated to function in electron transport from the active site "C" cluster to ferredoxin. The structure suggests coupling between CO bound to the nickel and H_2O/OH^- bound to the exogenous iron in the C=O bond-forming step leading to the oxidized product CO_2. The structure also shows a gas channel extending from the "C" cluster to the protein surface with the potential to interface with the β component containing the "A" cluster, thus catalyzing acetyl-CoA cleavage and carbonyl group conversion to CO [68–71]. Although the structure is unknown, spectroscopic investigations indicate that the "A" cluster is comprised of a 4Fe-4S center bridged to a binuclear Ni-Ni site [70,72], similar to the homolog from an acetate-producing species from the domain *Bacteria* that synthesizes acetyl-CoA [73]. A mechanism is proposed in which transfer of an electron from "C" to "A" maintains the reduced catalytically active Ni(I) redox state of "A" [74]. Both kinetic and EPR approaches support the fact that alterations in the Ni coordination environment of the "A" cluster promote C−C bond cleavage, dependent on changes in the protein conformation from the open to closed state [75,76]. Moreover, CO is proposed to be an inhibitor of C-C bond cleavage; thus, control over C−C bond cleavage in concert with containment of CO in the gas channel explains the requirement for tight coupling of the decarbonylation reaction for efficient transfer of CO to "C" for oxidation [75]. The $\gamma\delta$ component transfers the methyl group of acetyl-CoA to THSPt, involving a corrinoid cofactor and an iron-sulfur cluster [55,77–79], although it has yet to be determined which of the two subunits interact with THSPt. Spectroscopic EPR analyses indicate that the corrinoid cofactor is maintained in the base-off state with a E'_0 of -486 mV for the $Co^{2+/1+}$ couple that facilitates reduction of Co^{2+} to Co^{1+} required for methylation of the corrinoid [77]. The analysis also identified a 4Fe-4S cluster with a midpoint potential close to the $Co^{2+/1+}$ couple, suggesting that the cluster is involved in reducing Co^{2+}.

The conversion of acetate to CH_4 and CO_2 provides only a marginal amount of energy available for growth ($\Delta G^{0\prime} = -36$ kJ/CH_4) that is spent on the ATP consumed in the activation to acetyl-CoA (Figure 4), which illustrates the importance of cells maximizing thermodynamic efficiency. A theoretical analysis of acetate-grown *M. barkeri* indicates that transfer of CH_4 and CO_2 into the gaseous phase contributes to the driving force of growth [80]. Thus it has been proposed that a carbonic anhydrase (Cam) from *M. thermophila* is located outside the cell membrane, where it converts CO_2 to membrane-impermeable HCO_3^- (Reaction 10, Figure 4), thereby facilitating removal of CO_2 from the cytoplasm [81]. Cam from *M. thermophila* is the archetype of an independently evolved class (γ class) of carbonic anhydrases that contains Fe^{2+} in the active site, contrary to all prokaryotic carbonic anhydrases, which contain zinc [82,83]. Structural and biochemical analyses [84] support a two-step ping pong mechanism, shown in Equations (6) and (7), where E represents enzyme residues, M is metal, and B is the buffer.

$$E\text{-}Fe^{2+}\text{-}OH^- + CO_2 = E\text{-}Fe^{2+}\text{-}HCO_3^- \tag{6a}$$

$$E\text{-}Fe^{2+}\text{-}HCO_3^- + H_2O = E\text{-}Fe^{2+}\text{-}H_2O + HCO_3^- \tag{6b}$$

$$E\text{-}Fe^{2+}\text{-}H_2O = H^+\text{-}E\text{-}Fe^{2+}\text{-}OH^- \tag{7a}$$

$$H^+\text{-}E\text{-}Fe^{2+}\text{-}OH^- + B = E\text{-}Fe^{2+}\text{-}OH^- + BH^+ \tag{7b}$$
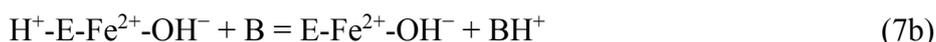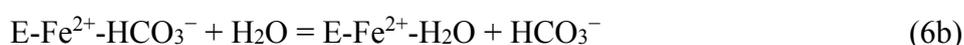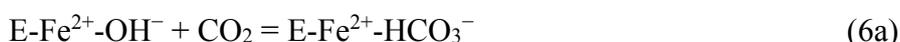
In Step 1 a lone pair of electrons on the metal-bound hydroxide attacks CO_2-producing metal-bound bicarbonate (Equation (6a)), which is subsequently displaced by water (Equation (6b)). In Step 2 a proton is extracted from the metal-bound water (Equation (7a)), and then transferred to the buffer (Equation (7b)).

ATP is synthesized by a chemiosmotic mechanism. Ferredoxin accepts electrons derived from the oxidation of the carbonyl group of acetyl-CoA by CODH/ACS in both H_2-dependent and H_2-independent acetotrophic methanogens. Both types also obtain energy for growth by coupling electron transfer from ferredoxin to the heterodisulfide CoM-S-S-CoB, with translocation of ions generating a gradient that drives ATP synthesis catalyzed by an A_1A_0-type ATP synthase [85–88]. The reduced ferredoxin of H_2-dependent species donates electrons to a membrane-bound hydrogenase (Ech), evolving H_2 and translocating protons [89–91]. It is proposed that a hydrogenase (Vho) reoxidizes H_2 and donates electrons to a quinone-like electron carrier called methanophenazine (MP) [92]. The MP donates electrons to the heterodisulfide reductase HdrDE concomitant with translocation of two protons contributing to the gradient. An additional two protons are translocated by the Vho. However, most acetotrophic methanogens are H_2 independent [93–104] and likely evolved a different strategy for oxidizing ferredoxin and reducing CoM-S-S-CoB typified by *M. acetivorans* [105,106] (Figure 4). The genome does not encode a functional Ech; instead, acetate-grown cells preferentially synthesize a Rnf complex [87] similar to the six-subunit Rnf complexes in microbes from the domain *Bacteria* [107–113]. A Δrnf strain is unable to grow with acetate, confirming that the complex is essential [114]. Unlike all other characterized Rnf complexes, the contiguous genes encoding the six-subunit core complex from *M. acetivorans* are co-transcribed with a gene encoding a multiheme cytochrome *c* abundant in membranes of acetate-grown cells [87]. A topology model [39] predicts roles for each of the six core subunits and cytochrome *c* wherein MP mediates electron transfer between cytochrome *c* and HdrDE, translocating a pair of protons [39]. It has been shown that the Rnf complex translocates Na^+ (Reaction 7),

joining the Na^+ translocating methyl transfer (Reaction 4) [114]. Thus, both Na^+ and H^+ gradients are generated during growth with acetate. Notably, the A_1A_0-type ATP synthase of *M. acetivorans* is dependent on both Na^+ and H^+ gradients [115]. A multisubunit Na^+/H^+ antiporter (Mrp) is proposed to adjust the Na^+/H^+ ratio (Reaction 8) optimal for ATP synthesis by the A_1A_0-type ATP synthase (Reaction 9) [116].

A genome-wide analysis of *Methanosaeta thermophila* has revealed genes encoding enzymes catalyzing reactions in the pathway of acetate to CH_4 identical to *Methanosarcina* species except for the activation of acetate to acetyl-CoA, which is catalyzed by an AMP- and PP_i-forming acetyl-CoA synthetase [117–120]. The synthetase has a several-fold lower K_m for acetate (0.4 mM) than acetate kinase from *M. thermophila* (22 mM) [118,121], a result consistent with *Methanosaeta* species dominating over *Methanosarcina* species in environments where acetate is in low concentrations. Genes encoding Ech and Rnf are absent in the genome of *Methanosaeta*, suggesting an unknown alternative electron transport pathway and mechanism for energy conservation [117].

3.2. Acetotrophic Energy-Converting Pathways Reducing Exogenous Electron Acceptors

Acetate-utilizing prokaryotes from the domain *Archaea* also obtain energy by anaerobic respiration. *Ferroglobus placidus* and *Geoglobus ahangari* are hyperthermophiles growing at 85 °C by oxidizing acetate to CO_2, only with Fe(III) serving as the electron acceptor [122]. *Geoglobus acetivorans* is another hyperthermophile growing optimally at 81 °C and utilizing acetate in addition to formate, pyruvate, fumarate, malate, propionate, butyrate, succinate, glycerol, stearate, palmitate, peptone, and yeast extract as electron donors for Fe(III) reduction. The organism is also able to grow with H_2 as the electron donor and Fe(III) as an electron acceptor without the need for organic substances [123]. Hyperthermophilic *Thermococcus* species have also been implicated in oxidizing acetate and reducing Fe(III) [124].

4. Concluding Remarks

Although heterotrophic acetate-producing hyperthermophiles are abundantly documented, no hyperthermophilic acetoclastic methanogen has been described that presents a disconnect in the ecology of these two metabolic groups for which cognate mesophiles function syntrophically in anaerobic microbial food chains, converting complex biomass to CH_4. One possibility is that hyperthermophilic temperatures are a thermodynamic barrier to the conversion of acetate to CH_4. Additionally, although it appears that heterotrophic organisms from the domain *Archaea* (Thaumarchaea) proliferate in mesothermal anaerobic environments [125], isolates and details of their metabolism are largely unknown. Thus, the finding that anaerobic respiratory acetate-oxidizing species are found in hyperthermophilic environments suggests the possibility that acetate-producing heterotrophs like *P. furiosus* supply acetate in a two-component microbial food chain converting complex organics to CO_2.

Conflicts of Interest

The author declares no conflict of interest.

References

1. Whitman, W.B.; Coleman, D.C.; Wiebe, W.J. Prokaryotes: The Unseen Majority. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6578–6583.
2. Berg, I.A.; Kockelkorn, D.; Ramos-Vera, W.H.; Say, R.F.; Zarzycki, J.; Hugler, M.; Alber, B.E.; Fuchs, G. Autotrophic Carbon Fixation in Archaea. *Nat. Rev. Microbiol.* **2010**, *8*, 447–460.
3. Thauer, R.K. Functionalization of Methane in Anaerobic Microorganisms. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 6712–6713.
4. Sapra, R.; Bagramyan, K.; Adams, M.W. A Simple Energy-Conserving System: Proton Reduction Coupled to Proton Translocation. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 7545–7550.
5. Selig, M.; Xavier, K.B.; Santos, H.; Schonheit, P. Comparative Analysis of Embden-Meyerhof and Entner-Doudoroff Glycolytic Pathways in Hyperthermophilic Archaea and the Bacterium Thermotoga. *Arch. Microbiol.* **1997**, *167*, 217–232.
6. Perevalova, A.A.; Svetlichny, V.A.; Kublanov, I.V.; Chernyh, N.A.; Kostrikina, N.A.; Tourova, T.P.; Kuznetsov, B.B.; Bonch-Osmolovskaya, E.A. *Desulfurococcus Fermentans* Sp. Nov., A Novel Hyperthermophilic Archaeon from a Kamchatka Hot Spring, and Emended Description of the Genus *Desulfurococcus*. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 995–999.
7. Anderson, I.J.; Dharmarajan, L.; Rodriguez, J.; Hooper, S.; Porat, I.; Ulrich, L.E.; Elkins, J.G.; Mavromatis, K.; Sun, H.; Land, M.; *et al.* The Complete Genome Sequence of *Staphylothermus Marinus* Reveals Differences in Sulfur Metabolism among Heterotrophic Crenarchaeota. *BMC Genomics* **2009**, *10*, doi:10.1186/1471-2164-10-145.
8. Labes, A.; Schonheit, P. Sugar Utilization in the Hyperthermophilic, Sulfate-Reducing Archaeon *Archaeoglobus Fulgidus* Strain 7324: Starch Degradation to Acetate and CO_2 via a Modified Embden-Meyerhof Pathway and Acetyl-Coa Synthetase (Adp-Forming). *Arch. Microbiol.* **2001**, *176*, 329–338.
9. Mukund, S.; Adams, M.W. Glyceraldehyde-3-Phosphate Ferredoxin Oxidoreductase, a Novel Tungsten-Containing Enzyme with a Potential Glycolytic Role in the Hyperthermophilic Archaeon *Pyrococcus furiosus*. *J. Biol. Chem.* **1995**, *270*, 8389–8392.
10. Blamey, J.M.; Adams, M.W.W. Purification and characterization of pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon *Pyrococcus furiosus*. *Biochim. Biophys. Acta* **1993**, *1161*, 19–27.
11. Ma, K.; Hutchins, A.; Sung, S.J.; Adams, M.W. Pyruvate Ferredoxin Oxidoreductase from the Hyperthermophilic Archaeon, *Pyrococcus furiosus*, Functions as a Coa-Dependent Pyruvate Decarboxylase. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9608–9613.
12. Musfeldt, M.; Selig, M.; Schonheit, P. Acetyl Coenzyme a Synthetase (ADP Forming) from the Hyperthermophilic Archaeon *Pyrococcus furiosus*: Identification, Cloning, Separate Expression of the Encoding Genes, *Acdai* and *Acdbi*, in *Escherichia Coli*, and *in vitro* Reconstitution of the Active Heterotetrameric Enzyme from Its Recombinant Subunits. *J. Bacteriol.* **1999**, *181*, 5885–5888.

13. Glasemacher, J.; Bock, A.K.; Schmid, R.; Schonheit, P. Purification and Properties of Acetyl-Coa Synthetase (Adp-Forming), an Archaeal Enzyme of Acetate Formation and Atp Synthesis, from the Hyperthermophile *Pyrococcus furiosus*. *Eur. J. Biochem.* **1997**, *244*, 561–567.

14. Schafer, T.; Selig, M.; Schonheit, P. Acetyl-Coa Synthetase (Adp Forming) in Archaea, a Novel Enzyme Involved in Acetate Formation and Atp Synthesis. *Arch. Microbiol.* **1993**, *159*, 72–83.

15. Brasen, C.; Schmidt, M.; Grotzinger, J.; Schonheit, P. Reaction Mechanism and Structural Model of ADP-Forming Acetyl-Coa Synthetase from the Hyperthermophilic Archaeon *Pyrococcus furiosus*: Evidence for a Second Active Site Histidine Residue. *J. Biol. Chem.* **2008**, *283*, 15409–15418.

16. Lessner, D.J.; Li, L.; Li, Q.; Rejtar, T.; Andreev, V.P.; Reichlen, M.; Hill, K.; Moran, J.J.; Karger, B.L.; Ferry, J.G. An Unconventional Pathway for Reduction of CO_2 to Methane in Co-Grown *Methanosarcina acetivorans* Revealed by Proteomics. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17921–17926.

17. Rother, M.; Oelgeschlager, E.; Metcalf, W.M. Genetic and Proteomic Analyses of Co Utilization by *Methanosarcina acetivorans*. *Arch. Microbiol.* **2007**, *188*, 463–472.

18. Ferry, J.G. Fundamentals of Methanogenic Pathways That Are Key to the Biomethanation of Complex Biomass. *Curr. Opin. Biotechnol.* **2011**, *22*, 351–357.

19. Ferry, J.G. How to Make a Living Exhaling Methane. *Annu. Rev. Microbiol.* **2010**, *64*, 453–473.

20. Thauer, R.K.; Kaster, A.K.; Seedorf, H.; Buckel, W.; Hedderich, R. Methanogenic Archaea: Ecologically Relevant Differences in Energy Conservation. *Nat. Rev. Microbiol.* **2008**, *6*, 579–591.

21. Liu, Y.; Whitman, W.B. Metabolic, Phylogenetic, and Ecological Diversity of the Methanogenic Archaea. *Ann. N. Y. Acad. Sci.* **2008**, *1125*, 171–189.

22. Welte, C.; Deppenmeier, U. Re-Evaluation of the Function of the F_{420} Dehydrogenase in Electron Transport of *Methanosarcina Mazei*. *FEBS J.* **2011**, *278*, 1277–1287.

23. Vepachedu, V.R.; Ferry, J.G. Role of the Fused Corrinoid/Methyl Transfer Protein Cmta during Co-Dependent Growth of *Methanosarcina acetivorans*. *J. Bacteriol.* **2012**, *194*, 4161–4168.

24. Moller-Zinkhan, D.; Thauer, R.K. Anaerobic Lactate Oxidation to 3 CO_2 by *Archaeoglobus Fulgidus* via The Carbon Monoxide Dehydrogenase Pathway. Demonstration of the Acetyl-Coa Carbon-Carbon Cleavage Reaction in Cell Extracts. *Arch. Microbiol.* **1990**, *153*, 215–218.

25. Gorris, L.; Voet, A.; van der Drift, C. Structural Characteristics of Methanogenic Cofactors in the Non-Methanogenic Archaebacterium *Archaeoglobus fulgidus*. *Biofactors* **1991**, *3*, 29–35.

26. Schworer, B.; Breitung, J.; Klein, A.R.; Stetter, K.O.; Thauer, R.K. Formylmethanofuran: Tetrahydromethanopterin Formyltransferase and N^5,N^{10}-Methylenetetrahydromethanopterin Dehydrogenase from the Sulfate-Reducing *Archaeoglobus Fulgidus*: Similarities with the Enzymes from Methanogenic Archaea. *Arch. Microbiol.* **1993**, *159*, 225–232.

27. Kunow, J.; Linder, D.; Stetter, K.O.; Thauer, R.K. $F_{420}h_2$:Quinone Oxidoreductase from *Archaeoglobus Fulgidus*. Characterization of a Membrane-Bound Multisubunit Complex Containing Fad and Iron-Sulfur Clusters. *Eur. J. Biochem.* **1994**, *223*, 503–511.

28. Dai, Y.R.; Reed, D.W.; Millstein, J.H.; Hartzell, P.L.; Grahame, D.A.; Demoll, E. Acetyl-Coa Decarbonylase/Synthase Complex from *Archaeoglobus fulgidus*. *Arch. Microbiol.* **1998**, *169*, 525–529.

29. Ingram-Smith, C.; Smith, K.S. Amp-Forming Acetyl-Coa Synthetases in Archaea Show Unexpected Diversity in Substrate Utilization. *Archaea* **2007**, *2*, 95–107.

30. Henstra, A.M.; Dijkema, C.; Stams, A.J. *Archaeoglobus Fulgidus* Couples CO Oxidation to Sulfate Reduction and Acetogenesis with Transient Formate Accumulation. *Environ. Microbiol.* **2007**, *9*, 1836–1841.

31. Musfeldt, M.; Schonheit, P. Novel Type of Adp-Forming Acetyl Coenzyme a Synthetase in Hyperthermophilic Archaea: Heterologous Expression and Characterization of Isoenzymes from the Sulfate Reducer *Archaeoglobus fulgidus* and the Methanogen *Methanococcus jannaschii*. *J. Bacteriol.* **2002**, *184*, 636–644.

32. Battistuzzi, F.U.; Feijao, A.; Hedges, S.B. A Genomic Timescale of Prokaryote Evolution: Insights into the Origin of Methanogenesis, Phototrophy, and the Colonization of Land. *BMC Evol. Biol.* **2004**, *4*, doi:10.1186/1471-2148-4-44.

33. Ueno, Y.; Yamada, K.; Yoshida, N.; Maruyama, S.; Isozaki, Y. Evidence from Fluid Inclusions for Microbial Methanogenesis in the Early Archaean Era. *Nature* **2006**, *440*, 516–519.

34. Rothman, D.H.; Fournier, G.P.; French, K.L.; Alm, E.J.; Boyle, E.A.; Cao, C.; Summons, R.E. Methanogenic Burst in the End-Permian Carbon Cycle. *Proc. Natl. Acad. Sci. USA.* **2014**, *111*, 5462–5467.

35. Buss, K.A.; Cooper, D.R.; Ingram-Smith, C.; Ferry, J.G.; Sanders, D.A.; Hasson, M.S. Urkinase: Structure of Acetate Kinase, a Member of the Askha Superfamily of Phosphotransferases. *J. Bacteriol.* **2001**, *183*, 680–686.

36. Lindahl, P.A.; Chang, B. The Evolution of Acetyl-Coa Synthase. *Orig. Life Evol. Biosph.* **2001**, *31*, 403–434.

37. Gottschalk, G.; Thauer, R.K. The Na^+ Translocating Methyltransferase Complex from Methanogenic Archaea. *Biochim. Biophys. Acta* **2001**, *1505*, 28–36.

38. Shima, S.; Warkentin, E.; Thauer, R.K.; Ermler, U. Structure and Function of Enzymes Involved in the Methanogenic Pathway Utilizing Carbon Dioxide and Molecular Hydrogen. *J. Biosci. Bioeng.* **2002**, *93*, 519–530.

39. Suharti, S.; Wang, M.; de Vries, S.; Ferry, J.G. Characterization of the RNFB and RNFG Subunits of the RNF Complex from the Archaeon *Methanosarcina acetivorans*. *PLoS ONE* **2014**, *9*, E97966.

40. Gorrell, A.; Lawrence, S.H.; Ferry, J.G. Structural and Kinetic Analyses of Arginine Residues in the Active-Site of the Acetate Kinase from *Methanosarcina thermophila*. *J. Biol. Chem.* **2005**, *280*, 10731–10742.

41. Miles, R.D.; Gorrell, A.; Ferry, J.G. Evidence for a Transition State Analog, Mgadp-Aluminum Fluoride-Acetate, in Acetate Kinase from *Methanosarcina thermophila*. *J. Biol. Chem.* **2002**, *277*, 22547–22552.

42. Ingram-Smith, C.; Gorrell, A.; Lawrence, S.H.; Iyer, P.; Smith, K.; Ferry, J.G. Identification of the Acetate Binding Site in the *Methanosarcina thermophila* Acetate Kinase. *J. Bacteriol.* **2005**, *187*, 2386–2394.

43. Miles, R.D.; Iyer, P.P.; Ferry, J.G. Site-Directed Mutational Analysis of Active Site Residues in the Acetate Kinase from *Methanosarcina thermophila*. *J. Biol. Chem.* **2001**, *276*, 45059–45064.

44. Ingram-Smith, C.; Barber, R.D.; Ferry, J.G. The Role of Histidines in the Acetate Kinase from *Methanosarcina thermophila*. *J. Biol. Chem.* **2000**, *275*, 33765–33770.

45. Singh-Wissmann, K.; Miles, R.D.; Ingram-Smith, C.; Ferry, J.G. Identification of Essential Arginines in the Acetate Kinase from *Methanosarcina thermophila*. *Biochemistry* **2000**, *39*, 3671–3677.

46. Singh-Wissmann, K.; Ingram-Smith, C.; Miles, R.D.; Ferry, J.G. Identification of Essential Glutamates in the Acetate Kinase From *Methanosarcina thermophila*. *J. Bacteriol.* **1998**, *180*, 1129–1134.

47. Gorrell, A.; Ferry, J.G. Investigation of the *Methanosarcina thermophila* Acetate Kinase Mechanism by Fluorescence Quenching. *Biochemistry* **2007**, *46*, 14170–14176.

48. Ingram-Smith, C.; Wharton, J.; Reinholz, C.; Doucet, T.; Hesler, R.; Smith, K. The Role of Active Site Residues in Atp Binding and Catalysis in the *Methanosarcina thermophila* Acetate Kinase. *Life* **2015**, *5*, 861–871.

49. Iyer, P.P.; Lawrence, S.H.; Luther, K.B.; Rajashankar, K.R.; Yennawar, H.P.; Ferry, J.G.; Schindelin, H. Crystal Structure of Phosphotransacetylase from the Methanogenic Archaeon *Methanosarcina thermophila*. *Structure* **2004**, *12*, 559–567.

50. Abbanat, D.R.; Ferry, J.G. Synthesis of Acetyl-Coa by the Carbon Monoxide Dehydrogenase Complex from Acetate-Grown *Methanosarcina thermophila*. *J. Bacteriol.* **1990**, *172*, 7145–7150.

51. Raybuck, S.A.; Ramer, S.E.; Abbanat, D.R.; Peters, J.W.; Orme-Johnson, W.H.; Ferry, J.G.; Walsh, C.T. Demonstration of Carbon-Carbon Bond Cleavage of Acetyl Coenzyme a by Using Isotopic Exchange Catalyzed by the Co Dehydrogenase Complex from Acetate-Grown *Methanosarcina thermophila*. *J. Bacteriol.* **1991**, *173*, 929–932.

52. Terlesky, K.C.; Nelson, M.J.K.; Ferry, J.G. Isolation of an Enzyme Complex with Carbon Monoxide Dehydrogenase Activity Containing a Corrinoid and Nickel from Acetate-Grown *Methanosarcina thermophila*. *J. Bacteriol.* **1986**, *168*, 1053–1058.

53. Eggen, R.I.L.; Vankranenburg, R.; Vriesema, A.J.M.; Geerling, A.C.M.; Verhagen, M.F.J.M.; Hagen, W.R.; Devos, W.M. Carbon Monoxide Dehydrogenase from *Methanosarcina Frisia* Gö1. Characterization of the Enzyme and the Regulated Expression of Two Operon-Like *Cdh* Gene Clusters. *J. Biol. Chem.* **1996**, *271*, 14256–14263.

54. Jetten, M.S.M.; Hagen, W.R.; Pierik, A.J.; Stams, A.J.M.; Zehnder, A.J.B. Paramagnetic Centers and Acetyl-Coenzyme a CO Exchange Activity of Carbon Monoxide Dehydrogenase from *Methanothrix soehngenii*. *Eur. J. Biochem.* **1991**, *195*, 385–391.

55. Grahame, D.A. Substrate and Cofactor Reactivity of A Carbon Monoxide Dehydrogenase Corrinoid Enzyme Complex. Stepwise Reduction of Iron Sulfur and Corrinoid Centers, the Corrinoid $Co^{2+/1+}$ Redox Midpoint Potential, and Overall Synthesis of Acetyl-Coa. *Biochemistry* **1993**, *32*, 10786–10793.

56. Grahame, D.A.; Demoll, E. Substrate and Accessory Protein Requirements and Thermodynamics of Acetyl-Coa Synthesis and Cleavage in *Methanosarcina barkeri*. *Biochemistry* **1995**, *34*, 4617–4624.

57. Jetten, M.S.M.; Stams, A.J.M.; Zehnder, A.J.B. Purification and Characterization of an Oxygen-Stable Carbon Monoxide Dehydrogenase of *Methanothrix soehngenii*. *FEBS Lett.* **1989**, *181*, 437–441.

58. Eggen, R.I.L.; Geerling, A.C.M.; Jetten, M.S.M.; Devos, W.M. Cloning, Expression, and Sequence Analysis of the Genes for Carbon Monoxide Dehydrogenase of *Methanothrix soehngenii*. *J. Biol. Chem.* **1991**, *266*, 6883–6887.

59. Jetten, M.S.M.; Pierik, A.J.; Hagen, W.R. Epr Characterization of a High-Spin System in Carbon Monoxide Dehydrogenase from *Methanothrix soehngenii*. *Eur. J. Biochem.* **1991**, *202*, 1291–1297.

60. Terlesky, K.C.; Ferry, J.G. Ferredoxin Requirement for Electron Transport from the Carbon Monoxide Dehydrogenase Complex to a Membrane-Bound Hydrogenase in Acetate-Grown *Methanosarcina thermophila*. *J. Biol. Chem.* **1988**, *263*, 4075–4079.

61. Terlesky, K.C.; Ferry, J.G. Purification and Characterization of a Ferredoxin from Acetate-Grown *Methanosarcina thermophila*. *J. Biol. Chem.* **1988**, *263*, 4080–4082.

62. Fischer, R.; Thauer, R.K. Ferredoxin-Dependent Methane Formation from Acetate in Cell Extracts of *Methanosarcina barkeri* (Strain Ms). *FEBS Lett.* **1990**, *269*, 368–372.

63. Ferry, J.G.; House, C.H. The Stepwise Evolution of Early Life Driven by Energy Conservation. *Mol. Biol. Evol.* **2006**, *23*, 1286–1292.

64. Martin, W.; Russell, M.J. On the Origin of Biochemistry at an Alkaline Hydrothermal Vent. *Philos. Trans. R. Soc. B* **2006**, *362*, 1887–1926.

65. Abbanat, D.R.; Ferry, J.G. Resolution of Component Proteins in an Enzyme Complex from *Methanosarcina thermophila* Catalyzing the Synthesis or Cleavage of Acetyl-Coa. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 3272–3276.

66. Clements, A.P.; Ferry, J.G. Cloning, Nucleotide Sequence, and Transcriptional Analyses of the Gene Encoding a Ferredoxin from *Methanosarcina thermophila*. *J. Bacteriol.* **1992**, *174*, 5244–5250.

67. Gong, W.; Hao, B.; Wei, Z.; Ferguson, D.J., Jr.; Tallant, T.; Krzycki, J.A.; Chan, M.K. Structure of the A_2e_2 Ni-Dependent Co Dehydrogenase Component of the *Methanosarcina barkeri* Acetyl-Coa Decarbonylase/Synthase Complex. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9558–9563.

68. Murakami, E.; Ragsdale, S.W. Evidence for Intersubunit Communication during Acetyl-Coa Cleavage by the Multienzyme Co Dehydrogenase/Acetyl-Coa Synthase Complex from *Methanosarcina thermophila* Evidence That the Beta Subunit Catalyzes C-C and C-S Bond Cleavage. *J. Biol. Chem.* **2000**, *275*, 4699–4707.

69. Grahame, D.A.; Demoll, E. Partial Reactions Catalyzed by Protein Components of the Acetyl-Coa Decarbonylase Synthase Enzyme Complex from *Methanosarcina barkeri*. *J. Biol. Chem.* **1996**, *271*, 8352–8358.

70. Funk, T.; Gu, W.W.; Friedrich, S.; Wang, H.X.; Gencic, S.; Grahame, D.A.; Cramer, S.P. Chemically Distinct Ni Sites in the A-Cluster in Subunit Beta of the Acetyl-Coa Decarbonylase/Synthase Complex from *Methanosarcina thermophila*: Ni L-Edge Absorption And X-Ray Magnetic Circular Dichroism Analyses. *J. Am. Chem. Soc.* **2004**, *126*, 88–95.

71. Gencic, S.; Grahame, D.A. Nickel in Subunit B of the Acetyl-Coa Decarbonylase/Synthase Multienzyme Complex in Methanogens. *J. Biol. Chem.* **2003**, *278*, 6101–6110.

72. Gu, W.W.; Gencic, S.; Cramer, S.P.; Grahame, D.A. The A-Cluster in Subunit Beta of the Acetyl-Coa Decarbonylase/Synthase Complex from *Methanosarcina thermophila*: Ni and Fe K-Edge Xanes and Exafs Analyses. *J. Am. Chem. Soc.* **2003**, *125*, 15343–15351.

73. Ragsdale, S.W. Nickel and the Carbon Cycle. *J. Inorg. Biochem.* **2007**, *101*, 1657–1666.

74. Gencic, S.; Grahame, D.A. Two Separate One-Electron Steps in the Reductive Activation of the a Cluster in Subunit Beta of the Acds Complex in *Methanosarcina thermophila*. *Biochemistry* **2008**, *47*, 5544–5455.

75. Gencic, S.; Duin, E.C.; Grahame, D.A. Tight Coupling of Partial Reactions in the Acetyl-Coa Decarbonylase/Synthase (Acds) Multienzyme Complex from *Methanosarcina thermophila*: Acetyl C-C Bond Fragmentation at the a Cluster Promoted by Protein Conformational Changes. *J. Biol. Chem.* **2010**, *285*, 15450–15463.

76. Gencic, S.; Kelly, K.; Ghebreamlak, S.; Duin, E.C.; Grahame, D.A. Different Modes of Carbon Monoxide Binding to Acetyl-Coa Synthase and the Role of a Conserved Phenylalanine in the Coordination Environment of Nickel. *Biochemistry* **2013**, *52*, 1705–1716.

77. Jablonski, P.E.; Lu, W.P.; Ragsdale, S.W.; Ferry, J.G. Characterization of the Metal Centers of the Corrinoid/Iron- Sulfur Component of the Co Dehydrogenase Enzyme Complex from *Methanosarcina thermophila* by Epr Spectroscopy and Spectroelectrochemistry. *J. Biol. Chem.* **1993**, *268*, 325–329.

78. Grahame, D.A. Catalysis of Acetyl-Coa Cleavage and Tetrahydrosarcinapterin Methylation by a Carbon Monoxide Dehydrogenase-Corrinoid Enzyme Complex. *J. Biol. Chem.* **1991**, *266*, 22227–22233.

79. Maupin-Furlow, J.; Ferry, J.G. Characterization of the *Cdhd* and *Cdhe* Genes Encoding Subunits of the Corrinoid Iron-Sulfur Enzyme of the Co Dehydrogenase Complex from *Methanosarcina thermophila*. *J. Bacteriol.* **1996**, *178*, 340–346.

80. Liu, J.S.; Marison, I.W.; von Stockar, U. Microbial Growth by a Net Heat Up-Take: A Calorimetric and Thermodynamic Study on Acetotrophic Methanogenesis by *Methanosarcina barkeri*. *Biotechnol. Bioeng.* **2001**, *75*, 170–180.

81. Alber, B.E.; Ferry, J.G. A Carbonic Anhydrase from the Archaeon *Methanosarcina thermophila*. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 6909–6913.

82. Macauley, S.R.; Zimmerman, S.A.; Apolinario, E.E.; Evilia, C.; Hou, Y.; Ferry, J.G.; Sowers, K.R. The Archetype G-Class Carbonic Anhydrase (Cam) Contains Iron when Synthesized *in vivo*. *Biochemistry* **2009**, *48*, 817–819.

83. Tripp, B.C.; Bell, C.B.; Cruz, F.; Krebs, C.; Ferry, J.G. A Role for Iron in an Ancient Carbonic Anhydrase. *J. Biol. Chem.* **2004**, *279*, 6683–6687.

84. Zimmerman, S.A.; Ferry, J.G. Proposal for a Hydrogen Bond Network in the Active Site of the Prototypic G-Class Carbonic Anhydrase. *Biochemistry* **2006**, *45*, 5149–5157.

85. Hovey, R.; Lentes, S.; Ehrenreich, A.; Salmon, K.; Saba, K.; Gottschalk, G.; Gunsalus, R.P.; Deppenmeier, U. DNA Microarray Analysis of *Methanosarcina Mazei* Go1 Reveals Adaptation to Different Methanogenic Substrates. *Mol. Genet. Genomics* **2005**, *273*, 225–239.

86. Li, L.; Li, Q.; Rohlin, L.; Kim, U.; Salmon, K.; Rejtar, T.; Gunsalus, R.P.; Karger, B.L.; Ferry, J.G. Quantitative Proteomic and Microarray Analysis of the Archaeon *Methanosarcina acetivorans* Grown With Acetate *versus* Methanol. *J. Proteome Res.* **2007**, *6*, 759–771.

87. Li, Q.; Li, L.; Rejtar, T.; Lessner, D.J.; Karger, B.L.; Ferry, J.G. Electron Transport in the Pathway of Acetate Conversion to Methane in the Marine Archaeon *Methanosarcina acetivorans*. *J. Bacteriol.* **2006**, *188*, 702–710.

88. Pisa, K.Y.; Weidner, C.; Maischak, H.; Kavermann, H.; Muller, V. The Coupling Ion in the Methanoarchaeal ATP Synthases: H^+ vs. Na^+ in the A1A0 ATP Synthase from the Archaeon Methanosarcina mazei Gö1. *FEMS Microbiol. Lett.* **2007**, *277*, 56–63.

89. Hedderich, R. Energy-Converting [Nife] Hydrogenases from Archaea and Extremophiles: Ancestors of Complex I. *J. Bioenerg. Biomembr.* **2004**, *36*, 65–75.

90. Meuer, J.; Kuettner, H.C.; Zhang, J.K.; Hedderich, R.; Metcalf, W.W. Genetic Analysis of the Archaeon *Methanosarcina barkeri* Fusaro Reveals a Central Role for ECH Hydrogenase and Ferredoxin in Methanogenesis and Carbon Fixation. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5632–5637.

91. Heiden, S.; Hedderich, R.; Setzke, E.; Thauer, R.K. Purification of a Cytochrome-*B* Containing H_2-Heterodisulfide Oxidoreductase Complex from Membranes of *Methanosarcina barkeri*. *Eur. J. Biochem.* **1993**, *213*, 529–535.

92. Deppenmeier, U. The Membrane-Bound Electron Transport System of *Methanosarcina* Species. *J. Bioenerg. Biomembr.* **2004**, *36*, 55–64.

93. Shimizu, S.; Ueno, A.; Naganuma, T.; Kaneko, K. *Methanosarcina subterranea* sp. nov., a methanogenic archaeon isolated from a deep subsurface diatomaceous shale formation of northernmost japan. *Int. J. Syst. Evol. Microbiol.* **2015**, *65*, 1167–1171.

94. Wagner, D.; Schirmack, J.; Ganzert, L.; Morozova, D.; Mangelsdorf, K. *Methanosarcina soligelidi* sp. nov., a desiccation and freeze-thaw resistant methanogenic archaeon isolated from a siberian permafrost-affected soil. *Int. J. Syst. Evol. Microbiol.* **2013**, *64*, 3478–3484.

95. Von Klein, D.; Arab, H.; Volker, H.; Thomm, M. *Methanosarcina baltica*, sp. nov., a novel methanogen isolated from the Gotland Deep of the Baltic Sea. *Extremophiles* **2002**, *6*, 103–110.

96. Blotevogel, K.H.; Fischer, U. Transfer of *Methanococcus Frisius* to the Genus *Methanosarcina* as *Methanosarcina Frisia* Comb. Nov. *Int. J. Syst. Bacteriol.* **1989**, *39*, 91–92.

97. Zinder, S.H.; Sowers, K.R.; Ferry, J.G. *Methanosarcina thermophila* sp. nov., a Thermophilic, Acetotrophic, Methane-Producing Bacterium. *Int. J. Syst. Bacteriol.* **1985**, *35*, 522–523.

98. Shimizu, S.; Upadhye, R.; Ishijima, Y.; Naganuma, T. *Methanosarcina horonobensis* sp. nov., a methanogenic archaeon isolated from a deep subsurface miocene formation. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 2503–2507.

99. Lyimo, T.J.; Pol, A.; den Camp, H.J.O.; Harhangi, H.R.; Vogels, G.D. *Methanosarcina semesiae* sp. nov., a dimethylsulfide-utilizing methanogen from mangrove sediment. *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 171–178.

100. Cabrol, N.; Villemur, R.; Perrier, J.; Jacob, F.; Foullet, B.; Chambon, P. Isolation of a methanogenic bacterium, *Methanosarcina* sp. strain FR, for its abiilty to degrade high concentrations of perchloroethylene. *Can. J. Microbiol.* **1998**, *44*, 1142–1147.

101. Zhilina, T.N.; Zavarzin, G.A. Comparative cytology of *Methanosarcinae* and description of *Methanosarcina vacuolata* sp. nova. *Microbiology* **1979**, *48*, 223–228.

102. Sowers, K.R.; Baron, S.F.; Ferry, J.G. *Methanosarcina acetivorans* sp. nov., An Acetotrophic Methane-Producing Bacterium Isolated from Marine Sediments. *Appl. Environ. Microbiol.* **1984**, *47*, 971–978.

103. Ganzert, L.; Schirmack, J.; Alawi, M.; Mangelsdorf, K.; Sand, W.; Hillebrand-Voiculescu, A.; Wagner, D. *Methanosarcina spelaei* sp. nov., a Methanogenic Archaeon Isolated from a Floating Biofilm of a Subsurface Sulphurous Lake. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 3478–3484.

104. Simankova, M.V.; Parshina, S.N.; Tourova, T.P.; Kolganova, T.V.; Zehnder, A.J.; Nozhevnikova, A.N. *Methanosarcina lacustris* sp. nov., a New Psychrotolerant Methanogenic Archaeon from Anoxic Lake Sediments. *Syst. Appl. Microbiol.* **2001**, *24*, 362–367.

105. Li, Q.; Li, L.; Rejtar, T.; Lessner, D.J.; Karger, B.L.; Ferry, J.G. Electron Transport in the Pathway of Acetate Conversion to Methane in the Marine Archaeon *Methanosarcina acetivorans*. *J. Bacteriol.* **2006**, *188*, 702–710.

106. Wang, M.; Tomb, J.F.; Ferry, J.G. Electron Transport in Acetate-Grown *Methanosarcina acetivorans*. *BMC Microbiol.* **2011**, *11*, doi:10.1186/1471-2180-11-165.

107. Hreha, T.N.; Mezic, K.G.; Herce, H.D.; Duffy, E.D.; Bourges, A.; Pryshchep, S.; Juarez, O.; Barquera, B. The Complete Topology of the RNF Complex from *Vibrio Cholerae*. *Biochemistry* **2015**, *54*, 2443–2455.

108. Saeki, K.; Kumagai, H. The *Rnf* Gene Products in *Rhodobacter Capsulatus* Play an Essential Role in Nitrogen Fixation during Anaerobic Dmso-Dependent Growth in the Dark. *Arch. Microbiol.* **1998**, *169*, 464–467.

109. Muller, V.; Imkamp, F.; Biegel, E.; Schmidt, S.; Dilling, S. Discovery of a Ferredoxin: Nad$^+$-Oxidoreductase (RNF) in *Acetobacterium Woodii*: A Novel Potential Coupling Site in Acetogens. *Ann. N. Y. Acad. Sci.* **2008**, *1125*, 137–146.

110. Seedorf, H.; Fricke, W.F.; Veith, B.; Bruggemann, H.; Liesegang, H.; Strittmatter, A.; Miethke, M.; Buckel, W.; Hinderberger, J.; Li, F.; *et al.* The Genome of *Clostridium kluyveri*, a Strict Anaerobe with Unique Metabolic Features. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2128–2133.

111. Sarkar, A.; Kohler, J.; Hurek, T.; Reinhold-Hurek, B. A Novel Regulatory Role of the RNF Complex of *Azoarcus* SP. Strain Bh72. *Mol. Microbiol.* **2011**, *83*, 408–422.

112. Tremblay, P.L.; Zhang, T.; Dar, S.A.; Leang, C.; Lovley, D.R. The RNF Complex of *Clostridium Ljungdahlii* Is a Proton-Translocating Ferredoxin: Nad$^+$ Oxidoreductase Essential for Autotrophic Growth. *mBio* **2012**, *4*, doi:10.1128/mBio.00406-12.

113. Meyer, B.; Kuehl, J.V.; Price, M.N.; Ray, J.; Deutschbauer, A.M.; Arkin, A.P.; Stahl, D.A. The Energy-Conserving Electron Transfer System Used by *Desulfovibrio alaskensis* Strain G20 During Pyruvate Fermentation Involves Reduction of Endogenously Formed Fumarate and cytoplasmic and membrane-bound complexes, Hdr-Flox and RNF. *Environ. Microbiol.* **2014**, *16*, 3463–3486.

114. Schlegel, K.; Welte, C.; Deppenmeier, U.; Muller, V. Electron Transport During Aceticlastic Methanogenesis by *Methanosarcina acetivorans* Involves a Sodium-Translocating RNF Complex. *FEBS J.* **2012**, *279*, 4444–4452.

115. Schlegel, K.; Leone, V.; Faraldo-Gomez, J.D.; Muller, V. Promiscuous Archaeal ATP Synthase Concurrently Coupled to Na$^+$ And H$^+$ Translocation. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 947–952.

116. Jasso-Chavez, R.; Apolinario, E.E.; Sowers, K.R.; Ferry, J.G. MRPA Functions in Energy Conversion during Acetate-Dependent Growth of *Methanosarcina acetivorans*. *J. Bacteriol.* **2013**, *195*, 3987–3994.

117. Smith, K.S.; Ingram-Smith, C. *Methanosaeta*, the Forgotten Methanogen? *Trends Microbiol.* **2007**, *7*, 150–155.

118. Berger, S.; Welte, C.; Deppenmeier, U. Acetate Activation in *Methanosaeta Thermophila*: Characterization of the Key Enzymes Pyrophosphatase and Acetyl-Coa Synthetase. *Archaea* **2012**, *2012*, 1–10.

119. Jetten, M.S.M.; Stams, A.J.M.; Zehnder, A.J.B. Isolation and Characterization of Acetyl-Coenzyme a Synthetase from *Methanothrix soehngenii*. *J. Bacteriol.* **1989**, *171*, 5430–5435.

120. Eggen, R.I.L.; Geerling, A.C.M.; Boshoven, A.B.P.; Devos, W.M. Cloning, Sequence Analysis, and Functional Expression of the Acetyl Coenzyme a Synthetase Gene from *Methanothrix soehngenii* in *Escherichia Coli*. *J. Bacteriol.* **1991**, *173*, 6383–6389.

121. Aceti, D.J.; Ferry, J.G. Purification and Characterization of Acetate Kinase from Acetate-Grown *Methanosarcina thermophila*. *J. Biol. Chem.* **1988**, *263*, 15444–15448.

122. Tor, J.M.; Kashefi, K.; Lovley, D.R. Acetate Oxidation Coupled to Fe(III) Reduction in Hyperthermophilic Microorganisms. *Appl. Environ. Microbiol.* **2001**, *67*, 1363–1365.

123. Slobodkina, G.B.; Kolganova, T.V.; Querellou, J.; Bonch-Osmolovskaya, E.A.; Slobodkin, A.I. *Geoglobus Acetivorans* sp. nov., an Iron(III)-Reducing Archaeon from a Deep-Sea Hydrothermal Vent. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 2880–2883.

124. Slobodkin, A.; Campbell, B.; Cary, S.C.; Bonch-Osmolovskaya, E.; Jeanthon, C. Evidence for the Presence of Thermophilic Fe(III)-Reducing Microorganisms in Deep-Sea Hydrothermal Vents at 13 Degrees N (East Pacific Rise). *FEMS Microbiol. Ecol.* **2001**, *36*, 235–243.

125. Seyler, L.M.; Mcguinness, L.M.; Kerkhof, L.J. Crenarchaeal Heterotrophy in Salt Marsh Sediments. *ISME J.* **2014**, *8*, 1534–1543.