ARCHAEAL EVOLUTION

Sediment, methane and energy

Three recent metagenomic studies analyse methanogenesis-related genes in previously uncharacterized, sediment-inhabiting archaeal lineages. They elucidate the metabolic capacity encoded in the genomes of these lineages, yet how these organisms harness energy is still a mystery.

Joana C. Xavier and William F. Martin

Life is a chemical reaction. When the reaction is over, everything from microorganisms to mammals eventually finds its final resting place in sediment. This issue of *Nature Microbiology* features three metagenomic studies that examine microbial processes in sediment and their implications for ecology and evolution[1–3].

Wang et al.[1] scoured existing databases in search of genes for the last enzyme of the methanogenic pathway, methyl-CoM reductase (MCR). MCR has been the focus of much interest of late because it is not only the source of biological methane, but it is also the first enzyme in the pathway of anaerobic methane oxidation (AOM). Whether derived from H_2 and CO_2, acetate or methanol, methanogenesis is an exergonic reaction: it releases energy that the cell can harness in the form of ion gradients and ATP, the universal energy currency. How that process works is well-characterized[4]. By contrast, to go forward as a chemical reaction, the reverse reaction of methanogenesis, AOM, requires coupling to some other energy-releasing reaction to make the overall reaction exergonic[5]. Sulfate reduction is a common partner process to AOM in marine environments because seawater is rich in sulfate, though sulfate has to be activated to sulfite (sulfite reduction being the exergonic process). But in previous systems studies, AOM and sulfate (or sulfite) reduction were thought to occur in different organisms, being linked by syntrophic interactions: AOM usually occurs in archaea living in consortia with sulfate-reducing bacteria[1–5]. Whether sulfate-dependent AOM itself is actually coupled to ATP synthesis in archaea, is still unknown.

Borrell et al.[2] and Wang et al.[1] mined available datasets and identified metagenome-assembled genomes (MAGs) suggesting that AOM and sulfate reduction can occur in the same cell. McKay et al.[3] probed sediment in the Washburn Springs geothermal pool in Yellowstone National Park, in the United States, and found a previously uncharacterized archaeal MAG encoding genes for both AOM and sulfite reduction. If operative at the same time, this would offer a potential mechanism linking methane oxidation to ATP synthesis in cells performing AOM, although this has not been shown. Furthermore, Borrell et al.[2] and Wang et al.[1] find that MCR genes are more widely distributed among archaeal metagenomic lineages than previously thought, suggesting that AOM is more widespread, too. MCR is an important enzyme because members of this family are also implicated in anaerobic alkane oxidation, a recently discovered process that occurs in sediment and subsurface environments[6]. Such apparently alkane-specialized MCR variants are also more widespread among environmental archaeal lineages than previously recognized[1,2].

The three new reports not only have sediment, MAGs and methane in common, but also one particularly interesting archaeon, *Korarchaeum cryptofilum*, the only member of the Korarchaeota so far characterized from enrichment cultures[7]. The three studies find genes for enzymes of the methanogenic pathway in MAGs assembled for relatives of *K. cryptofilum*. Borrell et al.[2] suggest that a lineage named NM4, a relative of *K. cryptofilum*, contains genes for methanogenesis and may be able to grow as a methanogen (Fig. 1). McKay et al.[3] provide more data for their metagenomic korarchaeon, tentatively named *Candidatus Methanodesulfokores washburnensis*. As one of several possible growth modes, they suggest it is able to couple sulfite reduction to methane oxidation in one cell (Fig. 1). Wang et al.[1] identify a lineage that they call Korarchaeota WYZ-LMO9 that, based on its gene collection, should also be able to perform sulfite-reducing AOM (Fig. 1). These are all metagenomic inferences, however, and the question of how AOM might be connected to ATP synthesis, if it is connected at all, is still unknown. It remains a speculative possibility that AOM by itself is not an energy metabolic pathway, but fulfils some other function, for example providing one carbon units instead.

Let us recall that nobody has cultured any of these new metagenomic lineages. Moreover, despite three independent reports, there is still no guarantee that the sulfur reduction genes really do occur in the same genome (and cell) as the methanogenesis genes. Regardless of how deeply sequenced, MAGs stem from an environment harbouring thousands or millions of different strains (or more), not a culture harbouring one organism. The MAGs are stitched together by computers, not by analysing sequence overlap but by looking at properties like GC content. McKay et al.[3] set a good example by reporting how different binning and assembly procedures generate a range of *Ca. M. washburnensis* estimated MAG sizes that span 1.4–2.9 Mb, which is a considerable range of uncertainty. They also show that assembly issues in metagenomic data are important and can affect the phylogenetic placement of different lineages, resulting in distinct arrangements between korarchaeotes, asgard archaea and eukaryotes, with implications for elucidating the ancestry of the eukaryote host lineage (the cell that acquired the mitochondrion). Notably, we still do not know how these archaeal lineages being described through metagenomics actually grow, and this brings our focus to cultured cells.

K. cryptofilum was initially identified by environmental sequencing. Karl Stetter's group grew it to enrichment in laboratory cultures, imaged it and obtained a closed circular genome of 1,590,757 bp[7]. Based on these data, the authors proposed that *K. cryptofilum* was probably growing in the enrichment culture by substrate level phosphorylation through amino acid fermentations. The *K. cryptofilum* genome sequence obtained from growing cells was very rich in genes for peptidases, peptide importers, transaminases, 2-oxoacid oxidoreductases that generate acyl-CoA, and a hydrogenase for redox balance[7]. The ATP-generating enzyme family of these amino acid fermentations, ADP-forming acetyl-CoA synthase (ACD)[8], is characteristic for
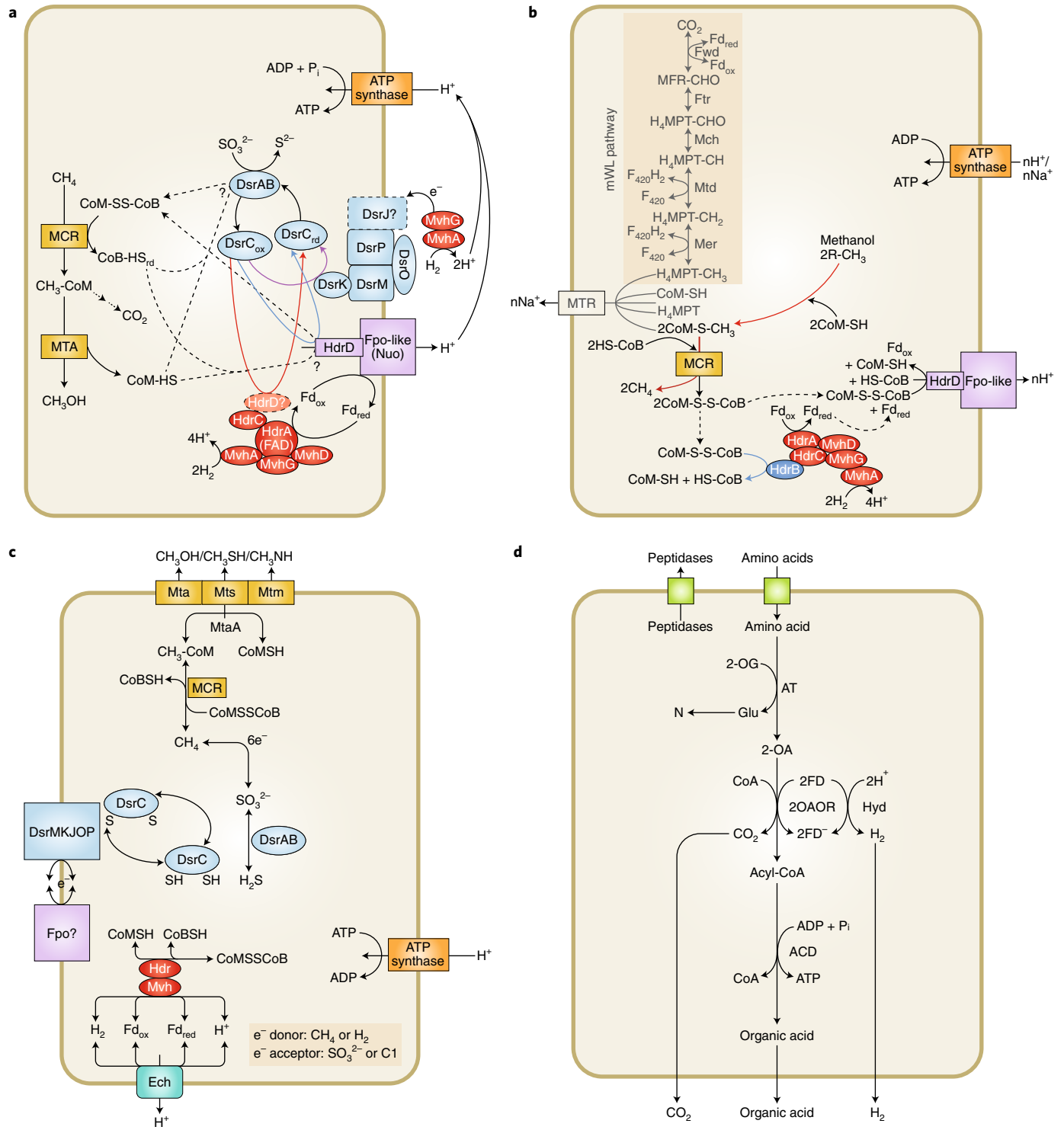
Fig. 1 | Proposed energy metabolisms in MAGs and cultured *K. cryptophilum*. a, Sulfite reduction with methane and hydrogen in *Ca. Methanodesulfokores washburnensis*, as proposed in ref. [3]. **b**, Methyl-dependent hydrogenotrophic methanogenesis in a MAG branching within the 'TACK' superphylum related to *K. cryptofilum*, as proposed in ref. [2]. Grey text indicates components missing in the genome. **c**, Metabolic scheme of Korarchaeota WYZ-LMO9 as proposed in ref. [1], where methane oxidation might be coupled to sulfite reduction or methylotrophic methanogenesis might involve hydrogen oxidation. **d**, Substrate level phosphorylation through amino acid fermentations as proposed for enrichment cultures of *K. cryptofilum*, adapted from ref. [7]. MTA, methyltransferase; Nuo, NADH–quinone oxidoreductase; MTR, N5-methyltetrahydromethanopterin–coenzyme M–methyltransferase complex; Ech, energy converting hydrogenase; AT, aminotransferase; 2OAOR, 2-oxoacid–ferredoxin oxidoreductase; Hyd, hydrogenase. Panels **a**–**c** are adapted from ref. [3], ref. [2] and ref. [1], respectively, Springer Nature Ltd.

archaea. ACD converts the amino-acid-derived thioester into an enzyme-bound acyl phosphate intermediate that phosphorylates ADP (ref. [8]). Amino acid fermentation pathways are simple but abundant[9], and for many fermenting archaea, the ACD reaction is the main source of net ATP synthesis[8,9].

The medium used for enrichment of *K. cryptofilum*[7] contained peptone and was gassed with N_2/CO_2 (80/20), but had no H_2 or other reductant capable of reducing CO_2, for example native metals[10], meaning that its carbon metabolism was heterotrophic. *K. cryptofilum* grew on peptone[7], which can serve as a source of carbon, energy, electrons and nitrogen. Sediment microorganisms should feel at home in peptone. Sediment contains cell mass, which includes DNA, RNA (recall that RNA fermentation pathways are well known[9]), lipids (although mainly fatty acids and isoprenes, which are unfermentable) and proteins. Importantly, as much as 90% of the DNA in sediment is not thought to be packed in cells — it is extracellular[11,12]. By weight, cells are about 3% DNA, 20% RNA, 10% lipids and about 50–60% protein[9]. All things being equal, for every 1 mg of prokaryotic DNA that is released into the environment, about 20 mg of protein is, too. Globally, that adds up to hundreds of megatonnes of protein per year released in sediment[11,12], suggesting

this substrate may be readily available to microorganisms. Investigations of mRNA abundance in sediment revealed that the most abundant functional class of expressed genes is for amino acid metabolism[13]. Clearly, amino acid fermentations are important in such environments. Though genes for MCR are abundant in sediment, their transcripts are scarce[13]. Why that is so remains a mystery, yet these data suggest that there is still much to learn about methane-generating and methane-degrading pathways in anaerobic sediment.

Methanogenesis is ancient. Several lines of evidence suggest that the first cells on Earth were H_2-dependent bacterial and archaeal autotrophs that lived at hydrothermal vents[9,10,14,15]. Under this scenario, sediment that accumulated around those first sites of anaerobic primary production was likely Earth's first heterotrophic environment. However, how the new, metagenomically characterized organisms living in these environments obtain energy is still a mystery: they might make methane, they might oxidize it or their ATP synthesis might involve other processes altogether, such as amino acid fermentation. In any case, like hydrothermal vents themselves[14,15], anaerobic sediments provide a window into ancient microbial ecology. ❐

Joana C. Xavier* and William F. Martin*
Institute for Molecular Evolution, University of Düsseldorf, Düsseldorf, Germany.
*e-mail: xavier@hhu.de; bill@hhu.de

References
1. Wang, Y., Wegener, G., Hou, J., Wang, F. & Xiao, X. *Nat. Microbiol.* https://doi.org/10.1038/s41564-019-0364-2 (2019).
2. Borrel, G. et al. *Nat. Microbiol.* https://doi.org/10.1038/s41564-019-0363-3 (2019).
3. McKay, L. J. et al. *Nat. Microbiol.* https://doi.org/10.1038/s41564-019-0362-4 (2019).
4. Thauer, R. K., Kaster, A. K., Seedorf, H., Buckel, W. & Hedderich, R. *Nat. Rev. Microbiol.* **6**, 579–591 (2008).
5. Milucka, J. et al. *Nature* **491**, 541–546 (2012).
6. Laso-Pérez, R. et al. *Nature* **539**, 396–401 (2016).
7. Elkins, J. G. et al. *Proc. Natl Acad. Sci. USA* **105**, 8102–8107 (2008).
8. Weisse, R. H., Faust, A., Schmidt, M., Schönheit, P. & Scheidig, A. J. *Proc. Natl Acad. Sci. USA* **113**, E519–E528 (2016).
9. Schönheit, P., Buckel, W. & Martin, W. F. *Trends Microbiol.* **24**, 12–25 (2016).
10. Varma, S. J., Muchowska, K. B., Chatelain, P. & Moran, J. *Nat. Ecol. Evol.* **2**, 1019–1024 (2018).
11. Dell'Anno, A. & Danovaro, R. *Science* **309**, 2179–2179 (2005).
12. Torti, A., Lever, M. A. & Jørgensen, B. B. *Mar. Genom.* **24**, 185–196 (2015).
13. Orsi, W. D., Edgcomb, V. P., Christman, G. D. & Biddle, J. F. *Nature* **499**, 205–208 (2013).
14. Baross, J. A. *Nature* **564**, 42–43 (2018).
15. Weiss, M. C. et al. *Nat. Microbiol.* **1**, 16116 (2016).