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Metabolic versatility in methanogens

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Methanogenesis is an anaerobic metabolism responsible for the generation of >90% of the methane formed on Earth today, with important implications for fuels production and global warming. Although methanogenic Archaea have been cultured for over 70 years, key insights regarding electron flow and energy conservation in methanogenesis have only recently emerged. Fundamental differences between two metabolic types of methanogenesis, hydrogenotrophic and methylotrophic, are now understood, with implications for metabolic versatility and the potential for engineering of methanogens to utilize new substrates. The development of model species with genetic and bioinformatic tools has advanced the field and holds potential for further characterizing and engineering of methanogenesis. Our understanding of a related pathway, anaerobic methane oxidation, is in its infancy.

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Introduction

Methanogenesis is a form of anaerobic respiration that is responsible for the global generation of approximately one billion tons of methane annually. The organisms that carry out this process, the methanogens, comprise a phylogenetically diverse group of *Euryarchaeota*, and can be classified into two groups, the hydrogenotrophs and the methylotrophs [1•]. The hydrogenotrophs are represented by five orders [1–3] and nearly all species rely on the reduction of CO_2 to CH_4. H_2 is the dominant electron donor but certain other electron sources can be used as well. The methylotrophs are represented by the order *Methanosarcinales*. In addition, a seventh order of methanogens has been proposed [4–6]. Many methylotrophs are capable of reducing CO_2 to CH_4, but the group is characteristically able to generate CH_4 from a variety of methyl compounds, or the methyl group of acetate. CO is a substrate as well. Hydrogenotrophic methanogenesis may be evolutionarily ancient, possibly having evolved around the same time as the emergence of life on Earth, while methylotrophic methanogenesis is thought to be evolutionarily recent, possibly having evolved within the last 500 million years [7–9]. Hydrogenotrophic and methylotrophic methanogenesis are additionally distinguished by their mechanisms of energy conservation, as described below.

Pathways of methanogenesis

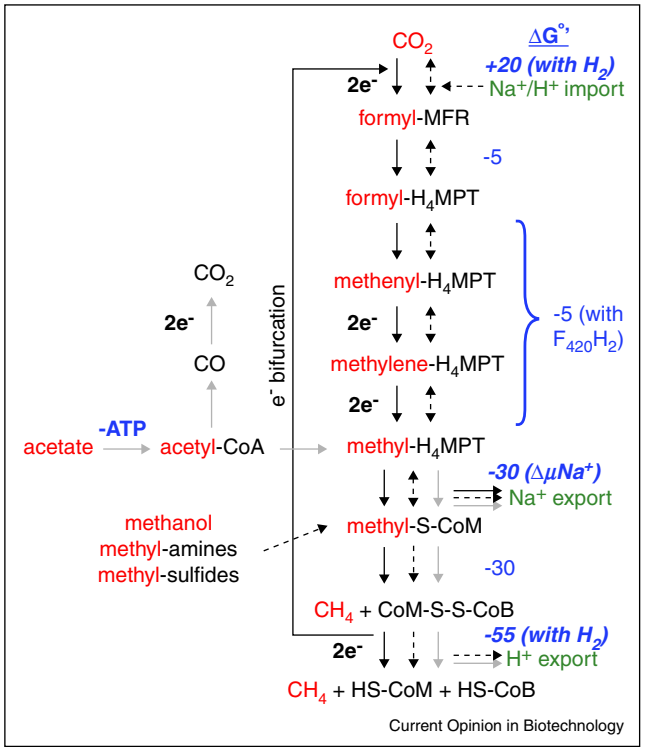
Methanogenesis can occur from CO_2, methyl compounds, or acetate. The pathways are illustrated in Figure 1. In methanogenesis from CO_2, first CO_2 is reduced to a formyl group covalently attached to the carrier molecule methanofuran (MFR). The formyl group is then transferred to the carrier tetrahydromethanopterin (H_4MPT) and a dehydration results in cyclization to generate methenyl-H_4MPT. The methenyl group undergoes a reduction to a methylene group, then another reduction to a methyl group. The methyl group is then transferred to the third carrier — the sulfhydryl-containing coenzyme M (HS-CoM) — concomitant with the export of Na^+ across the cell membrane [10]. The methyl group is finally reduced to CH_4 by the oxidation of HS-CoM together with another sulfhydryl-containing coenzyme, coenzyme B (HS-CoB), generating the heterodisulfide CoM-S-S-CoB. The heterodisulfide is then reduced to regenerate HS-CoM and HS-CoB.

In methanogenesis from methanol, methylamines, or methylsulfides, the substrates enter the pathway as methyl-S-CoM (Figure 1). Electrons for reduction of methyl-S-CoM to CH_4 come either from H_2, or from the oxidation of another methyl-S-CoM to CO_2 (methyl disproportionation). In methanogenesis from acetate, the methyl group enters the pathway as methyl-H_4MPT, and the carboxyl carbon is oxidized to provide electrons for methyl reduction (aceticlastic methanogenesis). CO can be oxidized to CO_2, providing electrons for methanogenesis, or can be converted to acetyl-CoA, leading to a partially acetogenic metabolism [11–13].

Hydrogenotrophic methanogenesis

Although some methylotrophic methanogens can use H_2, the term hydrogenotrophic refers to those methanogens that are restricted to CO_2 reduction using H_2 or certain alternative electron donors. Recent work has shown that hydrogenotrophic methanogens use electron bifurcation as a primary means of energy coupling. Increasingly recognized as an important mechanism in anaerobes, electron bifurcation couples the reduction of a high-potential

Figure 1



Pathways of methanogenesis. Reactions of hydrogenotrophic (solid black lines), methylotrophic (dashed black lines), and aceticlastic (grey lines) methanogenesis are shown. All pathways share the reductions of methyl-S-CoM and CoM-S-S-CoB. In methylotrophic methanogenesis, four methylated compounds enter at the level of methyl-S-CoM and one is oxidized to generate the electrons for the reduction of the other three. In aceticlastic methanogenesis, the methyl carbon of acetate enters at the level of methyl-H_4MPT and is reduced to methane using electrons from the oxidation of the carbonyl carbon. The number of electrons transferred in each redox reaction is listed next to the appropriate arrows. Numbers in blue are the energy yield of each reaction ($\Delta G^{\circ\prime}$, kJ/mol). Bold italicized numbers are for reactions important to energy conservation or energy depletion. MFR, methanofuran; H_4MPT, tetrahydromethanopterin; HS-CoM, coenzyme M; HS-CoB, coenzyme B; $F_{420}H_2$, reduced form of the electron carrying coenzyme F_{420}. ΔG values are from [1•,15••]. Figure adapted from [46].

substrate to the reduction of a low-potential substrate, conserving the energy from the former reduction to drive the latter reduction [1,14•,15••,16,17•]. In the case of hydrogenotrophic methanogenesis, electron bifurcation couples the final, heterodisulfide-reducing step to the initial reduction of CO_2 to formyl-MFR (Figure 1). The pathway based on electron bifurcation centers around the heterodisulfide reductase (Hdr) complex and is illustrated in Figure 2. Although the mechanistic details of electron bifurcation are unknown, a model is proposed in which electrons from hydrogen (or another electron donor such as formate) reduce a flavin contained in Hdr to generate $FADH_2$. This fully-reduced flavin is then oxidized stepwise, to the semiquinone FADH and then to the fully oxidized FAD, sending electrons equally to two reduction steps: the exergonic reduction of the heterodisulfide CoM-S-S-CoB to regenerate HS-CoM and HS-CoB, and the endergonic reduction of a ferredoxin (Fd). The reduced Fd is used in turn to reduce CO_2 to formyl-MFR. This coupling renders methanogenesis cyclic, and the pathway was recently named the Wolfe Cycle in honor of the many contributions of Ralph Wolfe to its elucidation [17•,18••]. In addition to the stoichiometric electron requirement for the reduction steps of the pathway, an anaplerotic supply of electrons is required at the CO_2 reduction step [18••]. A membrane-associated, energy-converting hydrogenase that consumes chemiosmotic membrane potential supplies these electrons through Fd reduction.

Methylotrophic methanogenesis

By contrast to hydrogenotrophic methanogenesis, electron bifurcation is not an integral part of methylotrophic methanogenesis [1•,19]. Instead, heterodisulfide reduction occurs via a membrane-bound electron transport chain that exports protons, generating a chemiosmotic membrane potential (Figure 1). When CO_2 is the carbon substrate, electrons for its reduction come entirely from a membrane-associated, energy-converting hydrogenase, at the expense of chemiosmotic membrane potential.

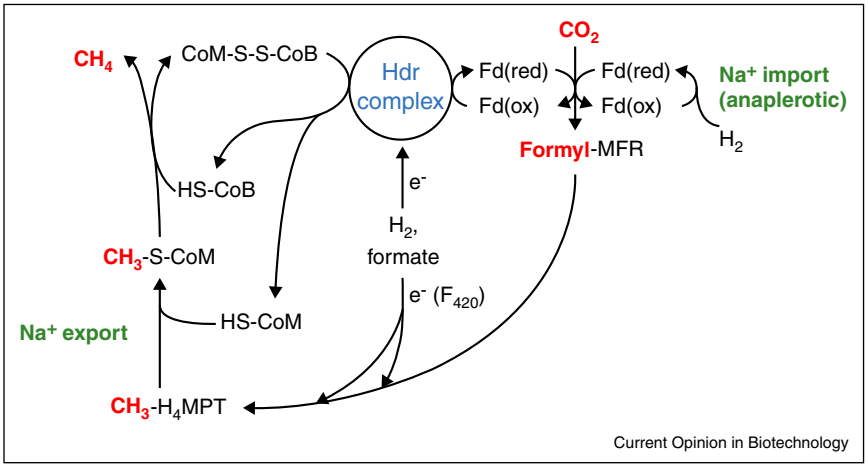
Reverse methanogenesis

In the last several years, a group of Archaea related to methanogens, the anaerobic methanotrophs (ANME), have been identified and implicated in the anaerobic oxidation of methane (AOM) to CO_2. Genome sequences suggest that the ANME organisms contain nearly all the enzymes of the methanogenic pathway and apparently run methanogenesis in reverse [20,21]. Although there is little data regarding the biochemistry of reverse methanogenesis, in one class of ANME organisms, methyl-S-CoM reductase has posttranslational modifications and a modified cofactor that are not found in the enzyme from methanogens [22]. How anaerobic methane oxidation is coupled to energy conservation is not understood. It was thought that AOM was dependent on the association of ANME organisms with bacterial partners that render the pathway thermodynamically feasible by reducing sulfate or nitrate. However, recent evidence suggests that in some cases AOM coupled to sulfate or nitrate reduction can be carried out solely by the ANME organism [21,23]. Although the study of ANME is in its infancy, these organisms highlight another of the diverse ways the methanogenic pathway can function.

Techniques in molecular biology for characterizing and engineering methanogens

Genetic techniques have been instrumental in elucidating the pathways of methanogenesis that occur *in vivo*. Currently, tools for introduction of DNA, genetic selection, and protein expression exist for two groups of methanogens: the *Methanosarcinales* and the *Methanococcales*, with a focus on species of *Methanosarcina* and *Methanococcus*

Figure 2



The Wolfe cycle of hydrogenotrophic methanogenesis. Electron bifurcation occurs at the heterodisulfide reductase (Hdr) complex, reducing the heterodisulfide CoM-S-S-CoM and ferredoxin (Fd). Reduced Fd is used for CO_2 reduction in the first step, rendering the pathway cyclical. A separate flow of electrons through F_{420} provides for intermediate reduction steps in the pathway.

(reviewed in [24,25]). In addition to transformation, conjugal transfer of DNA from *Escherichia coli* into *Methanococcus maripaludis* [26] was shown, suggesting that interdomain conjugal DNA transfer may be useful to genetically manipulate other methanogens that lack established genetic systems. Recently a transposon library was constructed for *M. maripaludis*, and for the first time a whole-genome functionality screen was conducted on a member of the methanogens [27•]. The ability to easily eliminate genes and screen phenotypes in both hydrogenotrophic and methylotrophic methanogens continues to show promise for the characterization of methanogenesis.

A broad understanding of methanogenesis depends on the development of accurate models for metabolism and gene regulation. Although it is difficult to map metabolic networks from a simple genetics approach, several models have been developed *in silico* describing how the genes and proteins important for methanogenesis from a variety of substrates interact. For *Methanosarcina* species, genome-scale metabolic models have been constructed that accurately predict growth and metabolic flux [28•,29], and in both *Methanosarcina* species and *M. maripaludis*, accurate models of metabolism during syntrophic growth have been established [30,31]. Also in *M. maripaludis*, operon structure has been determined over the entire genome, and an *e*nvironment and *g*ene *r*egulatory *i*nfluence *n*etwork (EGRIN) has been constructed using simple transcriptomic datasets to generate hypotheses regarding the regulation of various genes important to metabolism [32,33•]. The EGRIN model has already identified two transcription factors important for the regulation of core metabolic genes, and generates many more hypotheses about the interactions of other regulatory modules. Using these models to understand metabolic flux and its regulation will aid future engineering efforts with methanogens.

Pathways for alternative electron donors in methanogenesis: the hydrogenotrophs

Hydrogenotrophic methanogens are almost exclusively restricted to CO_2 as the carbon substrate for methanogenesis. This may be the result of the cyclical nature of the pathway (Figure 2). Electron bifurcation couples heterodisulfide reduction to the generation of low-potential electrons in the form of reduced Fd, and the necessity to productively utilize this reduced Fd to reduce CO_2 to formyl-MFR may limit the range of carbon substrates that may be used for methanogenesis. If CO_2 enters the pathway each time heterodisulfide reduction occurs, the introduction of additional C1 substrates would produce a surfeit of intermediates. Only one species, *Methanosphaera stadtmanae*, is thought to use reduced Fd other than to reduce CO_2 to formyl-MFR. In this case, H_2 is apparently produced from reduced Fd by an energy-converting hydrogenase, generating a chemiosmotic membrane potential, and allowing the organism to grow on H_2 and methanol. Whether hydrogenotrophic methanogens could be engineered to use an expanded range of carbon substrates for methanogenesis depends on whether a similar use of reduced Fd is feasible.

Although hydrogenotrophic methanogens may be metabolically restricted with regard to carbon substrates, recent work has shown that they are versatile in their use of electron donors. All species can use H_2, but many can substitute formate, and a few can use certain alcohols. Recent work has elucidated how electron donors enter

the pathway. First, the electron donor must reduce the electron carrier, coenzyme F_{420}, to generate $F_{420}H_2$, the reductant for the second (methenyl-H_4MPT-reducing) and third (methylene-H_4MPT-reducing) steps [1•]. Hydrogenases, formate dehydrogenases, and alcohol dehydrogenases that reduce F_{420} are known. Second, the electron donor must deliver electrons to the electron bifurcating center of the Hdr complex, which then provides electrons for the first (CO_2-reducing) and last (heterodisulfide-reducing) steps of the pathway. Previously $F_{420}H_2$ was thought to generate H_2, which then delivered electrons to Hdr via an Hdr-associated hydrogenase. However, it has now been shown that, at least in the case of formate, H_2 is not an essential intermediate, and formate dehydrogenase associates directly with Hdr [14•,18••,34•,35,36]. Any additional electron donors engineered for hydrogenotrophic methanogenesis would ideally reduce F_{420} and reduce Hdr, or be converted into a substrate that does.

In addition to the stoichiometric requirements, an anaplerotic input of electrons is required at the CO_2 reduction step to sustain the methanogenic cycle [18••]. This is provided by the membrane bound hydrogenase Eha, which uses H_2 to reduce Fd. However, H_2 is unnecessary even as an anaplerotic electron source if there is another means to reduce Fd [34•]. Recently CO was shown to serve in this capacity, and is thus a supplemental electron donor for hydrogenotrophic methanogenesis. In addition, although electron flow does not normally occur between F_{420} and Fd in hydrogenotrophic methanogenesis, a pathway was discovered that enables this to happen [34•]. NADPH produced from $F_{420}H_2$ reduces 1,3-diphosphoglycerate to glyceraldehyde-3-phosphate, which is then oxidized to 3-phosphoglycerate with the reduction of Fd. 1,3-Diphosphoglycerate is regenerated from 3-phosphoglycerate with the expenditure of ATP, completing the glyceraldehyde-3-phosphate:Fd oxidoreductase (GAPOR) cycle [37].

CH_4 generation from CO_2 can also occur when electrical current is supplied to cells: *Methanobacterium palustre* generates methane from the direct application of electrical energy [38]. Hence, although hydrogenotrophic methanogens were traditionally thought to utilize H_2 as the sole electron donor for CO_2 reduction to methane, recent studies demonstrate that hydrogenotrophs possess their own kind of metabolic diversity: broad utilization of electron donors other than H_2.

Pathways for alternative carbon donors in methanogenesis: the methylotrophs

By contrast to hydrogenotrophic methanogens, the pathway of methylotrophic methanogens is linear, and C1 substrates can in principle enter the pathway at any level of reduction. In addition to the long-known substrates methanol, methylamines, methylsulfides, and acetate, N,N,N-trimethylglycine (glycine betaine), N,N,N-trimethylethanolamine (choline) and N,N-dimethylethanolamine were recently reported as substrates for certain members of the Methanosarcinales [39,40]. The use of CO by *Methanosarcina acetivorans* in a partially acetogenic and formigenic metabolism further highlights the versatility of the methylotrophic methanogens [11–13]. The ability of methylotrophic methanogens to utilize acetate and methyl compounds suggests possibilities for the engineering of novel pathways of methanogenesis. Any pathway that produces acetate should theoretically generate CH_4 as a final product. For example, *Methanosarcina barkeri* can produce acetate from pyruvate fermentatively, and can produce CH_4 from acetate [41]. Indeed, *M. acetivorans* was recently engineered to contain a bacterial esterase resulting in the capacity to generate methane from the methyl groups of methyl-acetate and methyl-propionate [42••]. In the case of methyl-acetate, the acetate byproduct was then used in the aceticlastic reaction to produce methane.

The ability to engineer methylotrophs to use organics more complex than acetate raises the question: can the capacity to utilize large organic molecules like sugars be engineered in methanogens? CH_4 generation from glucose is thermodynamically favorable ($\Delta G^{\circ\prime} = -16.8$ kJ per mole electrons), but not as favorable as glucose fermentation to ethanol ($\Delta G^{\circ\prime} = -56.5$ kJ per mole electrons), suggesting an explanation for why this metabolism is not naturally occurring: methanogens generating CH_4 from glucose would be outcompeted by fermenters [43,44]. However, methanogenesis from glucose could be engineered by introducing systems for glucose transport, activation [45], and oxidation to pyruvate.

Conclusion

Methanogenic Archaea are capable of generating methane from a greater variety of small molecules than previously appreciated. In hydrogenotrophic methanogenesis, while the coupling of the heterodisulfide and CO_2 reducing steps may exclude other carbon substrates, alternative electron donors should be possible as long as the capacity exists to reduce F_{420}, the flavin of Hdr, and Fd. In the methylotrophs, the ability of methyl groups and acetate to enter the methanogenic pathway suggests possibilities for engineering the capacity to oxidize large organic molecules. Future efforts may aim to increase the already surprisingly broad substrate repertoire of these organisms, and increasingly accurate metabolic models will aid in directing flux through metabolic pathways of interest. Contrary to what was believed only a decade ago, the prospects for characterizing and engineering novel metabolic substrates for methanogenesis are bright.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R: **Methanogenic archaea: ecologically relevant differences in energy conservation**. *Nat Rev Microbiol* 2008, **6**:579-591.

Outlines the differences in energy conservation in hydrogenotrophic and methylotrophic methanogens, and their ecological consequences.

2. Sakai S, Takaki Y, Shimamura S, Sekine M, Tajima T, Kosugi H, Ichikawa N, Tasumi E, Hiraki AT, Shimizu A, Kato Y *et al.*: **Genome sequence of a mesophilic hydrogenotrophic methanogen *Methanocella paludicola*, the first cultivated representative of the order Methanocellales**. *PLoS One* 2011, **6**:e22898.

3. Sakai S, Imachi H, Hanada S, Ohashi A, Harada H, Kamagata Y: ***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* 2008, **58**:929-936.

4. Borrel G, Harris HM, Tottey W, Mihajlovski A, Parisot N, Peyretaillade E, Peyret P, Gribaldo S, O'Toole PW, Brugere JF: **Genome sequence of "candidatus *Methanomethylophilus alvus*" MX1201, a methanogenic archaeon from the human gut belonging to a seventh order of methanogens**. *J Bacteriol* 2012, **194**:6944-6945.

5. Borrel G, O'Toole PW, Harris HM, Peyret P, Brugere JF, Gribaldo S: **Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis**. *Genome Biol Evol* 2013, **5**:1769-1780.

6. Paul K, Nonoh JO, Mikulski L, Brune A: **"Methanoplasmatales," thermoplasmatales-related Archaea in termite guts and other environments, are the seventh order of methanogens**. *Appl Environ Microbiol* 2012, **78**:8245-8253.

7. Fournier GP, Gogarten JP: **Evolution of acetoclastic methanogenesis in *Methanosarcina* via horizontal gene transfer from cellulolytic clostridia**. *J Bacteriol* 2008, **190**:1124-1127.

8. Martin WF: **Hydrogen, metals, bifurcating electrons, and proton gradients: the early evolution of biological energy conservation**. *FEBS Lett* 2012, **586**:485-493.

9. Sousa FL, Thiergart T, Landan G, Nelson-Sathi S, Pereira IA, Allen JF, Lane N, Martin WF: **Early bioenergetic evolution**. *Philos Trans R Soc Lond B Biol Sci* 2013, **368**:20130088.

10. Gottschalk G, Thauer RK: **The Na^+-translocating methyltransferase complex from methanogenic archaea**. *Biochim Biophys Acta* 2001, **1505**:28-36.

11. Rother M, Metcalf WW: **Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon**. *Proc Natl Acad Sci U S A* 2004, **101**:16929-16934.

12. Oelgeschlager E, Rother M: **Carbon monoxide-dependent energy metabolism in anaerobic Bacteria and Archaea**. *Arch Microbiol* 2008, **190**:257-269.

13. Lessner DJ, Li L, Li Q, Rejtar T, Andreev VP, Reichlen M, Hill K, Moran JJ, Karger BL, Ferry JG: **An unconventional pathway for reduction of CO_2 to methane in CO-grown *Methanosarcina acetivorans* revealed by proteomics**. *Proc Natl Acad Sci U S A* 2006, **103**:17921-17926.

14. • Costa KC, Wong PM, Wang T, Lie TJ, Dodsworth JA, Swanson I, Burn JA, Hackett M, Leigh JA: **Protein complexing in a methanogen suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase**. *Proc Natl Acad Sci U S A* 2010, **107**:11050-11055.

Identifies the protein complex linking steps of methanogenesis by electron bifurcation and establishes the basis for direct use of formate as electron donor in hydrogenotrohic methanogenesis.

15. •• Kaster AK, Moll J, Parey K, Thauer RK: **Coupling of ferredoxin and heterodisulfide reduction via electron bifurcation in hydrogenotrophic methanogenic Archaea**. *Proc Natl Acad Sci U S A* 2011, **108**:2981-2986.

Biochemical demonstration that electron bifurcation occurs in a hydrogenotrophic methanogen.

16. Thauer RK, Kaster AK, Goenrich M, Schick M, Hiromoto T, Shima S: **Hydrogenases from methanogenic Archaea, nickel, a novel cofactor, and H_2 storage**. *Annu Rev Biochem* 2010, **79**:507-536.

17. • Thauer RK: **The Wolfe cycle comes full circle**. *Proc Natl Acad Sci U S A* 2012, **109**:15084-15085.

Provides a brief history of how the current model of hydrogenotrophic methanogenesis was developed.

18. •• Lie TJ, Costa KC, Lupa B, Korpole S, Whitman WB, Leigh JA: **Essential anaplerotic role for the energy-converting hydrogenase Eha in hydrogenotrophic methanogenesis**. *Proc Natl Acad Sci U S A* 2012, **109**:15473-15478.

Demonstrates the role of the energy converting hydrogenase Eha, and completes the model of the hydrogenotrophic methanogenic pathway.

19. Buan NR, Metcalf WW: **Methanogenesis by *Methanosarcina acetivorans* involves two structurally and functionally distinct classes of heterodisulfide reductase**. *Mol Microbiol* 2010, **75**:843-853.

20. Meyerdierks A, Kube M, Kostadinov I, Teeling H, Glockner FO, Reinhardt R, Amann R: **Metagenome and mRNA expression analyses of anaerobic methanotrophic Archaea of the ANME-1 group**. *Environ Microbiol* 2010, **12**:422-439.

21. Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW: **Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage**. *Nature* 2013, **500**:567-570.

22. Shima S, Krueger M, Weinert T, Demmer U, Kahnt J, Thauer RK, Ermler U: **Structure of a methyl-coenzyme M reductase from black sea mats that oxidize methane anaerobically**. *Nature* 2012, **481**:98-101.

23. Milucka J, Ferdelman TG, Polerecky L, Franzke D, Wegener G, Schmid M, Lieberwirth I, Wagner M, Widdel F, Kuypers MM: **Zero-valent sulphur is a key intermediate in marine methane oxidation**. *Nature* 2012, **491**:541-546.

24. Sarmiento F, Leigh JA, Whitman WB: **Genetic systems for hydrogenotrophic methanogens**. *Methods Enzymol* 2011, **494**:43-73.

25. Kohler PR, Metcalf WW: **Genetic manipulation of *Methanosarcina* spp.**. *Front Microbiol* 2012, **3**:259.

26. Dodsworth JA, Li L, Wei S, Hedlund BP, Leigh JA, de Figueiredo P: **Interdomain conjugal transfer of DNA from Bacteria to Archaea**. *Appl Environ Microbiol* 2010, **76**:5644-5647.

27. • Sarmiento F, Mrazek J, Whitman WB: **Genome-scale analysis of gene function in the hydrogenotrophic methanogenic archaeon *Methanococcus maripaludis***. *Proc Natl Acad Sci U S A* 2013, **110**:4726-4731.

The first analysis of gene essentiality across the genome of a methanogen.

28. • Gonnerman MC, Benedict MN, Feist AM, Metcalf WW, Price ND: **Genomically and biochemically accurate metabolic reconstruction of *Methanosarcina barkeri* Fusaro, iMG746**. *Biotechnol J* 2013, **8**:1070-1079.

An accurate, in-depth metabolic model for methanogenesis in a methylotroph.

29. Benedict MN, Gonnerman MC, Metcalf WW, Price ND: **Genome-scale metabolic reconstruction and hypothesis testing in the methanogenic archaeon *Methanosarcina acetivorans* C2A**. *J Bacteriol* 2012, **194**:855-865.

30. Stolyar S, Van Dien S, Hillesland KL, Pinel N, Lie TJ, Leigh JA, Stahl DA: **Metabolic modeling of a mutualistic microbial community**. *Mol Syst Biol* 2007, **3**:92.

31. Bizukojc M, Dietz D, Sun J, Zeng AP: **Metabolic modelling of syntrophic-like growth of a 1,3-propanediol producer, *Clostridium butyricum*, and a methanogenic archeon,**

***Methanosarcina mazei*, under anaerobic conditions**. *Bioprocess Biosyst Eng* 2010, **33**:507-523.

32. Yoon SH, Reiss DJ, Bare JC, Tenenbaum D, Pan M, Slagel J, Moritz RL, Lim S, Hackett M, Menon AL *et al.*: **Parallel evolution of transcriptome architecture during genome reorganization**. *Genome Res* 2011, **21**:1892-1904.

33. • Yoon SH, Turkarslan S, Reiss DJ, Pan M, Burn JA, Costa KC, Lie TJ, Slagel J, Moritz RL, Hackett M *et al.*: **A systems level predictive model for global gene regulation of methanogenesis in a hydrogenotrophic methanogen**. *Genome Res* 2013, **23**:1839-1851.
The first predictive model for metabolic gene regulation in a methanogen.

34. • Costa KC, Lie TJ, Jacobs MA, Leigh JA: **H_2-independent growth of the hydrogenotrophic methanogen *Methanococcus maripaludis***. *MBio* 2013, **4**.
Shows that hydrogenotrophic methanogenesis does not require H_2.

35. Lupa B, Hendrickson EL, Leigh JA, Whitman WB: **Formate-dependent H_2 production by the mesophilic methanogen *Methanococcus maripaludis***. *Appl Environ Microbiol* 2008, **74**:6584-6590.

36. Costa KC, Lie TJ, Xia Q, Leigh JA: **VhuD facilitates electron flow from H_2 or formate to heterodisulfide reductase in *Methanococcus maripaludis***. *J Bacteriol* 2013, **195**:5160-5165.

37. Park MO, Mizutani T, Jones PR: **Glyceraldehyde-3-phosphate ferredoxin oxidoreductase from *Methanococcus maripaludis***. *J Bacteriol* 2007, **189**:7281-7289.

38. Cheng S, Xing D, Call DF, Logan BE: **Direct biological conversion of electrical current into methane by electromethanogenesis**. *Environ Sci Technol* 2009, **43**:3953-3958.

39. Watkins AJ, Roussel EG, Parkes RJ, Sass H: **Glycine betaine as a direct substrate for methanogens (*Methanococcoides* spp.)**. *Appl Environ Microbiol* 2014, **80**:289-293.

40. Watkins AJ, Roussel EG, Webster G, Parkes RJ, Sass H: **Choline and N,N-dimethylethanolamine as direct substrates for methanogens**. *Appl Environ Microbiol* 2012, **78**:8298-8303.

41. Bock AK, Schönheit P: **Growth of *Methanosarcina barkeri* (Fusaro) under nonmethanogenic conditions by the fermentation of pyruvate to acetate: ATP synthesis via the mechanism of substrate level phosphorylation**. *J Bacteriol* 1995, **177**:2002-2007.

42. •• Lessner DJ, Lhu L, Wahal CS, Ferry JG: **An engineered methanogenic pathway derived from the domains Bacteria and Archaea**. *MBio* 2010, **1**:e00243-e310.
Demonstrates the ability to engineer methylotrophs to utilize novel organic molecules for methanogenesis.

43. Zinder SH: **Physiological ecology of methanogens**. In *Methanogenesis: Ecology, Physiology, Biochemistry & Genetics.* Edited by Ferry JG. New York: Chapman & Hall; 1993:128-206.

44. McInerney MJ, Beaty PS: **Anaerobic community structure from a nonequilibrium thermodynamic perspective**. *Can J Microbiol* 1988, **34**:487-493.

45. Jahreis K, Pimentel-Schmitt EF, Bruckner R, Titgemeyer F: **Ins and outs of glucose transport systems in eubacteria**. *FEMS Microbiol Rev* 2008, **32**:891-907.

46. Galagan JE, Nusbaum C, Roy A, Endrizzi MG, Macdonald P, FitzHugh W, Calvo S, Engels R, Smirnov S, Atnoor D *et al.*: **The genome of *Methanosarcina acetivorans* reveals extensive metabolic and physiological diversity**. *Genome Res* 2002, **12**:532-542.