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Syntrophy in anaerobic global carbon cycles

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Syntrophy is an essential intermediary step in the anaerobic conversion of organic matter to methane where metabolically distinct microorganisms are tightly linked by the need to maintain the exchanged metabolites at very low concentrations. Anaerobic syntrophy is thermodynamically constrained, and is probably a prime reason why it is difficult to culture microbes as these approaches disrupt consortia. Reconstruction of artificial syntrophic consortia has allowed uncultured syntrophic metabolizers and methanogens to be optimally grown and studied biochemically. The pathways for syntrophic acetate, propionate and longer chain fatty acid metabolism are mostly understood, but key steps involved in benzoate breakdown and cyclohexane carboxylate formation are unclear. Syntrophic metabolism requires reverse electron transfer, close physical contact, and metabolic synchronization of the syntrophic partners. Genomic analyses reveal that multiple mechanisms exist for reverse electron transfer. Surprisingly, the flagellum functions were implicated in ensuring close physical proximity and synchronization of the syntrophic partners.

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Introduction

Syntrophy can mean any type of crossfeeding of molecules between microbial species whereby a more restricted definition is applied when discussing anaerobic syntrophic metabolism. Here, anaerobic syntrophy is defined as a thermodynamically interdependent lifestyle where the degradation of a compound such as a fatty acid occurs only when degradation end products, usually hydrogen, formate, and acetate, are maintained at very low concentrations. This typically occurs in cooperation with a second microorganism, usually a methanogen, that consumes the product with high affinity (Table 1). For example, the degradation of butyrate with hydrogen and acetate production is thermodynamically unfavorable unless these metabolites are maintained at very low levels by methanogens. This anaerobic metabolism, especially when methanogenesis is the driver of the terminal electron accepting reactions, often involves consortia with tightly coupled syntrophic partnerships [1,2•,3]. Syntrophic interactions also occur in sulfate-reducing environments as evidenced by sulfate-reducing consortia involved in anaerobic methane oxidation [4].

Anaerobic syntrophy differs from other types of microbial metabolism like aerobic fatty acid metabolism or denitrification in that a consortium of interacting microbial species rather than a single microbial species is needed to mineralize organic compounds [5]. A wide range of compounds including alcohols, fatty and aromatic acids, amino acid, sugars, and hydrocarbons are syntrophically degraded [2•,3]. Syntrophy is essential for the complete conversion of natural polymers such as polysaccharides, proteins, nucleic acids, and lipids to CO_2 and CH_4 (Figure 1) [6]. Initially, fermentative bacteria hydrolyze the polymeric substrates such as polysaccharides, proteins, and lipids, and ferment the hydrolysis products to acetate and longer-chain fatty acids, propionate, alcohols, CO_2, formate, and H_2. These products plus some amino acids and aromatic compounds are then syntrophically metabolized to the methanogenic substrates, H_2, formate, and acetate [2•,3]. Lastly, two different groups of methanogens, the hydrogenotrophic methanogens and the acetotrophic methanogens, complete the process by converting acetate, formate, and hydrogen produced by other microorganisms to methane and carbon dioxide.

Syntrophic fatty and aromatic acid metabolism accounts for much of the carbon flux in methanogenic environments [2•,3]. Many aromatic compounds are converted to benzoyl-CoA, which is further metabolized by syntrophic consortia [3]. Drake et al. [7] coined the term ''intermediary ecosystem metabolism'' analogous to intermediary cellular metabolism to emphasize the importance of the intermediate steps that occur after polymer hydrolysis as the main drivers of methanogenesis. Our knowledge of intermediary ecosystem metabolism is incomplete because we have only limited information of the *in situ* occurrence and activity of key players. This is, in part, due to the difficulty in culturing and studying microorganisms involved in syntrophic metabolism.

From a thermodynamic point of view, anaerobic syntrophy represents an extreme lifestyle [8••]. Even when

Table 1

Reactions involved in syntrophic metabolism.

Reactions	$\Delta G^{\circ\prime}$[a] (kJ mol^{-1})	$\Delta G'$[b] (kJ mol^{-1})
Hydrogenotrophic Methanogenesis		
$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	−135.6	−38.6
Syntrophic Oxidations		
Propionate$^-$ + 3 H_2O → Acetate$^-$ + HCO_3^- + H^+ + 3 H_2	+76.1	−1.5
Butyrate$^-$ + 2 H_2O → 2 Acetate$^-$ + H^+ + 2 H_2	+48.6	−31.2
Benzoate$^-$ + 7 H_2O → 3 Acetate$^-$ + HCO_3^- + 3 H^+ + 3 H_2	+70.1	−56.6

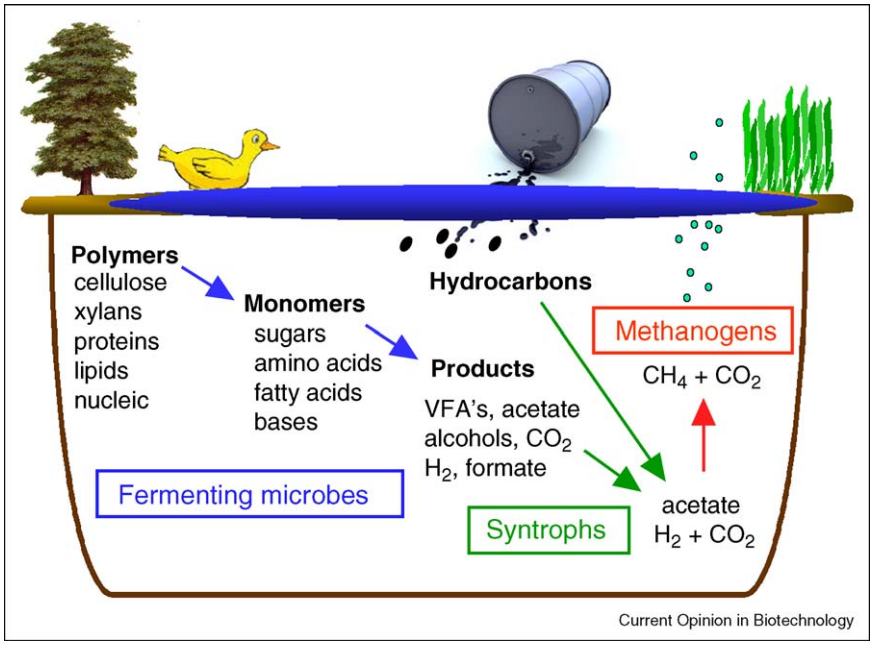
[a] Calculated from the data in Ref. [60].
[b] Calculated on the basis of the following conditions observed in methanogenic ecosystems: 10 Pa of H_2, 50 kPa of CH_4, 50 mM bicarbonate, 50 μM acetate, and 100 μM of each substrate.

hydrogen, formate, and acetate are low, the Gibbs free energy change for syntrophic metabolism is very close to the minimum increment of energy required for ATP synthesis, which is predicted to be about −15 to −20 kJ mol^{-1} [2•]. In some cases, syntrophic consortia grow at free energy changes of −10 kJ mol^{-1} or less [9•,10•]. Low energy yields mean that growth rates (<0.005 h^{-1}) and growth yields (2.6 g dry weight mole^{-1} propionate) are low [9•,10•]. Maintenance energy values for syntrophic metabolizers (0.1 to 7.5 kJ h^{-1} mol C^{-1}) are an order of magnitude below that predicted from the empirical relationship derived from maintenance energy values of diverse microorganisms grown at different temperatures [9•,10•]. The low maintenance energy requirements indicate that syntrophic bacteria are well adapted to an energetically stressed lifestyle. Mechanisms by which syntrophic consortia conserve energy when their thermodynamic driving force is very low are not well understood, but whole genome sequencing approaches are providing us with more insight into the metabolic capability of these organisms.

Syntrophy and culturing the uncultured

Only a small fraction of the total microbial community present in natural environments can be cultured [11]. Disruption of microbial consortia, by common isolation techniques, can cause difficulty when attempting to culture syntrophic metabolizers. This can be overcome by adding a pure culture of an established metabolic partner to isolation media in order to obtain a stable syntrophic coculture [12]. This approach has yielded some interesting surprises lately. The dominant sugar users in a lake sediment were not the typical fermentative anaerobes, but syntrophic metabolizers that could only be grown

Figure 1



Anaerobic routes for syntrophic decomposition of animal and plant derived biopolymers to methane, carbon dioxide, and water. Aromatic and aliphatic hydrocarbons are also used as syntrophic substrates (image credit, Saul Gravy/Photographer's Choice/Getty Images).

anaerobically and in co-culture with a hydrogen-using methanogen [13•]. Microorganisms that grow by syntrophic formate or methanol oxidation have also been obtained [14,15]. Members of the *Desulfotomaculum* cluster Ih are present in diverse methanogenic ecosystems including sediments, digestor sludges, and rice paddy soils [16••,17•,18]. Rather than using sulfate as an electron acceptor as suggested by their phylogeny, these organisms syntrophically metabolize propionate or aromatic acids in syntrophic association with methanogens [16••]. Stable isotope probing of paddy soils and freshwater marsh sediments implicated cluster Ih organisms (e.g., *Pelotomaculum* spp.) in the Firmicutes plus *Syntrophobacter* spp., and *Smithella* spp. in the Delta proteobacteria as propionate metabolizing syntrophs [17•,18]. 16S rRNA gene surveys and stable isotope labeling also associated new microbial lineages of Firmicutes and Delta proteobacteria with syntrophic fatty acid metabolism in digestor sludges [19•,20,21,22•]. In addition, non-acetogens (e.g., *Syntrophus* spp.) were linked to syntrophic acetate oxidation in freshwater marsh sediments [23]. Additional work is needed to confirm whether some of the microorganisms detected by cultivation-independent approaches are the primary syntrophic metabolizer or secondary consumers of carbon since pure culture representatives are not known to degrade these compounds syntrophically.

Many syntrophic methanogenic partners can also be difficult to culture (Figure 1). Culture-independent and stable isotopic analyses identified a novel lineage of methanogens called rice cluster I as the most active and abundant members of the methanogenic community in rice paddy soils [24]. Members of this group could not be cultured until an enrichment protocol was devised that included a syntrophic propionate degrader to allow continuous hydrogen production at very low partial pressures. This approach has also lead to the isolation of another novel methanogen [25••].

Genome sequences reveal unanticipated aspects of syntrophy

Recent genome sequencing analysis of model organisms provides insights into key biochemical aspects of the syntrophic lifestyle (Table S1). While the genome sizes are generally small, they suggest nutritional self-sufficiency with limited capacity for alternative metabolisms to either ferment or respire. Additionally, the genomes revealed unexpected features of metabolism such as multiple gene copies for many of the key enzymes for pathways leading to acetate formation from fatty and aromatic acids (see [8••,26•,27•] and genome sequences listed in Table S1). For instance, *Pelotomaculum thermopropionicum*, *Syntrophus aciditrophicus*, *Syntrophomonas wolfei* and *Syntrophobacter fumaroxidans* genomes contain multiple genes for fatty acid activation (acetyl-CoA synthetase (AMP-forming) genes) and β-oxidation (acyl-CoA dehydrogenase, enoyl-CoA dehydrogenase, and acetyl-CoA acetyltransferase (thiolase) genes) dispersed throughout the chromosome. *P. thermopropionicum* and *S. fumaroxidans* oxidize propionate by the methylmalonyl-CoA pathway (see below), but neither is known to use other fatty acids so the function of the β-oxidation genes in these two organisms is unclear. In contrast, *Escherichia coli* uses two sets of β-oxidation genes, one for aerobic and another for anaerobic fatty acid metabolism [28]. For each set, separate genes encode acetyl-CoA synthetase (AMP-forming), acyl-CoA dehydrogenase, and acetyl-CoA acetyltransferase (thiolase) activities, while a single gene encodes for enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities.

A key reaction during syntrophic metabolism is the electron transfer from $FADH_2$ and NADH to hydrogen or formate, which involves pathways independent from those used for fermentation and respiration. Genes for a membrane-bound, ion-translocating hydrogenase (*coo*), a high molecular weight *c*-type cytrochrome complex (*hmc*) and two hydrogenases (*hyd* and *hyn*) were upregulated during syntrophic compared to sulfate-limited growth of *Desulfovibrio vulgaris* with lactate. Mutations in these genes impaired or severely limited syntrophic but not sulfate-dependent growth [29••]. A membrane-bound, molybdopterin oxidoreductase is required for syntrophic, but not fermentative or respiratory growth of *Desulfovibrio desulfuricans* strain G20 [30]. *S. aciditrophicus* contains genes for a novel membrane Rnf-like complex suggestive of an ion-translocating NADH: ferredoxin oxidoreductase that may be involved in syntrophic electron transfer [8••]. *Pelobacter carbinolicus*, which grows syntrophically with alcohols, contains genes for this Rnf-like complex, but in *Pelobacter propionicus*, which does not grow syntrophically, these genes are absent [26•].

Unusual features of syntrophic carbon metabolism

Several strategies for syntrophic acetate and propionate metabolism exist [1,2•,31]. In each case end products such as formate or hydrogen are released for immediate removal by their syntrophic partner. *Geobacter sulfurreducens* oxidizes acetate by the tricarboxylic acid cycle [26•] while *Thermacetogenium phaeum* uses the Wood–Ljungdahl pathway [32]. Apparently, *T. phaeum* employs the same pathway for acetate synthesis and its oxidation as several key enzymes of this pathway (acetyl-CoA synthase, carbon monoxide dehydrogenase, and formate dehydrogenase) were detected under pure culture conditions and in coculture with methanogens. Thus, *T. phaeum* can switch from syntrophic acetate oxidation to homoacetogenic acetate formation [32]. The reversibility of this pathway suggests highly efficient energy conservation at near-equilibrium conditions. Syntrophic propionate degraders such as *Syntrophobacter* spp. and *P. thermopropionicum*

degrade propionate by the methylmalonyl-CoA pathway, which involves the activation of propionate to propionyl-CoA by a CoA transferase, and the cosynthesis of methylmalonyl-CoA from oxaloacetate by a transcarboxylase [1,2•,33]. Methylmalonyl-CoA is then rearranged to form succinyl-CoA, which is oxidized via fumarate, oxaloacetate and pyruvate to acetate. In contrast, the syntrophic propionate degrader *Smithella propionica* uses a newly discovered pathway to ferment propionate that includes the condensation of two molecules of propionate to form a six-carbon intermediate which is ultimately cleaved to form acetate and butyrate [34]. However, the intermediates and enzymes involved in this novel pathway are not yet known.

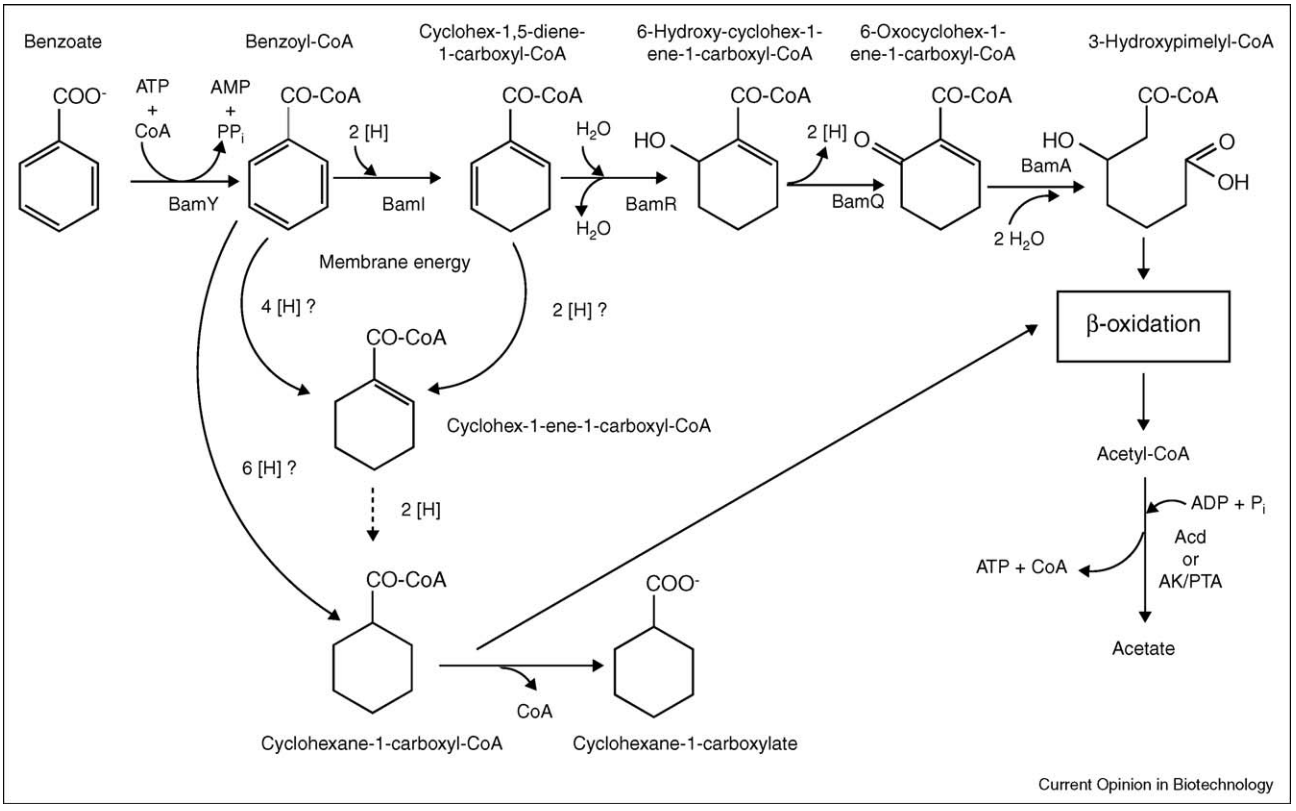
In *S. wolfei*, the β-oxidation of butyrate generates two acetyl-CoA molecules, one of which is used to make ATP by the action of phosphotransacetylase and acetate kinase [35]. The second acetyl-CoA is used for the activation of butyrate to butyryl-CoA by an energy-neutral transfer of the CoA group from acetyl-CoA. This is in contrast to the energy-intensive acetyl-CoA synthetase used by most bacteria, which hydrolyzes ATP to AMP and pyrophosphate. *S. aciditrophicus* has very low phosphotransacetylase and acetate kinase activities [36] and apparently forms ATP from ADP, phosphate and acetyl-CoA using a distinct acetyl-CoA synthetase. Nine genes for ADP-using, acetyl-CoA synthetases are present in the chromosome, which were apparently acquired by horizontal gene transfer from archaea [8••].

Syntrophic benzoate degradation (Table 1) is an enigma because it is unclear how known substrate-level phosphorylation and ion-translocating reactions provide sufficient energy for the activation of benzoate, the reduction of benzoyl-CoA, and the production of hydrogen or formate by reverse electron transfer. Yet the bacterium still generates sufficient net ATP to support growth. Some have argued that syntrophic benzoate reduction involves a four- or six-electron reduction that is energy yielding [37]. *Syntrophus aciditrophicus* transiently accumulates cyclohex-1-ene-1-carboxylate and up to 260 μM of cyclohexane carboxylate during syntrophic benzoate metabolism [36]. This intermediate could be formed by four- or six-electron reduction of benzoyl-CoA (Figure 2). However, genomic analyses of *S. aciditrophicus* revealed the presence of genes similar to those discovered in *Geobacter metallireducens*, [38] which are believed to encode for a novel type of benzoyl-CoA reductase. This enzyme probably requires membrane energy to reduce benzoyl-CoA to cyclohex-1,5-diene carboxyl-CoA rather than ATP as for the ATP-using benzoyl-CoA reductase found in denitrifiers and photosynthetic bacteria [8••,38] (Figure 2). A fluorinated metabolite with two double bonds, either 1-carboxyl-3-fluoro-2,6-cyclohexadiene or 1-carboxyl-3-fluoro-3,6-cyclohexadiene, was detected in fluorobenzoate-degrading cultures [39]. *S. aciditrophicus* contains enzymes needed to convert cyclohex-1,5-diene carboxyl-CoA to 6-hydroxycyclohex-1-ene carboxyl-CoA, and for 6-oxocyclohex-1-ene carboxyl-CoA conversion to 3-hydroxypimelyl-CoA [40•,41]. Thus, it appears that *S. aciditrophicus* uses an energy-intensive, two-electron reduction reaction to convert benzoyl-CoA to cyclohex-1,5-diene carboxyl-CoA. The latter is then metabolized via 6-oxocyclohex-1-ene carboxyl-CoA to 3-hydroxypimelyl-CoA (Figure 2). It is not clear how net energy is conserved during syntrophic benzoate metabolism.

In addition to the bioenergetic enigma of energy acquisition, there is still much that we do not understand regarding benzoate metabolism in *S. aciditrophicus*. It is unclear why cyclohexane carboxylate accumulates to a high concentration during syntrophic benzoate utilization [36]. In pure culture, *S. aciditrophicus* can ferment benzoate to acetate and cyclohexane carboxylate [42] and use benzoate as an electron acceptor to form cyclohexane carboxylate with crotonate as the electron donor [43•]. The type of enzymatic machinery needed for alicyclic acid formation in *S. aciditrophicus* is unknown as is the process for its regulation. Another interesting feature of *S. aciditrophicus* is its ability to form cyclohexane carboxylate when grown with crotonate alone [44]. *S. aciditrophicus* uses a previously undiscovered pathway for the synthesis of cyclohexane carboxylate from acetate intermediates derived from crotonate, likely by reversing the route used for anaerobic benzoate oxidation. Previously, the only known way to form cyclohexane carboxylate was by the dehydration and reduction of shikimate, the classic precursor used for the formation of aromatic amino acids. The mechanisms used to form cyclohexane carboxylate by *S. aciditrophicus* maybe similar to those used to form naphthoic acids that are commonly detected in many biodegraded oils and petroleum contaminated sites [45,46].

Syntrophy is also important in controlling the flux of methane from gas hydrates and the quality of the Earth's oil resources. In sulfate-reducing sediments, anaerobic methane-oxidizing archaea frequently form tightly linked consortia with sulfate-reducing Delta proteobacteria [4]. The quantitative importance of syntrophy is illustrated by the presence of heavy oil, extra heavy oil and bitumen deposits, which comprise about 70% of the world's oil resources. These deposits were formed after syntrophic methanogenic consortia metabolized the lighter alkane and aromatic fractions [45,47,48••]. Recently, a syntrophic hydrocarbon-degrading bacterium, *Desulfoglaeba alkanexedens* was isolated from oil storage and production facilities [49]. *D. alkanexedens* can also grow in pure culture by sulfate-dependent alkane oxidation. Other studies implicate *Syntrophus* spp. in methanogenic alkane degradation [48••,50]. Syntrophic bacteria related to those in *Syntrophomonaceae* and in the phylum Synergistetes accounted for 27% of the bacterial phylotypes detected in fluids

Figure 2



Proposed pathway for syntrophic benzoate metabolism adapted from McInerney et al. [8••]. The enzymes involved are: BamY, benzoyl-CoA ligase; BamI, benzoyl-CoA reductase; BamR, cyclohex-1,5-diene-1-carboxyl-CoA hydratase; BamQ, 6-hydroxycyclohexane-1-carboxyl-CoA dehydrogenase; BamA, 6-oxocyclohexane-1-carboxyl-CoA hydrolase. Gene product designations are from [38,40•,41]. Reactions with question marks are postulated routes for cyclohex-1-ene-1-carboxyl-CoA and cyclohexane carboxyl-CoA formation, but enzymatic evidence is lacking. ATP synthesis from acetyl-CoA could occur by archaeal-like acetyl-CoA synthetases (ADP-forming) (Acd) or by enzymes similar to phosphotransacetylase (Pta) and acetate kinase (AK). Reducing equivalents, [H].

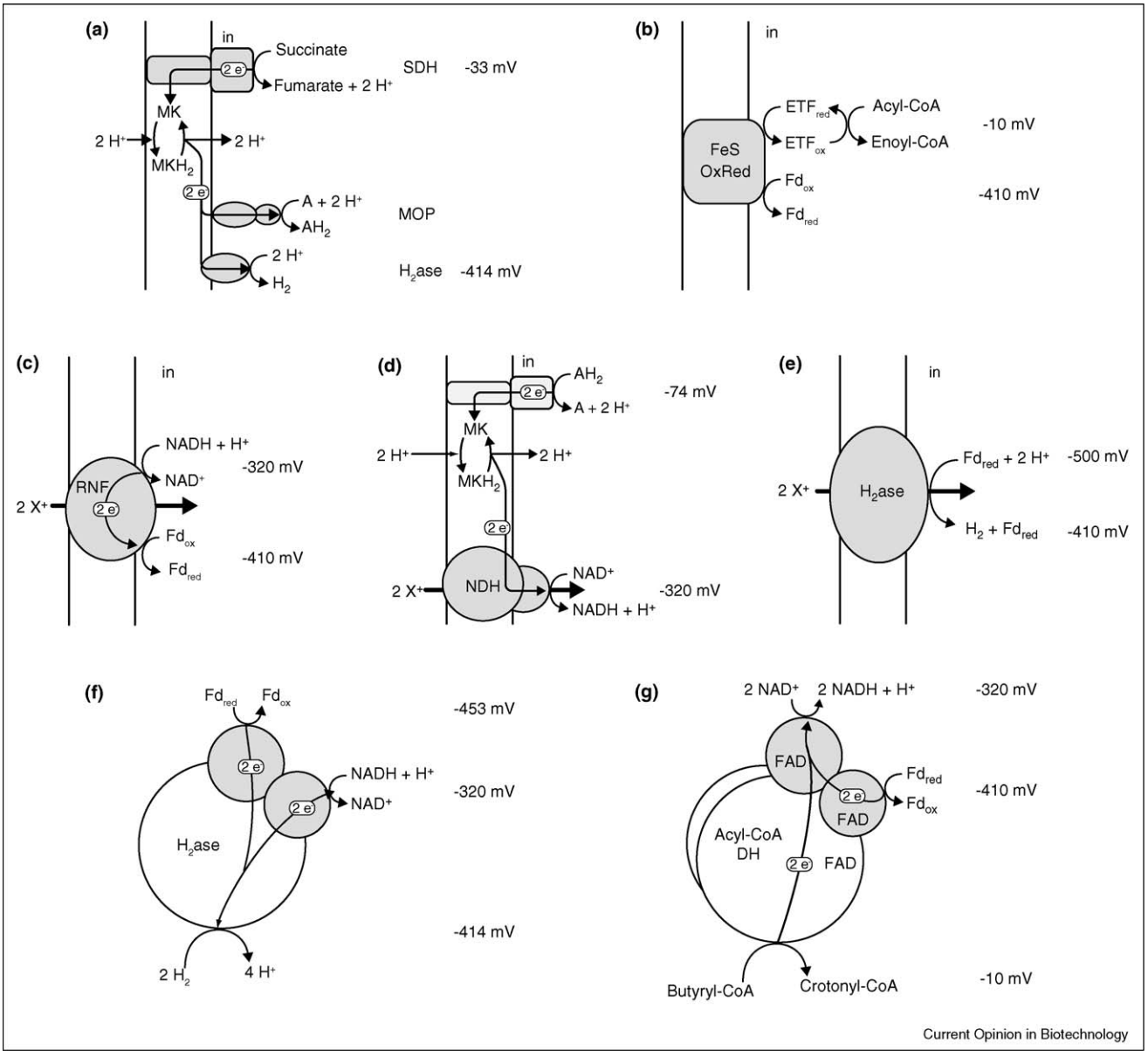
extracted from an off shore oil reservoir [51]. Introduction of syntrophic alkane degrading consortia to oil reservoirs may be a novel approach to recover the energy of entrapped hydrocarbons in the form of methane [52•].

Multi-species interactions

Many syntrophic consortia form highly organized, multicellular structures with the partners in close physical proximity to each other [1]. Filamentous structures connecting the syntrophic partners have been observed by electron microscopy [53•,54]. Scanning tunneling microscopy showed that these structures were electron transmissive in *P. thermopropionicum*, suggesting that they act as nanowires that transfer electrons directly between partners without the need for interspecies hydrogen or formate transfer [53•]. These syntrophic metabolizers contain genes for flagella and pili, but lack the *pilA* gene that encodes for the nanowire [55]. On the other hand, they have multiple formate dehydrogenase and hydrogenase genes and, in contrast to *Geobacter* spp. and *Shewnella* spp., they lack the genes for the inner and outer membrane cytochromes believed to be needed to transfer electrons to nanowires [8••,26•,27•]. Fluorescence microscopy using antibodies directed against the FliC (flagellin protein) of *P. thermopropionicum* showed that the filaments observed in *P. thermopropionicum*- *Methanothermobacter thermoautotrophicus* cocultures were flagella [56••]. The addition of FliD (flagellar cap protein), but not FliC, of *P. thermopropionicum* to pure cultures of *M. thermoautotrophicus* accelerated methanogenesis and altered the expression of about 50 *M. thermoautotrophicus* genes [56••]. The function of the flagellum in syntrophy seems to ensure close physical proximity and to synchronize the metabolism of the syntrophic partners. These studies and others [1,2•] argue for the importance of hydrogen and formate transfer rather than electron conductivity in syntrophic metabolism.

How do the syntrophic partners sense their surroundings and regulate their metabolism? Many genes with PAS domains are physically linked with genes for important catabolic functions in *P. thermopropionicum*

Figure 3



Possible models for reversed electron transfer during syntrophic metabolism. A, Reverse quinone loop; B, Iron-sulfur oxidoreductase; C, Rnf complex; D, NADH dehydrogenase Complex I (NDH); E, Ion-translocating hydrogenase complex; F, soluble, electron-bifurcating, butyryl-CoA dehydrogenase: electron transfer flavoprotein complex; and G, soluble, electron-bifurcating, NADH-oxidizing hydrogenase complex. Abbreviations: FAD, flavin adenine dinucleotide; Fd, ferredoxin, A and AH_2, oxidized and reduced forms of putative redox intermediates. Values are $E^{\circ\prime}$ in mV [60].

and *S. aciditrophicus* [8••,27•]. Genes with PAS domains are often used to sense environmental stimuli and their proximity to catabolic genes suggest important catabolic pathways may be regulated by environmental stimuli and/or global internal stimuli rather than by specific substrates [27•]. Codon usage analysis of diverse bacteria found that protein-coding sequences of *P. thermopropionicum* grouped with more distantly related phylogenic groups of syntrophic metabolizers including *Syntrophomonas wolfei*, *S. aciditrophicus* and *Desulfovibrio desulfuricans* but not with sugar-using Fimicutes. This suggests that syntrophic metabolizers may have evolved by interacting with niche-associated microbes [27•].

Bioenergetics and reverse electron transfer

Syntrophic metabolism involves production of hydrogen (E' of $\sim$ -294 mV at 1 Pa H_2) or formate ($E'$of -288 mV at 10 μM formate) from high potential electron donors such as acyl-CoA intermediates (E' of -10 mV) or succinate

(E' of +33 mV). Such redox reactions are thermodynamically unfavorable, e.g., large negative $\Delta E'$ changes, and can occur only with energy input by a process called reverse electron transfer [1,8••]. Several studies have demonstrated that hydrogen production from butyrate, benzoate and glycolate required ATP input or the presence of a proton gradient [1,2•]. From these studies, it is clear that reverse electron transfer is needed but the biochemical machinery involved has not been elucidated. Multiple mechanisms for reverse electron transfer during syntrophic metabolism have been deduced from genomic analyses (Figure 3) [1,8••,27•]. *S. wolfei*, *S. aciditrophicus*, *P. thermopropionicum* and *Syntrophobacter fumaroxidans* contain menaquinones, which could function as the electron carrier between a membrane-associated acyl-CoA dehydrogenase or succinate dehydrogenase complexes and membrane-associated hydrogenases, formate dehydrogenases or other membrane redox complexes (Figure 3A) [1,8••,27•]. Mutation of a predicted, membrane-bound, molybdopterin oxidoreductase disrupted hydrogen oxidation and syntrophic growth of *Desulfovibrio desulfuricans* strain G20, possibly by disrupting electron flow to menaquinones [30]. Genes for electron transfer flavoproteins (ETF), which transfer electrons from acyl-CoA intermediates to membrane complexes, are adjacent to those for a predicted membrane-bound oxidoreductase in *S. aciditrophicus* (Figure 3B) [8••]. *S. aciditrophicus* and *P. carbinolicus* contain genes for a membrane-bound complex called Rnf, which could couple the unfavorable reduction of ferredoxin with NADH as the electron donor to the translocation of proton or sodium ions (Figure 3C) [8••,26•]. Other possibilities for reverse electron transfer include NADH dehydrogenase complex I and membrane-bound, ion-translocating hydrogenases found in *Geobacter sulfurreducens*, *Pelobacter carbinolicus*, and *Desulfovibrio desulfuricans* (Figure 3D and E) [26•].

While reverse electron transfer was thought to occur only in membrane respiratory chains, two soluble enzyme have been purified that use the energy of a favorable redox reaction to drive an unfavorable redox reaction by a process called electron bifurcation [57,58••] (Figure 3F and G). *Thermotoga maritimae* contains a hydrogenase complex that couples the favorable production of hydrogen from reduced ferredoxin with the unfavorable production of hydrogen from NADH (Figure 3G)(equation 1) [58••]. *S. wolfei* and *S. fumaroxidans* genomes contain homologs to this novel hydrogenase complex.

$$\mathrm{NADH} + \mathrm{Ferredoxin_{red}} + 3\mathrm{H^+} \rightarrow \mathrm{H_2} + \mathrm{NAD^+} + \mathrm{Fd_{ox}} \quad (1)$$

Another electron bifurcation reaction is documented in *Clostridium kluyveri* that ferments ethanol and acetate to butyrate and small amounts of H_2. A soluble enzyme complex in *C. kluyveri* couples the energetically favorable reduction of crotonyl-CoA to butyryl-CoA by NADH with the unfavorable reduction of ferredoxin (Fd) by NADH (Figure 3F) (equation 2) [57]:

$$\mathrm{Crotonyl\text{-}CoA} + \mathrm{Fd_{ox}} + 2\mathrm{NADH} + 2\mathrm{H^+} \rightarrow \mathrm{Butyryl\text{-}CoA} + \mathrm{Fd_{red}} + 2\mathrm{NAD^+} \quad (2)$$

The reverse of this reaction could be used to drive reverse electron transfer during syntrophic fatty acid oxidation. However, it is not clear how reduced ferredoxin is generated during syntrophic fatty acid metabolism. Recently, a NADH:acceptor oxidoreductase was partially purified from cell-free extracts of *S. wolfei* that functions as a bifurcating hydrogenase: butyryl-CoA dehydrogenase complex, avoiding the involvement of Fd as electron mediator [59].

Conclusions and biotechnological applications

Global cycling of carbon in anaerobic environments requires complex communities of metabolically coupled microorganisms that are highly adapted to their environmental niche. Relative to our current understanding of the biochemical pathways used by many aerobic microorganisms for carbon mineralization, little is yet known about the key steps in anaerobic food chains that require syntrophic metabolism. Aromatic ring reduction by syntrophic metabolizers and strict anaerobes involves a novel benzoyl-CoA reductase not found in aerobes or facultative anaerobes. Syntrophic aromatic metabolism also involves the formation of alicyclic compounds such as cyclohexane carboxylate by unknown enzymes. Syntrophic propionate metabolism occurs by the well-studied methyl-malonyl-CoA pathway found in many anaerobes, but also by a novel pathway involving the formation of a six-carbon intermediate by unknown reactions. Continued improvements in our ability to culture syntrophs, track metabolite fluxes, and to identify and characterize new biochemistries are beginning to provide a more comprehensive picture of intermediary ecosystem metabolism and the factors that control the flux of organic matter to methane. This information is critically needed if we are to accurately predict the consequences of global climate change and to develop new sustainable biofuel technologies. Recent advances in the study of model synthetic co-cultures using genomic, proteomic, and biochemical tools have revealed unanticipated features of metabolism and energy conservation. Important catabolic processes may be regulated by environmental and/or intercellular stimuli rather than by specific substrates. The flagellum may play an important role in the establishment and synchronization of contact-dependent syntrophic consortia. Syntrophic metabolizers have evolved a number of solutions to solve the problem of reverse electron transfer. One of these, the soluble, electron-bifurcating hydrogenase system [58••], provides a model to develop biomimetic systems for biohydrogen production. Unlocking the remaining secrets of syntrophy

should lead to the development of more sustainable and carbon-friendly energy sources in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.copbio.2009.10.001.

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- • of special interest
- •• of outstanding interest

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