How to Make a Living by Exhaling Methane

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Key Words

Archaea, anaerobic, energy conservation, electron transport carbon cycle

Abstract

Methane produced in the biosphere is derived from two major pathways. Conversion of the methyl group of acetate to CH_4 in the aceticlastic pathway accounts for at least two-thirds, and reduction of CO_2 with electrons derived from H_2, formate, or CO accounts for approximately one-third. Although both pathways have terminal steps in common, they diverge considerably in the initial steps and energy conservation mechanisms. Steps and enzymes unique to the CO_2 reduction pathway are confined to methanogens and the domain *Archaea*. On the other hand, steps and enzymes unique to the aceticlastic pathway are widely distributed in the domain *Bacteria*, the understanding of which has contributed to a broader understanding of prokaryotic biology.

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INTRODUCTION

Most of the human population exhales traces of CH_4 that, like the CO_2 of mitochondria, are the product of respiration, albeit anaerobic, by gut methanogens inhaling H_2 and CO_2 (95). H_2 and CO_2 are produced by other anaerobes decomposing complex organic matter in the gut, where CH_4 is absorbed into the bloodstream and exchanged in the lungs (117).

Like humans, Earth's biosphere can be considered a single living-breathing organism (70) where gases including CH_4 play an essential role (**Figure 1**). The chief organs are the domains *Eukarya*, *Bacteria*, and *Archaea*, the balanced interactions of which are essential to sustain Earth's life. Each year the biosphere inhales ~70 gigatons of CO_2 transformed by photosynthesis into complex organic matter while exhaling O_2. Microbes in aerobic environments, primarily from *Bacteria*, digest the organic matter consuming O_2 and producing CO_2. The organic matter also enters diverse O_2-free habitats such as the lower intestinal tract of humans, wetlands, rice paddy soils, and the rumen of livestock, where anaerobic microbes digest the organic matter, exhaling CO_2 and CH_4. In freshwater anaerobic environments, the process involves three metabolic groups of anaerobes from *Bacteria* and *Archaea*. The fermentative group digests the complex organic matter primarily to butyrate, propionate, acetate, formate, H_2, and CO_2. The acetogenic group digests the butyrate and propionate to formate, acetate, CO_2, and H_2. The third group consists of methane-producing species (methanogens) from *Archaea* and is subdivided into two groups. The CO_2-reducing group obtains energy for growth by oxidizing formate or H_2 and reducing CO_2 to CH_4. Acetotrophic methanogens split acetate in the aceticlastic pathway, oxidizing the carbonyl group and reducing the methyl group to CH_4. Formation of CH_4 from acetate accounts for approximately two-thirds of the approximately one gigaton Earth's biosphere exhales each year (108), with the remaining one-third derived mostly from the reduction of CO_2 with H_2 or formate and smaller amounts from the methyl groups of methanol, methylamines, and dimethylsulfide. The CH_4 from anaerobic habitats diffuses into aerobic zones where O_2-requiring methanotrophic microbes oxidize it to CO_2. Consortia of anaerobic microbes that convert CH_4 to CO_2 while reducing either sulfate, nitrate, manganese, or iron have been described (8, 90, 110).

Human intervention in the natural process of methanogenesis influences the ecology of Earth in both detrimental and beneficial ways. Increases in livestock, rice cultivation, and other human activities in recent decades have enhanced the amount of atmospheric CH_4 that is severalfold more effective than CO_2 in radiating energy back to Earth's surface. However, the process also holds promise for conversion of waste and renewable photosynthate to CH_4, contributing to the growing need for alternatives to fossil fuels with no net release of CO_2 to the atmosphere. Finally, growing

Methanogen: a microorganism that produces methane

Anaerobe: a microorganism with energy-yielding metabolism independent of O_2 proliferating in anaerobic O_2-free environments

***Archaea*:** one of the three highest taxonomic classifications of life to which methane-producing species belong

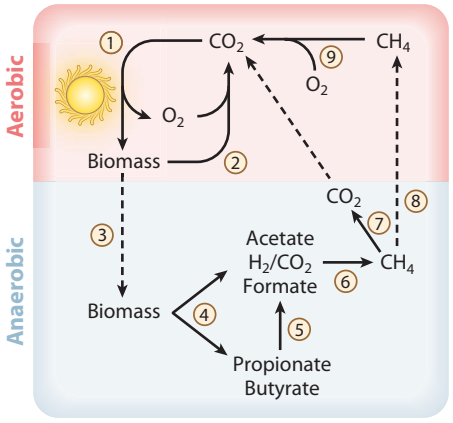

Figure 1

The global carbon cycle. Aerobic O_2-requiring conversions are shown in the top panel and anaerobic conversions in the bottom panel. Step 1: Fixation of CO_2 into organic matter. Step 2: Decomposition of organic matter to CO_2 by O_2-requiring microbes. Step 3: Deposition of organic matter into anaerobic environments. Step 4: Decomposition of complex biomass by fermentative microbes. Step 5: Conversion of volatile fatty acids by obligate H_2- and formate-producing syntrophic acetogens. Step 6: H_2- and formate-dependent reduction of CO_2 to CH_4 and conversion of the methyl group of acetate to CH_4 by methanogens. Step 7: Anaerobic oxidation of CH_4. Step 8: Diffusion of CH_4 into aerobic zones. Step 9: Aerobic oxidation of CH_4 by O_2-requiring methylotrophs.

evidence suggests methanogenic microbes evolved close to the time of the origin of life, providing a window to the early evolution of Earth's biosphere (7, 112). Furthermore, the finding of CH_4 on Mars has prompted speculation of a biological source with implications for extraterrestrial origins of methanogenic microbes (23, 80). This review presents the biochemistry and molecular biology underpinning the energy-yielding metabolism of acetotrophic and CO_2-reducing methanogens responsible for biologically produced CH_4 with corresponding influence on the evolution and contemporary ecology of Earth's biosphere.

MICROBIOLOGY

Methanogens are exclusive to the domain *Archaea*, the kingdom *Euryarchaeota*, and the five orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*. Further descriptions of the systematics and characteristics of methanogen taxa have been recently reviewed (21, 69). *Methanosarcinaceae* is the most metabolically versatile of the two families that compose the *Methanosarcinales*. All species in the *Methanosarcinaceae* obtain energy for growth by producing CH_4 from dismutation of the methyl group of methanol and methylamines. Members of the genus *Methanosarcina* also obtain energy by the reduction of CO_2 with H_2 or CO as the electron donor and by the conversion of acetate to CH_4 and CO_2. The other family, *Methanosaetaceae*, is composed of three species within the single genus *Methanosaeta* that only convert acetate to CH_4 and CO_2 for growth. *Methanosarcina* and *Methanosaeta* are the only genera that grow and produce CH_4 from acetate, although at least two-thirds of CH_4 in the biosphere is derived from this substrate. The other four orders obtain energy for growth only by the reduction of CO_2 to CH_4 referred to as obligate CO_2-reducing species. An exception is the genus *Methanosphaera*, which reduces the methyl group of methanol to CH_4 with H_2 (26). A sixth order, *Methanocellales*, was recently proposed based on an isolate from a rice paddy soil bacteria, *Methanocella paludicola*, that obtains energy for growth only by the reduction of CO_2 with H_2 or formate (94).

REACTIONS COMMON TO ALL METHANOGENIC PATHWAYS

Two major energy-yielding pathways account for all but a fraction of CH_4 produced by Earth's anaerobic biosphere. Approximately one-third originates from the reduction of CO_2 with electrons derived from oxidation of either H_2, formate, or CO in the CO_2 reduction pathway (Equations 1a–c). At least two-thirds derives from the methyl group of acetate by the aceticlastic pathway, in which acetate is cleaved into methyl and carbonyl groups, with the latter oxidized to provide the electron pair for reduction of the former to CH_4 (Equation 2).

Fermentative species: an anaerobe obtaining energy for growth by oxidizing substrates and reducing metabolic intermediates to formate, H_2, acetate, or other end products

Acetotrophic: refers to a microorganism able to utilize acetate as an energy source

Aceticlastic pathway: pathway in which acetate is split into methyl and carbonyl groups, with the former reduced to methane

HS-CoM: coenzyme M

HS-CoB: coenzyme B

H_4SPT: tetrahydrosarcinapterin

H_4MPT: tetrahydromethanopterin

In environments where H_2-, formate-, or CO-utilizing acetogenic anaerobes proliferate, the great majority of CH_4 is produced by the aceticlastic pathway. In some anaerobic habitats, methanol or methylamines are minor substrates for methanogens obtaining energy by dismutation of the substrate in which methyl groups are oxidized to CO_2, providing electrons for reduction of methyl groups to CH_4 (Equation 3).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad 1a.$$

$$4HCO_2H \rightarrow 3CO_2 + CH_4 + 2H_2O \quad 1b.$$

$$4CO + 2H_2O \rightarrow 3CO_2 + CH_4 \quad 1c.$$

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad 2.$$

$$4R\text{-}CH_3 + 2H_2O \rightarrow 4RH + 3CH_4 + CO_2 \quad 3.$$

$$(R = \text{-SH, -OH, -NH}_2\text{, -NHCH}_3\text{, -N(CH}_3)_2 \text{ or -N(CH}_3)_3^+).$$

Figure 2 is a composite overview of all methanogenic pathways. Reactions 2, 3, and 4 involving coenzyme M (HS-CoM) and coenzyme B (HS-CoB) (**Figure 3**) are common to the aceticlastic and CO_2 reduction pathways that differ in reactions that generate the methyl group attached to the coenzyme tetrahydrosarcinapterin (H_4SPT) in the former pathway or the functional analog tetrahydromethanopterin (H_4MPT) (**Figure 3**) in the latter pathway. The H_4S(M)PT cofactors have structural and functional similarities to tetrahydrofolate, albeit only to the extent that they are C-1 carriers. The ^{5}*N*-methyl-H_4M(S)PT intermediate is formed in the aceticlastic and CO_2 reduction pathways by a series of reactions represented in Reactions 1 and 5, respectively. The methyl group of ^{5}*N*-methyl-H_4M(S)PT is transferred to HS-CoM (Reaction 2) followed by reductive demethylation of CH_3-S-CoM to CH_4 with electrons donated from HS-CoB (Reaction 3). The heterodisulfide CoM-S-S-CoB, a product of the demethylation reaction, is reduced to the corresponding sulfhydryl forms of the coenzymes in Reaction 4. Reactions 3 and 4 are also shared with the methylotrophic pathway, in which CH_3-S-CoM is generated from the substrate methyl group in Reaction 7. Thus, all methanogenic pathways that are coupled to electron transport pathways share Reactions 3 and 4, generating a proton gradient that drives ATP synthesis. The pathways also differ by the source of electrons reducing CoM-S-S-CoB to HS-CoB and HS-CoM (Reaction 4). In the aceticlastic pathway the electron pair derives from oxidation of the carbonyl group of acetate, and in the CO_2 reduction pathway the electron pair derives from oxidation of either H_2 or formate (Equations 1a and 1b). In the methylotrophic pathway, electrons for Reaction 4 derive from oxidation of CH_3-H_4SPT via reactions in Reaction 9, a reversal of reactions in Reaction 5 of the CO_2

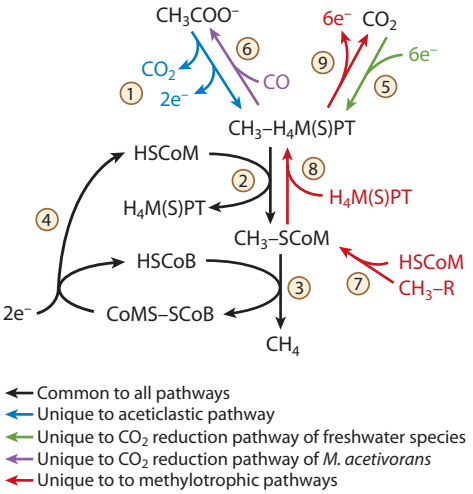

Figure 2

Composite overview of methanogenic pathways. Reactions 2, 3, and 4, which are common to all pathways, are in black. Step 1, unique to the aceticlastic pathway, is in blue. Step 5, unique to the CO_2 reduction pathway of freshwater species, is in green. Step 6, unique to the CO_2 reduction pathway of the marine isolate *Methanosarcina acetivorans*, is in purple. Steps 7, 8, and 9, unique to methylotrophic pathways, are in red, where R = -SH, -OH, -NH_2, -$NHCH_3$, -$N(CH_3)_2$, or -$N(CH_3)_3^+$. Enzymes catalyzing Reactions 2–4, common to all pathways, are methyl-tetrahydromethano(sarcina)-pterin:coenzyme M methyltransferase for Step 2; methyl-coenzyme M methylreductase for Step 3; and heterodisulfide reductase for Step 4. Adapted from Reference 18a.

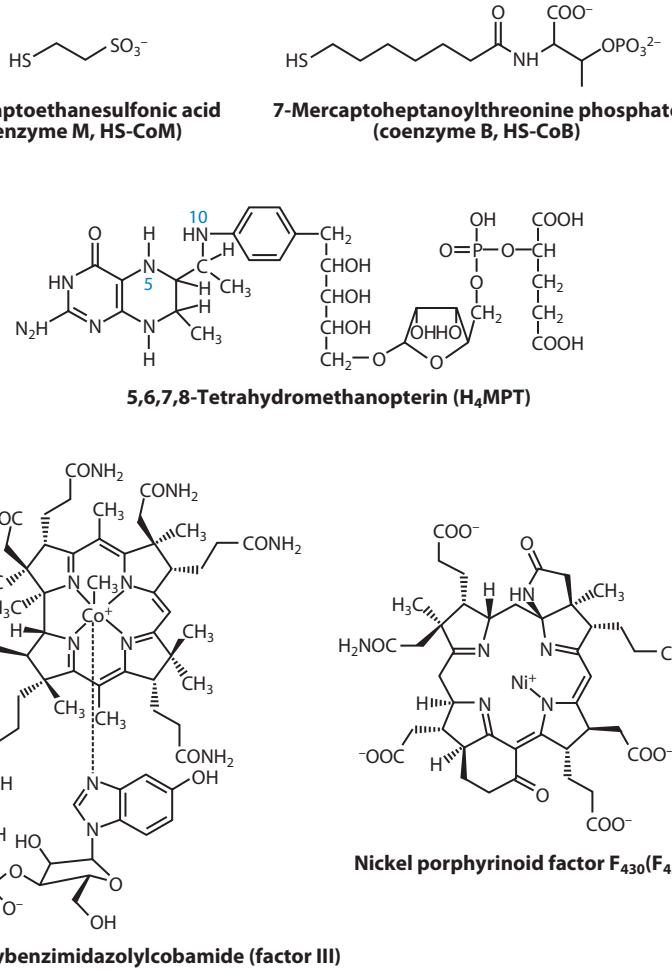

Figure 3

Coenzymes and cofactors participating in reactions common to all methanogenic pathways. *Methanosarcina* species synthesize tetrahydrosarcinapterin (H_4SPT), which serves the same function as tetrahydromethanopterin (H_4MPT) and has a similar structure except for a terminal α-linked glutamate (113).

reduction pathway. The CH_3-H_4SPT is generated by Reaction 8 catalyzed by the same enzyme catalyzing Reaction 2.

Methyl-H_4M(S)PT:coenzyme M methyltransferase (Mtr) catalyzes Reaction 2 (**Figure 2**) and its reverse (Reaction 8), which is unique to the methylotrophic pathway. The eight nonidentical subunits (MtrABCDEFGH) of the membrane-bound complex couple the exergonic methyl transfer to generation of a sodium ion gradient (high outside) in all methanogenic pathways, except the methylotrophic pathway, for which a sodium gradient (high outside) drives the endergonic Reaction 8 (**Figure 2**) (41). The MtrA subunit contains cofactor Factor III (**Figure 3**), which is predicted to extend into the cytoplasm, where the MtrH subunit catalyzes transfer of the methyl group of CH_3-H_4M(S)PT to Factor III of MtrA to form a carbon-cobalt bond in the upper axial position. The MtrE subunit is proposed to

transfer the methyl group from Factor III to HS-CoM, resulting in loss of the lower axial ligand and thus inducing a conformational change in MtrA that is transmitted to the MtrE subunit driving the translocation of sodium (32).

Methyl-CoM reductase (Mcr) catalyzes the reductive demethylation of CH_3-S-CoM to CH_4 with electrons donated by HS-CoB (**Figure 2**, Reaction 3). The heterodisulfide CoM-S-S-CoB is also a product of the reaction that is reduced to the corresponding active sulfhydryl forms of the coenzymes in Reaction 4 (**Figure 2**). The crystal structure of the homodimeric Mcr from *Methanothermobacter marburgensis* (*Methanobacterium thermoautotrophicum* strain Marburg) reveals two active site F_{430} cofactors (**Figure 3**) separated by 50 Å and embedded between interlinked subunits $\alpha\alpha'\beta\gamma$ and $\alpha'\alpha\beta'\gamma'$, indicating two independent active sites (18). Two mechanisms have been proposed that diverge in how the C-S bond of CH_3-S-CoM is cleaved. In mechanism I, the nickel of Ni^IF_{430} attacks the sulfur atom of CH_3-S-CoM, producing a $^\bullet CH_3$ radical and CoM-S-$Ni^{II}F_{430}$ (14, 88). The methyl radical then abstracts a hydrogen from HS-CoB, producing $^\bullet$S-CoB and CH_4. The $^\bullet$S-CoB radical reacts with CoM-S-$Ni^{II}F_{430}$, producing the anion radical CoM-S-$S^\bullet$-CoB^- that donates the extra electron to $Ni^{II}F_{430}$, regenerating the active Ni^IF_{430} species and yielding CoM-S-S-CoB. In mechanism II, a nucleophilic attack of nickel in Ni^IF_{430} on CH_3-S-CoM generates CH_3-$Ni^{III}F_{430}$ and HS-CoM. Transfer of an electron from HS-CoM to CH_3-$Ni^{III}F_{430}$ produces the $^\bullet$S-CoM radical and CH_3-$Ni^{II}F_{430}$. In the final step, protonolysis of CH_3-$Ni^{II}F_{430}$ produces CH_4. The $^\bullet$S-CoM thiyl radical is coupled with $^-$S-CoB to form the radical anion CoM-S-$S^\bullet$-CoB^- and is followed by one-electron transfer to $Ni^{II}F_{430}$ to yield active Ni^IF_{430} and CoM-S-S-CoB.

Heterodisulfide reductase (Hdr) is essential to all methanogenic pathways catalyzing Reaction 4 (**Figure 2**), which is the terminal step in electron transport coupled to generation of a proton gradient that drives ATP synthesis. A two-subunit enzyme (HdrDE) that functions in the methylotrophic pathway of *Methanosarcina* species (47, 60) and a three-subunit enzyme (HdrABC) that functions in obligate CO_2-reducing methanogens (43) have been biochemically characterized. The HdrDE type has been identified and characterized from acetate-grown cells of the freshwater isolates *Methanosarcina thermophila* (98) and *M. barkeri* (46) and the marine isolate *M. acetivorans* (65), and the results are consistent with a role for HdrDE in the aceticlastic pathway. Cytochrome *b*, present in the HdrE subunit of the *M. barkeri* enzyme (47), is proposed to transfer electrons to the catalytic subunit HdrD (40). Sequence analysis of the HdrABC iron-sulfur flavoprotein identifies motifs for binding the FAD and 4Fe-4S clusters in HdrA and the 4Fe-4S clusters in HdrC (45). Sequence conservation of HdrD with HdrBC is consistent with these subunits harboring the catalytic site. It was concluded from spectroscopic investigations of HdrDE and HdrABC that a novel 4Fe-4S cluster is the active site reducing CoM-S-S-CoB in two one-electron steps (44). In the mechanism, a thiyl radical generated by the initial one-electron reduction of CoM-S-S-CoB is stabilized through reduction and coordination of the thiol to the 4Fe-4S cluster. The specific subunit harboring the active site cluster was not identified; however, an investigation of heterologously produced HdrB from *M. marburgensis* (40) revealed a 4Fe-4S cluster with spectroscopic properties similar to the previously proposed active site iron-sulfur cluster (44). Site-directed mutagenesis of HdrB located the four cysteine ligands of the 4Fe-4S cluster in a unique $CX_{31-39}CCX_{35-36}CXXC$ motif that is also conserved in HdrD (40). Finally, the iron-sulfur clusters in the HdrC subunit are proposed to shuttle electrons from HdrA to HdrB.

Figure 4 contrasts reactions unique to the aceticlastic and CO_2 reduction pathways that converge on the common intermediate methyl-H_4M(S)PT, which is further metabolized to CH_4 by reactions universal to both pathways (**Figure 2**).

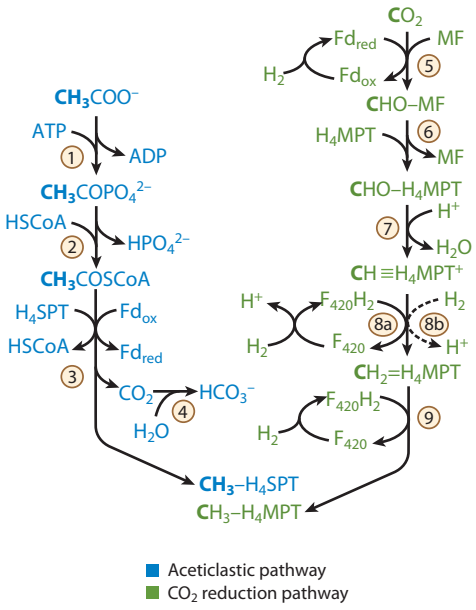

Figure 4

Comparison of reactions leading to CH_3-H_4M(S)PT in the aceticlastic and CO_2 reduction pathways. Enzymes catalyzing the reactions are acetate kinase for Reaction 1; phosphotransacetylase for Reaction 2; CO dehydrogenase/acetyl-CoA synthase for Reaction 3; carbonic anhydrase for Reaction 4; formyl-methanofuran dehydrogenase for Reaction 5; formyl-methanofuran:tetrahydromethanost-(sarcina)pterin formyltransferase for Reaction 6; cyclohydrolase for Reaction 7; methylene-tetrahydromethano(sarcina)pterin dehydrogenase for Reaction 8; and methylene-tetrahydromethano-(sarcina)pterin reductase for Reaction 9.

THE ACETICLASTIC PATHWAY

Reactions Leading to CH_3-H_4SPT

Methyl-H_4SPT is synthesized by Reactions 1–4 (**Figure 4**) in the aceticlastic pathway of *Methanosarcina* species, and this is consistent with proteomic and transcriptomic analyses showing upregulation of genes encoding the associated enzymes when cultured with acetate versus methanol (50, 64). Understanding the enzymes catalyzing these reactions has broad significance outside of acetotrophic methanogens, as homologs are widespread in diverse anaerobes from *Bacteria*. Indeed, homologs of acetate kinase and phosphotransacetylase catalyzing Reactions 1 and 2 (**Figure 4**) function in the primary energy-yielding pathways of fermentative and acetogenic species converting acetyl-CoA to ATP and producing acetate, which is the major intermediate in anaerobic microbial food chains (**Figure 1**). Investigations of acetate kinase from *Escherichia coli*, discovered in 1944 by Fritz Lipmann, were among the earliest mechanistic studies of enzymes (67). The first crystal structure was from the acetotrophic methanogen *M. thermophila* and revealed features suggesting acetate kinase is the founding member of the acetate and sugar kinase/Hsc70/actin (ASKHA) superfamily of phosphotransferases (13). The structure of the homodimeric enzyme cocrystallized with ADP, acetate, Al^{3+}, and F^- shows a linear array of ADP-AlF_3-acetate in the active site cleft that supports a direct in-line transfer mechanism in which AlF_3 mimics the meta-phosphate transition state (31, 77).

Phosphotransacetylase catalyzes Reaction 2 (**Figure 2**) in the aceticlastic pathway of *Methanosarcina* species, which together with acetate kinase is also the primary energy-yielding pathway in fermentative and acetogenic anaerobes from *Bacteria*. The crystal structure of the enzyme from *M. thermophila* complexed with HS-CoA reveals the active site supporting a catalytic mechanism wherein nucleophilic attack of $^-$S-CoA on the carbonyl carbon of acetyl phosphate yields acetyl-CoA (51, 62).

The enzyme central to aceticlastic pathways cleaves the C-C and C-S bonds of acetyl-CoA, transferring the methyl group to H_4SPT and oxidizing the carbonyl group to CO_2 with transfer of electrons to a 2x[4Fe-4S] ferredoxin (22, 105, 106) (**Figure 4**, Reaction 3). The enzyme was first named CO dehydrogenase/acetyl-CoA synthase (Cdh) because of the bifunctional character catalyzing synthesis or cleavage of acetyl-CoA and oxidation of CO derived from the carbonyl group of acetyl-CoA. The same complex is also referred to as acetyl-CoA decarbonylase/synthase (ACDS) and therefore in this

Cdh: carbon monoxide dehydrogenase/acetyl-CoA synthase

ACDS: acetyl-CoA decarbonylase/synthase

review is designated Cdh/ACDS. A diversity of anaerobes from *Bacteria* and *Archaea* synthesize Cdh/ACDS homologs that function in acetate-utilizing, energy-yielding pathways and pathways for generating acetyl-CoA from a methyl group and CO_2 for cell biosynthesis (35). Of ancient origin, primitive ancestors of Cdh/ACDS likely played a central role in the early evolution of life (20, 66, 73).

EPR: electron paramagnetic resonance

A two-subunit CO dehydrogenase has been purified and characterized from an acetate-utilizing species of the genus *Methanosaeta* (formerly *Methanothrix*) (10) that catalyzes the exchange of CO with the carbonyl group of acetyl-CoA, similar to the Cdh/ACDS of *Methanosarcina* species supporting acetyl-CoA cleavage activity (17, 53–55); however, the majority of mechanistic investigations have been on the five-subunit ($\alpha\beta\gamma\delta\varepsilon$) CdhABCDE complexes from the acetate-utilizing species *M. thermophila* and *M. barkeri* that are resolvable into three components (1, 37, 107). The $\alpha\varepsilon$ subunits compose the CdhAE component, the $\gamma\delta$ subunits compose the CdhDE component, and the β subunit composes the CdhC component. The CdhC component contains the A cluster, which catalyzes acetyl-CoA cleavage to yield a methyl and a carbonyl group (27, 29, 36, 81). The crystal structure has not been reported; however, various spectroscopic analyses suggest the A cluster is composed of a 4Fe-4S center bridged to a binuclear Ni-Ni site (27, 38) similar to the homolog from *Moorella thermoacetica*, an acetate-producing species from *Bacteria* (91). The CdhDE component contains a corrinoid cofactor accepting the methyl group from the CdhC component with transfer to H_4SPT (33, 34, 52, 74). Sequence analyses and characterization of the CdhD and CdhE subunits overproduced independently in *E. coli* identified an iron-sulfur cluster located in the CdhE subunit. Both the CdhD and CdhE subunits bind a corrinoid cofactor (74). However, it has yet to be determined which of the two subunits interacts with H_4SPT. The CdhAE component oxidizes CO derived from the carbonyl group of acetyl-CoA by the CdhC component and reduces ferredoxin (1). The crystal structure of the CdhAE component from *M. barkeri* (30) reveals a $\alpha_2\varepsilon_2$ configuration with 4x[4Fe-4S] clusters and a Ni-Fe-S C cluster in the α subunit consistent with previous EPR spectroscopic results (58). Two of the 4Fe-4S clusters are proposed to function in electron transport from the active site C cluster to ferredoxin. The C cluster is a pseudocubane $NiFe_3S_4$ cluster bridged to an exogenous iron atom. A gas channel is also identified in the structure extending from the C cluster to the protein surface in a direction that has potential to transport CO from the CdhC component.

The conversion of acetate to CH_4 and CO_2 provides only a marginal amount of energy available for growth ($\Delta G^{\circ\prime} = -36$ kJ/CH_4). A calorimetric and thermodynamic analysis of acetate-grown *M. barkeri* indicates that transfer of CH_4 and CO_2 into the gaseous phase adds to the driving force of growth (68). A carbonic anhydrase (Cam) characterized from *M. thermophila* is postulated to be outside the cell membrane, where it converts CO_2 to membrane-impermeable HCO_3^- (**Figure 4**, Reaction 4), facilitating removal of CO_2 from the cytoplasm (3). Cam is the archetype of an independently evolved class of carbonic anhydrases (γ class) with a novel left-handed β-helical fold (111). Homologs are widely distributed in *Bacteria* and *Archaea*, suggesting functions in prokaryotes in addition to that for acetotrophic methanogens (101). Homologs of Cam are also found in the mitochondria of plants, further underscoring the importance of this novel class of carbonic anhydrase in biology (87). Contrary to all prokaryotic carbonic anhydrases that contain zinc, Cam from *M. thermophila* contains Fe^{2+} in the active site, establishing it as the physiologically relevant metal, the first for any carbonic anhydrase (72, 111). Crystal structures and kinetic analyses of the wild-type and site-specific amino acid replacement variants have identified active site residues and a hydrogen bonding network essential for catalysis, leading to a proposed mechanism (118). The two-step ping-pong mechanism is shown in Equations 4a,b

and 5a,b, where E represents enzyme residues, M is metal, and B is buffer.

$$E\text{-}M^{2+}\text{-}OH^- + CO_2 = E\text{-}M^{2+}\text{-}HCO_3^- \quad 4a.$$

$$E\text{-}M^{2+}\text{-}HCO_3^- + H_2O = E\text{-}M^{2+}\text{-}H_2O + HCO_3^- \quad 4b.$$

$$E\text{-}M^{2+}\text{-}H_2O = H^+\text{-}E\text{-}M^{2+}\text{-}OH^- \quad 5a.$$

$$H^+\text{-}E\text{-}M^{2+}\text{-}OH^- + B = E\text{-}M^{2+}\text{-}OH^- + BH^+. \quad 5b.$$

In step 1 (Equations 4a, 4b), a lone pair of electrons on the metal-bound hydroxide attack carbon dioxide producing metal-bound bicarbonate that is displaced by water. In step 2 (Equations 5a, 5b), a proton is extracted from the metal-bound water that is transferred to buffer. The reader is referred to a recent review on the γ class of carbonic anhydrases in all three domains of life (19).

The genomic sequence of *Methanosaeta thermophila* (100) indicates that core reactions converting the methyl group of acetate to CH_4 are similar to those of freshwater and marine *Methanosarcina* species, except for activation of acetate to acetyl-CoA, which is catalyzed by AMP-forming acetyl-CoA synthetase. The synthetase has a severalfold-lower K_m for acetate than for acetate kinase, which is the basis for *Methanosaeta* species dominating *Methanosarcina* species in environments where acetate concentrations are low (100).

Electron Transport and Energy Conservation

All acetotrophic methanogens obtain energy for growth by coupling electron transfer from ferredoxin to CoM-S-S-CoB with translocation of ions, generating a gradient (high outside) that drives ATP synthesis by the proton-translocating archaeal A_1A_0-type ATP synthase abundant in acetate-grown *Methanosarcina* species (50, 64, 65, 89). Ferredoxin donates electrons to a membrane-bound hydrogenase (Ech) evolving H_2 and translocating protons in the freshwater isolate *M. barkeri* (**Figure 5*a***) (42, 76). Isolation of a H_2:CoM-S-S-CoB oxidoreductase complex from acetate-grown *M. barkeri* is consistent with a role for Ech and H_2 as intermediates in electron transport (46). The Vho hydrogenase oxidizes H_2 and donates electrons to a quinone-like electron carrier methanophenazine (**Figure 6**) that mediates electron transfer to the heterodisulfide reductase HdrDE while translocating protons that contribute to the gradient (**Figure 5*a***). The VhoAG subunits of the Vho hydrogenase catalyze the oxidation of H_2, whereas the VhoC subunit contains a *b*-type heme proposed to donate electrons to methanophenazine (16). An additional two protons are translocated by the Vho hydrogenase, resulting in four protons translocated by the H_2:CoMS-SCoB oxidoreductase system.

The sodium gradient generated in the exergonic Reaction 2 in **Figure 2** ($\Delta G^{\circ\prime} = -30$ kJ) by the membrane-bound methyltransferase Mtr could drive ATP synthesis in conjunction with a sodium/proton antiporter and the proton-translocating A_1A_0-type ATP synthase (89). Yet without Mtr, the proposed electron transport pathway from H_2 to CoM-S-S-CoB (**Figure 5*a***) features three proton-translocating segments with an obligatory two protons translocated by methanophenazine; however, it is unlikely that the amount of available energy ($\Delta G^{\circ\prime} = -36$ kJ) can support all three coupling sites. Thus, a soluble electron transport chain originating with ferredoxin that supplies methanophenazine or HdrDE with electrons may be necessary to bypass membrane-bound electron transport in order to adjust thermodynamic efficiency in response to fluctuating levels of growth substrate and available energy, particularly when acetate is limiting as is likely the case in the native environment. Indeed, the finding that a *ech* deletion mutant of *M. barkeri* retains a portion of the wild-type ferredoxin:CoM-S-S-CoB oxidoreductase activity supports this idea (116). Furthermore, deletion of the coenzyme F_{420}-dependent Frh hydrogenase and the F_{420} dehydrogenase prevents growth of *M. barkeri* with acetate, suggesting an alternative electron

F_{420}: coenzyme F_{420}

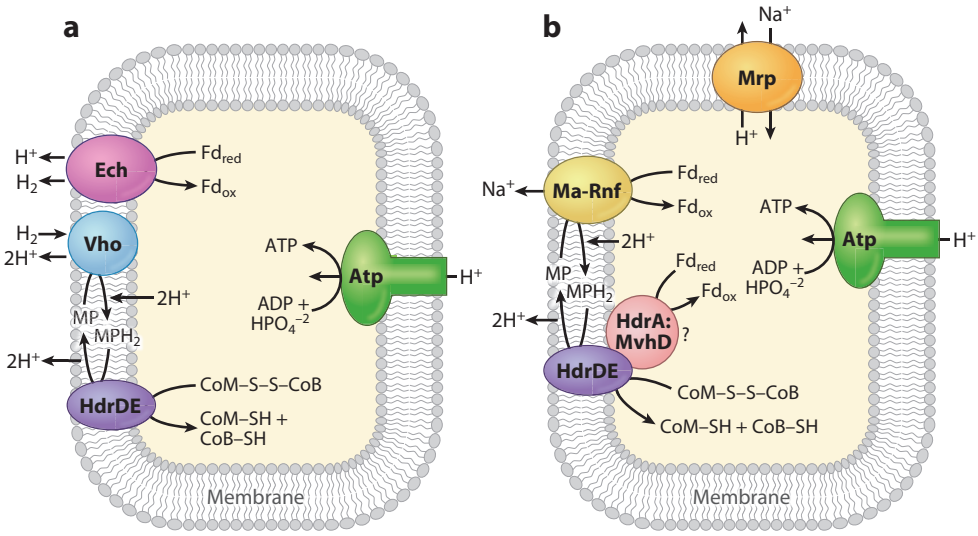

Figure 5

Electron transport pathways in acetotrophic *Methanosarcina* species. (*a*) Freshwater species. (*b*) Marine species. In both pathways, reduced ferredoxin (Fd_{red}) is the product of the oxidation of the carbonyl group of acetyl-CoA catalyzed by the CO dehydrogenase/acetyl-CoA synthase (**Figure 4**). Fd_{ox}, ferredoxin; HS-CoM, coenzyme M; MP, methanophenazine; HS-CoB, coenzyme B; Ech, H_2-evolving hydrogenase; Vho, H_2-uptake hydrogenase; Ma-Rnf, *M. acetivorans* Rnf; HdrDE, heterodisulfide reductase; HdrA:MvhD, heterodisulfide reductase subunit A:hydrogenase subunit D fusion protein; Mrp, multiple resistance/pH regulation Na^+/H^+ antiporter; Atp, H^+-translocating A_1A_0 ATP synthase.

transport pathway involving coenzyme F_{420} and either of these two enzymes (**Figure 6**) (59).

M. acetivorans, the only acetotrophic methanogen isolated from a marine environment, evolved a different strategy (**Figure 5*b***) for oxidizing ferredoxin and reducing CoM-S-S-CoB (102). The genome does not encode a functional Ech, and biochemical and genetic evidence suggests H_2 is not an obligatory intermediate (28, 39, 82). It has been suggested that avoiding H_2 is advantageous for *M. acetivorans* because sulfate-reducing species that dominate the marine environment outcompete methanogens for H_2, potentially disrupting electron transport (65).

Proteomic analyses (65) indicate that acetate-grown *M. acetivorans* preferentially synthesizes subunits encoded by a six-gene cluster with sequence identity to the Rnf complex (first described in *Rhodobacter capsulatus*), where it supplies reduced ferredoxin to nitrogenase (93, 97). Gene knockouts confirm that the complex is essential for growth of *M. acetivorans* on acetate (12). The six-gene cluster is cotranscribed with a gene encoding a cytochrome *c* prominent in membranes of acetate-grown cells (65). It is proposed that ferredoxin donates electrons to the *M. acetivorans* Rnf complex containing cytochrome *c*, and that methanophenazine mediates electron transfer between the cytochrome and HdrDE, translocating a pair of protons outside the cell membrane (**Figure 5*b***). Another potential role for the Rnf complex is the generation of a sodium gradient (high outside) analogous to that proposed for Rnf homologs in some species from *Bacteria* (9, 11). A multisubunit sodium/proton antiporter (Mrp) abundant in acetate-grown *M. acetivorans* could exchange the sodium gradient for a proton gradient that drives ATP synthesis by the proton-translocating A_1A_0 ATP synthase (**Figure 5*b***).

Genetic analyses support that HdrDE is essential for acetotrophic growth of

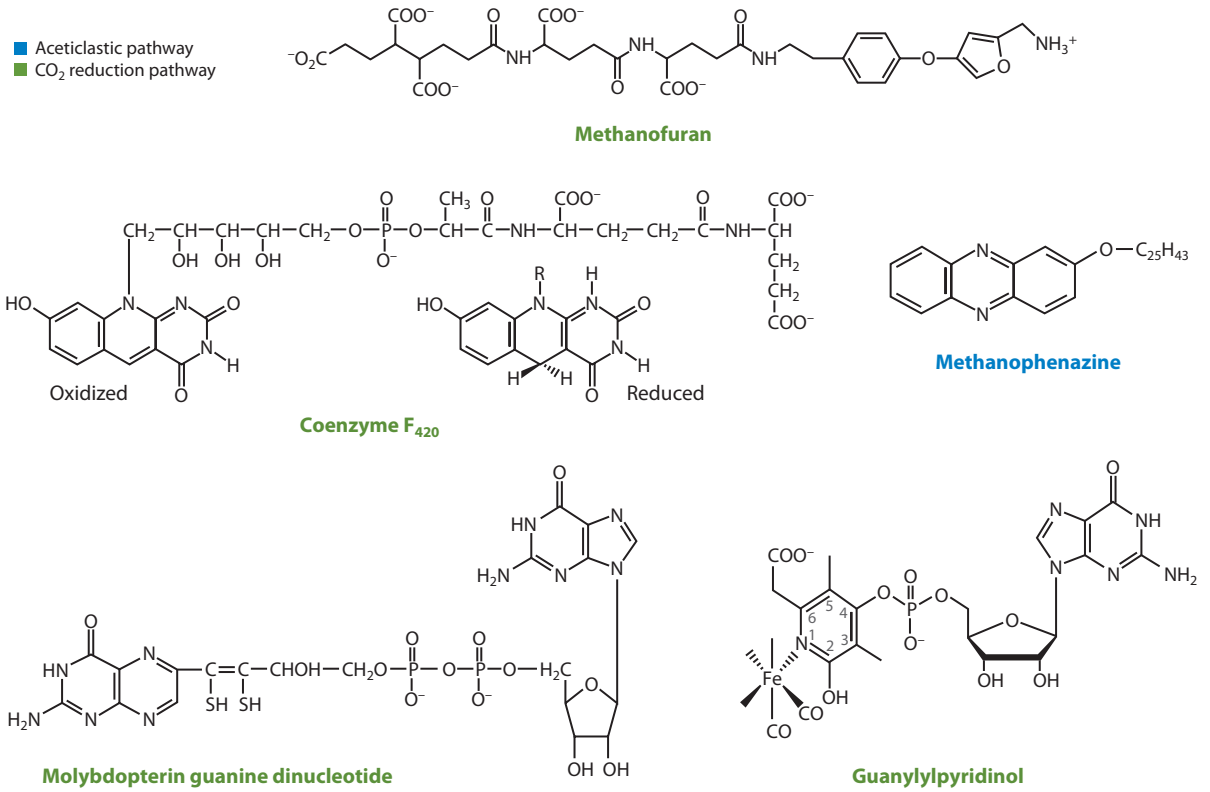

Figure 6

Coenzymes and cofactors unique to either the aceticlastic or the CO_2 reduction pathway.

M. acetivorans (12); however, the authors also present genetic evidence that a HdrA homolog of the HdrA subunit is involved in the soluble HdrABC-type heterodisulfide reductase operable in obligate CO_2-reducing methanogens. The HdrA subunit from obligate CO_2 reducers interfaces with, and accepts electrons from, the MvhD subunit of the MvhAGD hydrogenase, delivering electrons to the catalytically active HdrBC subunits. The HdrA homolog in *M. acetivorans* is a fusion of HdrA and MvhD (HdrA:MvhD) homologs of obligate CO_2-reducing species that is also abundant in acetate-grown *M. acetivorans*, supporting a role in acetotrophic growth (64). The HdrA:MvhD of *M. acetivorans* is encoded monocistronic and distant from genes encoding HdrBC, consistent with HdrA:MvhD functioning independently from HdrABC. What then could be the function of HdrA:MvhD in the aceticlastic pathway of *M. acetivorans*? An attractive possibility is that HdrA:MvhD associates with HdrDE, the functional equivalent of HdrBC, coupling oxidation of ferredoxin and reduction of CoM-S-S-CoB in a soluble system that bypasses membrane-bound ion-translocating pathways (**Figure 5*b***). Similar to electron transport in freshwater *Methanosarcina* species, multiple coupling sites for generating ion gradients in *M. acetivorans* are doubtful given the marginal amount of energy available for converting acetate to CH_4 and CO_2. The branched electron transport could allow for adjusting the thermodynamic efficiency, particularly when acetate is limiting as in the natural environment. Genes encoding Ech and Rnf are absent in the genome of the freshwater isolate *Methanosaeta thermophila*,

suggesting an unknown alternative electron transport pathway and mechanism for energy conservation in this species (100).

CO_2-reducing pathway: pathway in which CO_2 is reduced to CH_4 with electrons derived from oxidation of a reduced substrate such as H_2 or formate

THE CO_2 REDUCTION PATHWAY

Reactions Leading to CH_3-H_4M(S)PT

The reactions in the pathway converting CO_2 to CH_3-H_4MPT for obligate CO_2-reducing species are shown in **Figure 4** (Reactions 5–9). The reactions are similar for freshwater *Methanosarcina* species, except H_4SPT is used in place of H_4MPT and the mechanism for Reaction 5 is distinct. The oxidation of either H_2, CO, or formate provides the three electron pairs required for Reactions 5, 8, and 9. In freshwater species, CO is first oxidized to H_2 and CO_2 (83), whereas the marine isolate *M. acetivorans* evolved a unique CO_2-reducing pathway to avoid H_2 as an intermediate (63). Formate is not a substrate for *Methanosarcina* species, and the only formate dehydrogenases that have been characterized are from the obligate CO_2-reducing species *Methanobacterium formicicum* (96) and *Methanococcus vannielii* (57) that reduce coenzyme F_{420}. F_{420} is a flavin analog (**Figure 6**) with a carbon replacing the N^5 atom of the isoalloxazine ring that donates and accepts a hydride ion similar to NAD(P)H. Formic hydrogenlyase systems from obligate CO_2-reducing species are composed of formate dehydrogenase and F_{420}-dependent hydrogenase converting formate to H_2 and CO_2; however, whether formate is first oxidized to H_2 during growth on formate has not been resolved (6, 48, 71). The formate dehydrogenase from *M. formicicum* contains at the active site a molybdopterin cofactor (**Figure 6**) (56, 75) that is also required for Reaction 5 (2) (**Figure 4**).

In Reaction 5 (CO_2 + methanofuran + $2Fd_{red}$ + $2H^+$ → $2Fd_{ox}$ + formylmethanofuran + H_2O), CO_2 attaches to methanofuran (MF) (**Figure 6**) and then is reduced to formyl-MF catalyzed by formyl-MF dehydrogenase with ferredoxin (Fd) as the electron donor. Reaction 5 is endergonic, requiring an input of energy, particularly with the low partial pressures of H_2 encountered in the native environment (109). The mechanisms for reduction of ferredoxin with H_2 are different between obligate CO_2-reducing species (103) and freshwater *Methanosarcina* species (16). In freshwater *Methanosarcina* species, ferredoxin is reduced by the membrane-bound Ech complex dependent on the proton gradient (high outside) generated by electron transport from H_2 to CoM-S-S-CoB (42). The 4Fe-4S clusters of the Ech from *M. barkeri* were assigned to subunits EchC and EchF (24) for which the EPR signals are pH dependent (61), consistent with a role in proton translocation. The mechanism for reducing ferredoxin in Reaction 5 for obligate CO_2-reducing species has not been established, although a hypothesis has been advanced wherein the exergonic H_2-dependent reduction of CoM-S-S-CoB by the soluble MvhAGD hydrogenase-HdrABC heterodisulfide reductase complex is coupled to reduction of ferredoxin ($2H_2$ + CoM-S-S-CoB + Fd_{ox} = HS-CoM + HS-CoB + Fd_{red}^{2-} + $2H^+$, $\Delta G^{\circ\prime}$ = -39 kJ mol^{-1}) (109). In the mechanism, bifurcation of the electrons occurs at the flavin of the HdrA subunit accepting electrons from the Mvh hydrogenase. If H_2 is not an intermediate during growth with formate as the electron donor, a flavin-containing $F_{420}H_2$ dehydrogenase could be required for flavin-mediated electron bifurcation, for which a candidate has yet to be identified. The high demand for CO_2 by formyl-MF dehydrogenase in the CO_2 reduction pathway suggests a mechanism for transport and conversion of bicarbonate to CO_2 that is analogous to photosynthetic prokaryotes and plants that require carbonic anhydrase ($CO_2 + H_2O = HCO_3^- + H^+$) for this function (15, 79). Genes encoding carbonic anhydrases are found in all genomes sequenced from CO_2-reducing species, and a β type has been characterized from *Methanothermobacter thermoautotrophicus* (formerly *Methanobacterium thermoautotrophicum*), consistent with the proposed role (99, 104).

Reaction 6 (formyl-MF + H_4MPT → N^5-formyl-H_4MPT + MF) is catalyzed

by formylmethanofuran:tetrahydromethanopterin formyltransferase, and Reaction 7 (N^5-formyl-H_4MPT + H^+ → N^5, N^{10}-methenyl-H_4MPT$^+$ + H_2O) is catalyzed by methenyltetrahydromethanopterin cyclohydrolase. Reaction 8 is catalyzed by two mechanistically distinct methylenetetrahydromethanopterin dehydrogenases. The F_{420}-dependent enzyme (Mtd) catalyzes Reaction 8a (N^5, N^{10}-methenyl-H_4MPT$^+$ + $F_{420}H_2$ → N^5, N^{10}-methylene-H_4MPT + F_{420} + H^+), and the enzyme (Hmd) catalyzing Reaction 8b (N^5, N^{10}-methenyl-H_4MPT$^+$ + H_2 → N^5, N^{10}-methylene-H_4MPT + H^+) utilizes H_2 as the electron donor. The reduction of F_{420} by H_2 that supplies Mtd is catalyzed by hydrogenases with properties distinct from the Ech hydrogenase (4, 5, 25, 59, 114). Hmd is a homodimeric hydrogenase containing an iron atom in a cofactor (**Figure 6**) that is a derivative of pyridone (49, 84). The enzyme catalyzes the stereospecific transfer of a hydride ion from H_2 into the methylene carbon of the product (115). The enzyme contains no iron-sulfur clusters or nickel, in contrast to all known hydrogenases, and therefore may be of increasing importance in cells growing under nickel-deficient conditions. Reaction 9 (N^5, N^{10}-methylene-H_4MPT + $F_{420}H_2$ → N^5-methyl-H_4MPT + F_{420}) is catalyzed by methylenetetrahydromethanopterin reductase.

Electron Transport and Energy Conservation in Freshwater *Methanosarcina* and Obligate CO_2-Reducing Species

The reactions yielding the greatest energy for ATP synthesis in freshwater species are the H_2:CoM-S-S-CoB oxidoreductase systems (−39 kJ mol^{-1}), which differ between *Methanosarcina* and obligate CO_2-reducing species. In *Methanosarcina mazei* the system is the same as that functioning in the aceticlastic pathway pumping protons that drive the proton-translocating A_1A_0-type ATP synthase (**Figure 5*a***). However, obligate CO_2-reducing species do not contain cytochromes or methanophenazine with no obvious mechanism for generating an ion gradient coupled to electron transport. The H_2:CoM-S-S-CoB oxidoreductase system instead comprises the cytoplasmic F_{420}-nonreducing hydrogenase MvhAGD tightly bound to the soluble HdrABC type of heterodisulfide reductase (103). There is no obvious mechanism for energy conservation by this complex and conclusive evidence has yet to be reported. Thus, the only recognizable mechanism for ATP synthesis is the sodium gradient generated by the membrane-bound methyl-H_4MPT:coenzyme M methyltransferase complex (**Figure 2**, Reaction 2) driving a sodium-translocating A_1A_0-ATP synthase (109).

Electron Transport and Energy Conservation in the Marine Species *M. acetivorans*

On the basis of biochemical and quantitative proteomic analyses, a CO_2 reduction pathway (**Figure 7**) for the marine isolate *M. acetivorans* has been proposed that is similar to that of other *Methanosarcina* and obligate CO_2-reducing species but with novel exceptions in electron transport and ATP synthesis (63). The only electron donor for reduction of CO_2 to CH_4 by *M. acetivorans* is CO. In the proposed pathway, CO is oxidized by the Cdh/ACDS complex that reduces ferredoxin (Equation 6), a portion of which is reoxidized with transfer of electrons to coenzyme F_{420} (Equation 7).

$$7CO + 7H_2O + 7Fd = 7Fd^{2-} + 7CO_2 + 14H^+ \quad 6.$$

$$5Fd^{2-} + 5F_{420} = 5F_{420}H_2 + 5Fd \quad 7.$$

The reduction of $2CO_2$ to $2CH_3$-H_4SPT with $2Fd^{2-}$ and $4F_{420}H_2$ (**Figure 7**) involves enzymes and coenzymes identical to those of the pathway of freshwater methanogens except for the first step (**Figure 4**, Reaction 5), where electrons for reduction of ferredoxin derive directly from CO rather than from the Ech hydrogenase or the MvhAGD-HdrABC heterodisulfide complex as is the case for freshwater *Methanosarcina* or obligate CO_2-reducing

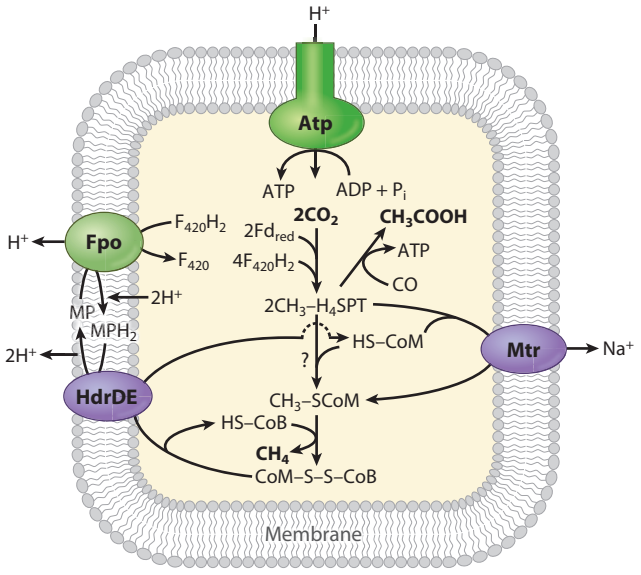

Figure 7

Electron transport and energy conservation during CO-dependent growth of *Methanosarcina acetivorans*. **Reactants and products are in bold**. Fd_{red}, reduced ferredoxin; Fpo, F_{420} dehydrogenase; F_{420}, coenzyme F_{420}; H_4SPT, tetrahydrosarcinapterin; HS-CoM, coenzyme M; Mtr, CH_3-H_4SPT:coenzyme M methyltransferase; MP, methanophenazine; HS-CoB, coenzyme B; HdrDE, heterodisulfide reductase; Atp, proton-translocating A_1A_0 ATP synthase.

species. Unlike all other CO_2-reducing pathways, a combination of quantitative proteomic and biochemical evidence (63) supports that a $F_{420}H_2$ dehydrogenase complex (FpoA-O) functions to supply electrons to the heterodisulfide reductase in CO-grown *M. acetivorans* (**Figure 7**). The $F_{420}H_2$ dehydrogenase complex was first discovered in the electron transport pathway of methanol conversion to CH_4 in *Methanosarcina* species (21), including *M. acetivorans* (64), wherein methanophenazine mediates electron transfer between $F_{420}H_2$ dehydrogenase and HdrDE pumping protons the same as during acetotrophic growth of *Methanosarcina* species (**Figure 7**). Thus, it is proposed that methanophenazine functions similarly in CO-grown *M. acetivorans* (**Figure 7**). The $F_{420}H_2$ dehydrogenase complex in methanol-grown *Methanosarcina* species pumps protons, suggestive of the $F_{420}H_2$ dehydrogenase complex and further contributing to the electrochemical potential that drives ATP synthesis in CO-grown *M. acetivorans* (**Figure 7**) (63). The participation of F_{420} as an electron carrier requires an enzyme for the transfer of electrons from ferredoxin to F_{420} (Equation 7) that has yet to be identified.

The membrane-bound, sodium-translocating methyltransferase (Mth) complex that transfers the methyl group from CH_3-H_4SPT to HS-CoM is downregulated in CO-grown *M. acetivorans*, whereas it is upregulated in methanol- and acetate-grown cells, suggesting a reduced involvement of this enzyme (63). On the other hand, three genes annotated to encode corrinoid-containing proteins are upregulated in CO-grown versus methanol- and acetate-grown *M. acetivorans*, consistent with a role during growth with CO (63). The predicted proteins contain two domains, one with a corrinoid-binding motif and the other with sequence identity to HS-CoM-dependent methyltransferases, suggesting a role in the transfer of a methyl group to HS-CoM. Thus, it is postulated (63) that the upregulated corrinoid proteins participate in transfer of the methyl group from methyl-H_4SPT to HS-CoM via a soluble sodium-independent pathway. The proposal does not rule out that both pathways function simultaneously or that either is essential. Assuming the sodium gradient drives ATP synthesis, an attractive hypothesis is that both the membrane-bound, sodium-translocating and soluble reactions function to adjust thermodynamic efficiency in response to fluctuating levels of growth substrate. Under laboratory conditions where cells are routinely cultured with greater than 0.5 atmosphere of CO and abundant available energy, the postulated soluble methyl transfer may be dispensable, explaining normal growth of mutants deleted of genes encoding the corrinoid proteins (85).

The formation of acetate as an end product (**Figure 7**) is yet another novel feature of energy conservation in the CO_2 reduction pathway in *M. acetivorans* (63, 86, 92). Quantitative proteomic analyses (63) indicate a role for the Cdh/ACDS complex in the synthesis of acetyl-CoA from the methyl group of CH_3-H_4SPT,

CO, and CoA-SH. The acetyl group of acetyl-CoA is further converted to acetate by phosphotransacetylase and acetate kinase catalyzing the synthesis of ATP. Thus, ATP is synthesized via both substrate-level and chemiosmotic mechanisms. Formate and dimethylsulfide are also produced in minor amounts with no obvious pathway for ATP synthesis (78, 86).

Notably, H_2 is not an intermediate in either the aceticlastic or the CO_2 reduction pathway for methanogenesis by the marine isolate *M. acetivorans*, in contrast to freshwater *Methanosarcina* species. It has been suggested (63, 65) that *M. acetivorans* evolved pathways in this manner to avoid loss of reductant to H_2-utilizing, sulfate-reducing microbes that outcompete methanogens for H_2 in marine environments (119).

CONCLUSIONS AND PERSPECTIVES

Clearly, considerable information has accumulated in the recent past regarding the energy-yielding metabolism of acetotrophic and CO_2-reducing methanogenic species; however, much remains to be learned. In particular, a basic understanding of the mechanism of energy conservation in freshwater CO_2-reducing species is ripe for investigation. Note that no identifiable electron transport mechanism for energy conservation has been identified for obligate CO_2-reducing species, although evolved in *Methanosarcina* species. However, it has been argued that nature evolved different energy conservation mechanisms in response to the concentration of electron donors (e.g., H_2) in the environment that maximized either growth rate or efficiency (109). Nonetheless, only hypotheses for electron transport and energy conservation in obligate CO_2-reducing species have been advanced, and they have yet to be tested. On the other hand, identification of enzyme complexes that function in energy-yielding pathways of *Methanosarcina* species has opened clear avenues of investigation with implications for further understanding of electron transport and energy conservation in species from *Bacteria*. Advances in determining the crystal structures of membrane proteins will certainly facilitate discovery. The recent genomic sequencing of several methanogenic species has revealed hypothetical proteins of unknown function, some of which are likely to be involved in energy-conserving functions. Robust genetic systems recently developed for methanogenic species will surely accelerate the discovery of novel proteins and their function. Another understudied area is the regulation of genes in energy-conserving pathways, particularly regulatory proteins, which will benefit from genetic approaches now within reach.

Substrate-level ATP synthesis: transfer of phosphate to ADP, forming ATP involving an activated intermediate

Chemiosmotic ATP synthesis: transfer of phosphate to ADP, forming ATP catalyzed by ATP synthase driven by an ion gradient

SUMMARY POINTS

1. Methane-producing microbes are terminal organisms of an anaerobic microbial food chain, converting complex biomass in diverse anaerobic environments that is integral to the global carbon cycle.
2. Methane is a product of the energy-yielding metabolism of species restricted to *Archaea*.
3. At least two-thirds of the methane produced derives from the methyl group of acetate and the remaining one-third derives primarily from the reduction of CO_2. Both pathways have in common the reductive demethylation of methyl-coenzyme M to CH_4, which is the most exergonic.
4. Methane-producing species are divided according to their mechanism for energy conservation, which is exclusively dependent on electron transport in all but one species.

5. The metabolically diverse genus *Methanosarcina* generates an ion gradient coupled to electron transport in the reductive demethylation reaction of both pathways that energize ATP synthase.
6. The mechanism of energy conservation in species only reducing CO_2 is largely unknown, although hypotheses have been advanced.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Abbanat DR, Ferry JG. 1991. Resolution of component proteins in an enzyme complex from *Methanosarcina thermophila* catalyzing the synthesis or cleavage of acetyl-CoA. *Proc. Natl. Acad. Sci. USA* 88:3272–76
2. Acharya P, Warkentin E, Ermler U, Thauer RK, Shima S. 2006. The structure of formylmethanofuran:tetrahydromethanopterin formyltransferase in complex with its coenzymes. *J. Mol. Biol.* 357:870–79
3. Alber BE, Ferry JG. 1994. A carbonic anhydrase from the archaeon *Methanosarcina thermophila*. *Proc. Natl. Acad. Sci. USA* 91:6909–13
4. Alex LA, Reeve JN, Orme-Johnson WH, Walsh CT. 1990. Cloning, sequence determination, and expression of the genes encoding the subunits of the nickel-containing 8-hydroxy-5-deazaflavin reducing hydrogenase from *Methanobacterium thermoautotrophicum* H. *Biochemistry* 29:7237–44
5. Baron SF, Ferry JG. 1989. Purification and properties of the membrane-associated coenzyme F_{420}-reducing hydrogenase from *Methanobacterium formicicum*. *J. Bacteriol.* 171:3846–53
6. Baron SF, Ferry JG. 1989. Reconstitution and properties of a coenzyme F_{420}-mediated formate hydrogenlyase system in *Methanobacterium formicicum*. *J. Bacteriol.* 171:3854–59
7. Battistuzzi FU, Feijao A, Hedges SB. 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BMC Evol. Biol.* 4:44
8. Beal EJ, House CH, Orphan VJ. 2009. Manganese- and iron-dependent marine methane oxidation. *Science* 325:184–87
9. Boiangiu CD, Jayamani E, Brugel D, Herrmann G, Kim J, et al. 2005. Sodium ion pumps and hydrogen production in glutamate fermenting anaerobic bacteria. *J. Mol. Microbiol. Biotechnol.* 10:105–19
10. Boone DR, Kamagata Y. 1998. Rejection of the species *Methanothrix soehngenii*[VP] and the genus *Methanothrix*[VP] as *nomina confusa*, and transfer of *Methanothrix thermophila*[VP] to the genus *Methanosaeta*[VP] as *Methanosaeta thermophila* comb. nov. Request for an opinion. *Int. J. Syst. Bacteriol.* 48:1079–80
11. Bruggemann H, Baumer S, Fricke WF, Wiezer A, Liesegang H, et al. 2003. The genome sequence of *Clostridium tetani*, the causative agent of tetanus disease. *Proc. Natl. Acad. Sci. USA* 100:1316–21
12. Buan NR, Metcalf WW. 2010. Methanogenesis by *Methanosarcina acetivorans* involves two structurally and functionally distinct classes of heterodisulfide reductase. *Mol. Microbiol.* 75:843–53
13. Buss KA, Cooper DR, Ingram-Smith C, Ferry JG, Sanders DA, Hasson MS. 2001. Urkinase: structure of acetate kinase, a member of the ASKHA superfamily of phosphotransferases. *J. Bacteriol.* 183:680–86
14. Chen SL, Pelmenschikov V, Blomberg MR, Siegbahn PE. 2009. Is there a Ni-methyl intermediate in the mechanism of methyl-coenzyme M reductase? *J. Am. Chem. Soc.* 131:9912–13
15. Cot SS, So AK, Espie GS. 2008. A multiprotein bicarbonate dehydration complex essential to carboxysome function in cyanobacteria. *J. Bacteriol.* 190:936–45

16. Deppenmeier U. 2004. The membrane-bound electron transport system of *Methanosarcina* species. *J. Bioenerg. Biomembr.* 36:55–64
17. Eggen RIL, Geerling ACM, Jetten MSM, Devos WM. 1991. Cloning, expression, and sequence analysis of the genes for carbon monoxide dehydrogenase of *Methanothrix soehngenii*. *J. Biol. Chem.* 266:6883–87
18. Ermler U, Grabarse W, Shima S, Goubeaud M, Thauer RK. 1997. Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. *Science* 278:14572
18a. Ferry JG. 2008. Acetate-based methane production. In *Bioenergy*, ed. JD Wall, CS Harwood, A Demain, pp. 155–70. Washington, DC: ASM Press
19. Ferry JG. 2010. The gamma class of carbonic anhydrase. *Biochim. Biophys. Acta* 1804:374–81
20. Ferry JG, House CH. 2006. The stepwise evolution of early life driven by energy conservation. *Mol. Biol. Evol.* 23:1286–92
21. Ferry JG, Kastead KA. 2007. Methanogenesis. In *Archaea: Molecular Cell Biology*, ed. R Cavicchioli, pp. 288–314. Washington, DC: ASM Press
22. Fischer R, Thauer RK. 1990. Ferredoxin-dependent methane formation from acetate in cell extracts of *Methanosarcina barkeri* (strain MS). *FEBS Lett.* 269:368–72
23. Formisano V, Atreya S, Encrenaz T, Ignatiev N, Giuranna M. 2004. Detection of methane in the atmosphere of Mars. *Science* 306:1758–61
24. Forzi L, Koch J, Guss AM, Radosevich CG, Metcalf WW, Hedderich R. 2005. Assignment of the [4Fe-4S] clusters of Ech hydrogenase from *Methanosarcina barkeri* to individual subunits via the characterization of site-directed mutants. *FEBS J.* 272:4741–53
25. Fox JA, Livingston DJ, Orme-Johnson W-H, Walsh CT. 1987. 8-hydroxy-5-deazaflavin-reducing hydrogenase from *Methanobacterium thermoautotrophicum*. 1. Purification and characterization. *Biochemistry* 26:4219–27
26. Fricke WF, Seedorf H, Henne A, Kruer M, Liesegang H, et al. 2006. The genome sequence of *Methanosphaera stadtmanae* reveals why this human intestinal archaeon is restricted to methanol and H_2 for methane formation and ATP synthesis. *J. Bacteriol.* 188:642–58
27. Funk T, Gu WW, Friedrich S, Wang HX, Gencic S, et al. 2004. 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.* 126:88–95
28. Galagan JE, Nusbaum C, Roy A, Endrizzi MG, Macdonald P, et al. 2002. The genome of *M. acetivorans* reveals extensive metabolic and physiological diversity. *Genome Res.* 12:532–42
29. Gencic S, Grahame DA. 2003. Nickel in subunit β of the acetyl-CoA decarbonylase/synthase multienzyme complex in methanogens. *J. Biol. Chem.* 278:6101–10
30. Gong W, Hao B, Wei Z, Ferguson DJ Jr, Tallant T, et al. 2008. Structure of the $\alpha_2\,\varepsilon_2$ Ni-dependent CO dehydrogenase component of the *Methanosarcina barkeri* acetyl-CoA decarbonylase/synthase complex. *Proc. Natl. Acad. Sci. USA* 105:9558–63
31. Gorrell A, Lawrence SH, Ferry JG. 2005. Structural and kinetic analyses of arginine residues in the active site of the acetate kinase from *Methanosarcina thermophila*. *J. Biol. Chem.* 280:10731–42
32. Gottschalk G, Thauer RK. 2001. The Na^+ translocating methyltransferase complex from methanogenic archaea. *Biochim. Biophys. Acta* 1505:28–36
33. Grahame DA. 1991. Catalysis of acetyl-CoA cleavage and tetrahydrosarcinapterin methylation by a carbon monoxide dehydrogenase-corrinoid enzyme complex. *J. Biol. Chem.* 266:22227–33
34. Grahame DA. 1993. 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* 32:10786–93
35. Grahame DA. 2003. Acetate C-C bond formation and decomposition in the anaerobic world: the structure of a central enzyme and its key active-site metal cluster. *Trends Biochem. Sci.* 28:221–24
36. Grahame DA, Demoll E. 1996. Partial reactions catalyzed by protein components of the acetyl-CoA decarbonylase synthase enzyme complex from *Methanosarcina barkeri*. *J. Biol. Chem.* 271:8352–58
37. Grahame DA, Stadtman TC. 1987. Carbon monoxide dehydrogenase from *Methanosarcina barkeri*. Disaggregation, purification, and physicochemical properties of the enzyme. *J. Biol. Chem.* 262:3706–12

38. Gu WW, Gencic S, Cramer SP, Grahame DA. 2003. 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.* 125:15343–51
39. Guss AM, Mukhopadhyay B, Zhang JK, Metcalf WW. 2005. Genetic analysis of *mch* mutants in two *Methanosarcina* species demonstrates multiple roles for the methanopterin-dependent C-1 oxidation/reduction pathway and differences in H_2 metabolism between closely related species. *Mol. Microbiol.* 55:1671–80
40. Hamann N, Mander GJ, Shokes JE, Scott RA, Bennati M, Hedderich R. 2007. A cysteine-rich CCG domain contains a novel [4Fe-4S] cluster binding motif as deduced from studies with subunit B of heterodisulfide reductase from *Methanothermobacter marburgensis*. *Biochemistry* 46:12875–85
41. Harms U, Weiss DS, Gartner P, Linder D, Thauer RK. 1995. The energy conserving N^5-methyltetrahydromethanopterin: Coenzyme M methyltransferase complex from *Methanobacterium thermoautotrophicum* is composed of eight different subunits. *Eur. J. Biochem.* 228:640–48
42. Hedderich R. 2004. Energy-converting [NiFe] hydrogenases from Archaea and extremophiles: ancestors of complex I. *J. Bioenerg. Biomembr.* 36:65–75
43. Hedderich R, Berkessel A, Thauer RK. 1990. Purification and properties of heterodisulfide reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). *Eur. J. Biochem.* 193:255–61
44. Hedderich R, Hamann N, Bennati M. 2005. Heterodisulfide reductase from methanogenic archaea: a new catalytic role for an iron-sulfur cluster. *Biol. Chem.* 386:961–70
45. Hedderich R, Koch J, Linder D, Thauer RK. 1994. The heterodisulfide reductase from *Methanobacterium thermoautotrophicum* contains sequence motifs characteristic of pyridine-nucleotide-dependent thioredoxin reductases. *Eur. J. Biochem.* 225:253–61
46. Heiden S, Hedderich R, Setzke E, Thauer RK. 1993. Purification of a cytochrome *b* containing H_2-heterodisulfide oxidoreductase complex from membranes of *Methanosarcina barkeri*. *Eur. J. Biochem.* 213:529–35
47. Heiden S, Hedderich R, Setzke E, Thauer RK. 1994. Purification of a two-subunit cytochrome-*b*-containing heterodisulfide reductase from methanol-grown *Methanosarcina barkeri*. *Eur. J. Biochem.* 221:855–61
48. Hendrickson EL, Leigh JA. 2008. Roles of coenzyme F_{420}-reducing hydrogenases and hydrogen- and F_{420}-dependent methylenetetrahydromethanopterin dehydrogenases in reduction of F_{420} and production of hydrogen during methanogenesis. *J. Bacteriol.* 190:4818–21
49. Hiromoto T, Ataka K, Pilak O, Vogt S, Stagni MS, et al. 2009. The crystal structure of C176A mutated [Fe]-hydrogenase suggests an acyl-iron ligation in the active site iron complex. *FEBS Lett.* 583:585–90
50. Hovey R, Lentes S, Ehrenreich A, Salmon K, Saba K, et al. 2005. DNA microarray analysis of *Methanosarcina mazei* Go1 reveals adaptation to different methanogenic substrates. *Mol. Genet. Genomics* 273:225–39
51. Iyer PP, Lawrence SH, Luther KB, Rajashankar KR, Yennawar HP, et al. 2004. Crystal structure of phosphotransacetylase from the methanogenic archaeon *Methanosarcina thermophila*. *Structure* 12:559–67
52. Jablonski PE, Lu WP, Ragsdale SW, Ferry JG. 1993. 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.* 268:325–29
53. Jetten MSM, Hagen WR, Pierik AJ, Stams AJM, Zehnder AJB. 1991. Paramagnetic centers and acetyl-coenzyme A/CO exchange activity of carbon monoxide dehydrogenase from *Methanothrix soehngenii*. *Eur. J. Biochem.* 195:385–91
54. Jetten MSM, Pierik AJ, Hagen WR. 1991. EPR characterization of a high-spin system in carbon monoxide dehydrogenase from *Methanothrix soehngenii*. *Eur. J. Biochem.* 202:1291–97
55. Jetten MSM, Stams AJM, Zehnder AJB. 1989. Purification and characterization of an oxygen-stable carbon monoxide dehydrogenase of *Methanothrix soehngenii*. *FEBS Lett.* 181:437–41
56. Johnson JL, Bastian NR, Schauer NL, Ferry JG, Rajagopalan KV. 1991. Identification of molybdopterin guanine dinucleotide in formate dehydrogenase from *Methanobacterium formicicum*. *FEMS Microbiol. Lett.* 77:2–3
57. Jones JB, Stadtman TC. 1981. Selenium-dependent and selenium-independent formate dehydrogenases of *Methanococcus vannielii*. *J. Biol. Chem.* 256(2):656–63

58. Krzycki JA, Mortenson LE, Prince RC. 1989. Paramagnetic centers of carbon monoxide dehydrogenase from aceticlastic *Methanosarcina barkeri*. *J. Biol. Chem.* 264:7217–21
59. Kulkarni G, Kridelbaugh DM, Guss AM, Metcalf WW. 2009. Hydrogen is a preferred intermediate in the energy-conserving electron transport chain of *Methanosarcina barkeri*. *Proc. Natl. Acad. Sci. USA* 106:15915–20
60. Kunkel A, Vaupel M, Heim S, Thauer RK, Hedderich R. 1997. Heterodisulfide reductase from methanol-grown cells of *Methanosarcina barkeri* is not a flavoenzyme. *Eur. J. Biochem.* 244:226–34
61. Kurkin S, Meuer J, Koch J, Hedderich R, Albracht SP. 2002. The membrane-bound [NiFe]-hydrogenase (Ech) from *Methanosarcina barkeri*: unusual properties of the iron-sulphur clusters. *Eur. J. Biochem.* 269:6101–11
62. Lawrence SH, Luther KB, Schindelin H, Ferry JG. 2006. Structural and functional studies suggest a catalytic mechanism for the phosphotransacetylase from *Methanosarcina thermophila*. *J. Bacteriol.* 188:1143–54
63. Lessner DJ, Li L, Li Q, Rejtar T, Andreev VP, et al. 2006. An unconventional pathway for reduction of CO_2 to methane in CO-grown *Methanosarcina acetivorans* revealed by proteomics. *Proc. Natl. Acad. Sci. USA* 103:17921–26
64. Li L, Li Q, Rohlin L, Kim U, Salmon K, et al. 2007. Quantitative proteomic and microarray analysis of the archaeon *Methanosarcina acetivorans* grown with acetate versus methanol. *J. Proteome Res.* 6:759–71
65. Li Q, Li L, Rejtar T, Lessner DJ, Karger BL, Ferry JG. 2006. Electron transport in the pathway of acetate conversion to methane in the marine archaeon *Methanosarcina acetivorans*. *J. Bacteriol.* 188:702–10
66. Lindahl PA, Chang B. 2001. The evolution of acetyl-CoA synthase. *Orig. Life Evol. Biosph.* 31:403–34
67. Lipmann F. 1944. Enzymatic synthesis of acetyl phosphate. *J. Biol. Chem.* 155:55–70
68. Liu JS, Marison IW, von Stockar U. 2001. Microbial growth by a net heat up-take: a calorimetric and thermodynamic study on acetotrophic methanogenesis by *Methanosarcina barkeri*. *Biotechnol. Bioeng.* 75:170–80
69. Liu Y, Whitman WB. 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann. N. Y. Acad. Sci.* 1125:171–89
70. Lovelock J. 2000. *Gaia: A New Look at Life on Earth*. Oxford: Oxford Univ. Press
71. Lupa B, Hendrickson EL, Leigh JA, Whitman WB. 2008. Formate-dependent H_2 production by the mesophilic methanogen *Methanococcus maripaludis*. *Appl. Environ. Microbiol.* 74:6584–90
72. MacAuley SR, Zimmerman SA, Apolinario EE, Evilia C, Hou Y, et al. 2009. The archetype γ-class carbonic anhydrase (Cam) contains iron when synthesized in vivo. *Biochemistry* 48:817–19
73. Martin W, Russell MJ. 2006. On the origin of biochemistry at an alkaline hydrothermal vent. *Phil. Trans. R. Soc. B* 362:1887–926
74. Maupin-Furlow J, Ferry JG. 1996. 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.* 178:340–46
75. May HD, Schauer NL, Ferry JG. 1986. Molybdopterin cofactor from *Methanobacterium formicicum* formate dehydrogenase. *J. Bacteriol.* 166:500–4
76. Meuer J, Kuettner HC, Zhang JK, Hedderich R, Metcalf WW. 2002. 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* 99:5632–37
77. Miles RD, Gorrell A, Ferry JG. 2002. Evidence for a transition state analog, MgADP-aluminum fluoride-acetate, in acetate kinase from *Methanosarcina thermophila*. *J. Biol. Chem.* 277:22547–52
78. Moran JJ, House CH, Vrentas JM, Freeman KH. 2008. Methyl sulfide production by a novel carbon monoxide metabolism in *Methanosarcina acetivorans*. *Appl. Environ. Microbiol.* 74:540–42
79. Moroney JV, Bartlett SG, Samuelsson G. 2001. Carbonic anhydrases in plants and algae. *Plant Cell. Environ.* 24:141–53
80. Mumma MJ, Villanueva GL, Novak RE, Hewagama T, Bonev BP, et al. 2009. Strong release of methane on Mars in northern summer 2003. *Science* 323:1041–45
81. Murakami E, Ragsdale SW. 2000. 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.* 275:4699–707

82. Nelson MJK, Ferry JG. 1984. Carbon monoxide-dependent methyl coenzyme M methylreductase in acetotrophic *Methanosarcina* spp. *J. Bacteriol.* 160:526–32
83. O'Brien JM, Wolkin RH, Moench TT, Morgan JB, Zeikus JG. 1984. Association of hydrogen metabolism with unitrophic or mixotrophic growth of *Methanosarcina barkeri* on carbon monoxide. *J. Bacteriol.* 158:373–75
84. Obrist BV, Chen D, Ahrens A, Schunemann V, Scopelliti R, Hu X. 2009. An iron carbonyl pyridonate complex related to the active site of the [Fe]-hydrogenase (Hmd). *Inorg. Chem.* 48:3514–16
85. Oelgeschlager E, Rother M. 2009. In vivo role of three fused corrinoid/methyl transfer proteins in *Methanosarcina acetivorans*. *Mol. Microbiol.* 72:1260–72
86. Oelgeschlager E, Rother M. 2009. Influence of carbon monoxide on metabolite formation in *Methanosarcina acetivorans*. *FEMS Microbiol. Lett.* 292:254–60
87. Parisi G, Perales M, Fornasari M, Colaneri A, Schain N, et al. 2004. Gamma carbonic anhydrases in plant mitochondria. *Plant Mol. Biol.* 55:193–207
88. Pelmenschikov V, Siegbahn PE. 2003. Catalysis by methyl-coenzyme M reductase: a theoretical study for heterodisulfide product formation. *J. Biol. Inorg. Chem.* 8:653–62
89. Pisa KY, Weidner C, Maischak H, Kavermann H, Muller V. 2007. The coupling ion in the methanoarchaeal ATP synthases: H^+ versus Na^+ in the A_1A_0 ATP synthase from the archaeon *Methanosarcina mazei* Go1. *FEMS Microbiol. Lett.* 277:56–63
90. Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJ, Ettwig KF, et al. 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440:918–21
91. Ragsdale SW. 2007. Nickel and the carbon cycle. *J. Inorg. Biochem.* 101:1657–66
92. Rother M, Metcalf WW. 2004. Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. *Proc. Natl. Acad. Sci. USA* 101:16929–34
93. Saeki K, Kumagai H. 1998. The *rnf* gene products in *Rhodobacter capsulatus* play an essential role in nitrogen fixation during anaerobic DMSO-dependent growth in the dark. *Arch. Microbiol.* 169:464–67
94. Sakai S, Imachi H, Hanada S, Ohashi A, Harada H, Kamagata Y. 2008. *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order *Methanocellales* ord. nov. *Int. J. Syst. Evol. Microbiol.* 58:929–36
95. Samuel BS, Hansen EE, Manchester JK, Coutinho PM, Henrissat B, et al. 2007. Genomic and metabolic adaptations of *Methanobrevibacter smithii* to the human gut. *Proc. Natl. Acad. Sci. USA* 104:10643–48
96. Schauer NL, Ferry JG. 1986. Composition of the coenzyme F_{420}-dependent formate dehydrogenase from *Methanobacterium formicicum*. *J. Bacteriol.* 165:405–11
97. Schmehl M, Jahn A, Meyer zu Vilsendorf A, Hennecke S, Masepohl B, et al. 1993. Identification of a new class of nitrogen fixation genes in *Rhodobacter capsulatus*: a putative membrane complex involved in electron transport to nitrogenase. *Mol. Gen. Genet.* 241:602–15
98. Simianu M, Murakami E, Brewer JM, Ragsdale SW. 1998. Purification and properties of the heme- and iron-sulfur-containing heterodisulfide reductase from *Methanosarcina thermophila*. *Biochemistry* 37:10027–39
99. Smith KS, Ferry JG. 1999. A plant-type (beta-class) carbonic anhydrase in the thermophilic methanoarchaeon *Methanobacterium thermoautotrophicum*. *J. Bacteriol.* 181:6247–53
100. Smith KS, Ingram-Smith C. 2007. *Methanosaeta*, the forgotten methanogen? *Trends Microbiol.* 7:150–55
101. Smith KS, Jakubzick C, Whittam TC, Ferry JG. 1999. Carbonic anhydrase is an ancient enzyme widespread in prokaryotes. *Proc. Natl. Acad. Sci. USA* 96:15184–89
102. Sowers KR, Baron SF, Ferry JG. 1984. *Methanosarcina acetivorans* sp. nov., an acetotrophic methane-producing bacterium isolated from marine sediments. *Appl. Environ. Microbiol.* 47:971–78
103. Stojanowic A, Mander GJ, Duin EC, Hedderich R. 2003. Physiological role of the F_{420}-nonreducing hydrogenase (Mvh) from *Methanothermobacter marburgensis*. *Arch. Microbiol.* 180:194–203
104. Strop P, Smith KS, Iverson TM, Ferry JG, Rees DC. 2001. Crystal structure of the "cab"-type β-class carbonic anhydrase from the archaeon *Methanobacterium thermoautotrophicum*. *J. Biol. Chem.* 276:10299–305
105. Terlesky KC, Ferry JG. 1988. Ferredoxin requirement for electron transport from the carbon monoxide dehydrogenase complex to a membrane-bound hydrogenase in acetate-grown *Methanosarcina thermophila*. *J. Biol. Chem.* 263:4075–79

106. Terlesky KC, Ferry JG. 1988. Purification and characterization of a ferredoxin from acetate-grown *Methanosarcina thermophila*. *J. Biol. Chem.* 263:4080–82
107. Terlesky KC, Nelson MJK, Ferry JG. 1986. Isolation of an enzyme complex with carbon monoxide dehydrogenase activity containing a corrinoid and nickel from acetate-grown *Methanosarcina thermophila*. *J. Bacteriol.* 168:1053–58
108. Thauer RK. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson. *Microbiol.* 144:2377–406
109. Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* 6:579–91
110. Thauer RK, Shima S. 2007. Methane as fuel for anaerobic microorganisms. *Ann. N. Y. Acad. Sci.* 1125:158–70
111. Tripp BC, Bell CB, Cruz F, Krebs C, Ferry JG. 2004. A role for iron in an ancient carbonic anhydrase. *J. Biol. Chem.* 279:6683–87
112. Ueno Y, Yamada K, Yoshida N, Maruyama S, Isozaki Y. 2006. Evidence from fluid inclusions for microbial methanogenesis in the early archaean era. *Nature* 440:516–19
113. van Beelen P, Labro JFA, Keltjens JT, Geerts WJ, Vogels GD, et al. 1984. Derivatives of methanopterin, a coenzyme involved in methanogenesis. *Eur. J. Biochem.* 139:359–65
114. Vaupel M, Thauer RK. 1998. Two F_{420}-reducing hydrogenases in *Methanosarcina barkeri*. *Arch. Microbiol.* 169:201–5
115. Vogt S, Lyon EJ, Shima S, Thauer RK. 2008. The exchange activities of [Fe] hydrogenase (iron-sulfur-cluster-free hydrogenase) from methanogenic archaea in comparison with the exchange activities of [FeFe] and [NiFe] hydrogenases. *J. Biol. Inorg. Chem.* 13:97–106
116. Welte C, Kallnik V, Grapp M, Bender G, Ragsdale S, Deppenmeier U. 2010. Function of Ech hydrogenase in ferredoxin-dependent, membrane-bound electron transport in *Methanosarcina mazei*. *J. Bacteriol.* 192:674–80
117. Wolin MJ. 1981. Fermentation in the rumen and human large intestine. *Science* 213:1463–68
118. Zimmerman SA, Ferry JG. 2006. Proposal for a hydrogen bond network in the active site of the prototypic γ-class carbonic anhydrase. *Biochemistry* 45:5149–57
119. Zinder S. 1993. Physiological ecology of methanogens. In *Methanogenesis*, ed. JG Ferry, pp. 128–206. New York: Chapman & Hall