the conformational preferences and dynamics of transporters. Han *et al.* found that a cholesterol-based molecule could bind to SGLT1, and Niu *et al.* observed the interaction of the protein MAP17 with one of the helices in SGLT2, providing a starting point for addressing how the transporters might be regulated by other factors.

Finally, one sodium ion is absorbed for every glucose molecule in SGLT2, whereas in SGLT1 two sodium ions are absorbed[6]. Although the structures described by Han *et al.* and by Niu *et al.* pinpoint the sodium binding sites, a better dissection is needed of exactly how sodium-ion and sugar binding are intimately coupled to enable these differences. And with that, a new marathon begins.

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Biogeochemistry

A microbe that uses crude oil to make methane

Guillaume Borrel

A microorganism that dwells in an underground oil reservoir has been found to degrade various petroleum compounds and use them to produce methane through a previously unreported biochemical pathway. **See p.257**

Microbial communities tend to use the most energy-rich and most easily metabolized compounds that they have at their disposal. This leads to a progressive enrichment of compounds that are difficult to break down and that provide little energy, particularly in the absence of oxygen or other inorganic electron acceptors. Under these conditions, the use of hydrocarbons — molecules consisting of carbon and hydrogen, such as alkanes — has been thought to rely entirely on a collaboration (known as syntrophy) between bacteria that break down these compounds into acetate and molecular hydrogen (H_2), and microorganisms called methanogenic archaea that use the molecules to produce methane (CH_4), the simplest hydrocarbon[1–3]. On page 257, Zhou *et al.*[4] overturn this long-standing account of a division of labour in the methanogenic degradation of hydrocarbons by reporting that a single type of microorganism can degrade various large hydrocarbons into methane (Fig. 1).

Whereas many microorganisms can use a large range of substrates to obtain energy, methanogenic microorganisms (methanogens) are highly specialized. Most of them can obtain energy only by reducing carbon dioxide into methane using molecular hydrogen as an electron donor, and a few others can use acetate and methylated compounds (such as methanol and methylamines). Over the past five years, genomics studies have described several lineages of previously unknown and not-yet-cultured methanogenic microbes from various oxygen-free environments, including marine sediments, hot springs and subsurface oil reservoirs[5–8].

> **"The findings shed light on a previously unappreciated source of methane production."**

Although most of these newly discovered methanogens were inferred also to rely on simple compounds, mainly methylated compounds reduced by hydrogen[5,9], a great surprise was the prediction of a previously undescribed pathway for generating methane from multi-carbon alkanes and, possibly, long-chain fatty acids[5,10]. This metabolic pathway was initially predicted from genomes belonging to a class of uncultured archaea named *Candidatus* Methanoliparia[5], because members of this class mostly occur in oil reservoirs or environments contaminated with petroleum.

Zhou *et al.* collected crude oil from Shengli oilfield (northeast China), and found that *Ca.* Methanoliparia represented about half of the archaeal community in these samples. When the samples were incubated at between 35 °C and 65 °C, without oxygen or other inorganic electron acceptors, long-chain alkanes (linear chains of 13–38 carbons) were completely depleted, and a large amount of methane was produced. The authors similarly observed reductions in the levels of hydrocarbon molecules composed of a carbon ring bound to a long-chain alkyl group.

Because *Ca.* Methanoliparia are far from being the only microorganisms in these cultures, one might ask at this stage whether the production of methane from petroleum compounds might have resulted from the activity of conventional bacteria–archaea associations. Indeed, bacteria known to break down hydrocarbons were present, even if they represented only 4% of the total microbial community in the original culture. However, after several transfers of the culture into fresh medium, interspersed by weeks of incubation, the proportion of these bacteria dropped to less than 0.1%. By contrast, the abundance of *Ca.* Methanoliparia (around 40% of the microbial community) and the rate of methanogenic degradation of long-chain alkanes was maintained over these transfers.

Further supporting the absence of syntrophic associations involving *Ca.* Methanoliparia, Zhou *et al.* used microscopy to reveal that these archaea generally occurred alone and not in aggregates of multiple species (as had been shown previously[10]). Moreover, *Ca.* Methanoliparia genomes lack genes encoding enzymes and nanowire structures that are involved in electron transfer between syntrophic partners[5,10], corroborating the idea that *Ca.* Methanoliparia work alone. In *Ca.* Methanoliparia, as in the syntrophic partnerships between alkane-oxidizing bacteria and methanogenic archaea, electrons released by the alkane-oxidation pathway are used by the methanogenesis pathway. But, in *Ca.* Methanoliparia, the two pathways occur within one cell.

The predicted metabolic route for methanogenic degradation of long-chain hydrocarbons by *Ca.* Methanoliparia involves a combination of enzymes that are otherwise partitioned between syntrophic archaea and bacteria, as well as enzymes specific to *Ca.* Methanoliparia (Fig. 1). Among the enzymes that are used both in the *Ca.* Methanoliparia route and in the route mediated by syntrophic archaea and bacteria is the methyl-coenzyme M reductase

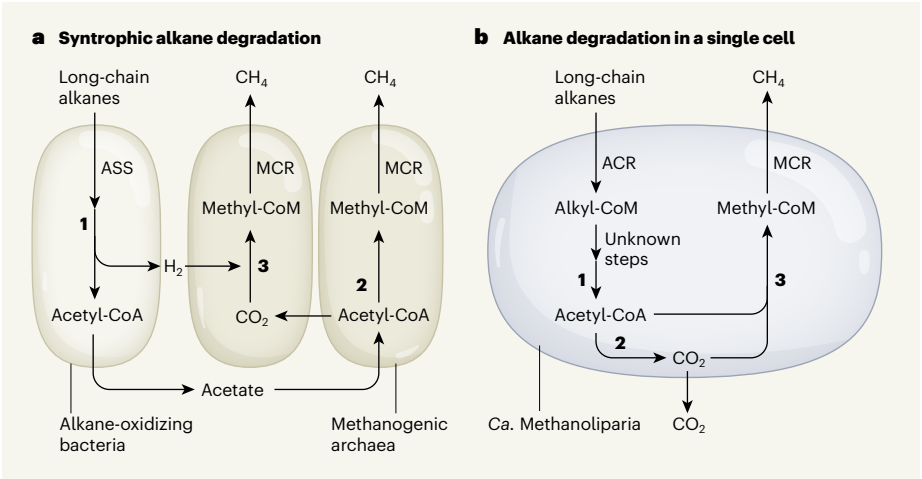

Figure 1 | Two microbial routes for the production of methane from hydrocarbon molecules. **a**, Hydrocarbons (molecules consisting only of hydrogen and carbon) are broken down and used to generate methane (CH_4) through a collaboration (syntrophy) between bacteria and archaea. In this process, long-chain alkanes are degraded into acetate and hydrogen (H_2) by alkane-oxidizing bacteria. One group of methanogenic archaea transforms acetate into carbon dioxide and CH_4, and another group makes methane using molecular hydrogen (H_2) and CO_2. **b**, Zhou *et al.*[4] show that archaeal microorganisms of the class *Candidatus* Methanoliparia can couple hydrocarbon degradation and methane generation. Several enzymes (MCR and those mediating steps 1, 2 and 3) and intermediate compounds (acetyl-coenzyme A (acetyl-CoA) and methyl-coenzyme M (methyl-CoM)) are similar between *Ca.* Methanoliparia and the syntrophic microbes. By contrast, the initial step of alkane breakdown is mediated by different enzymes: the ACR complex in *Ca.* Methanoliparia (which produces an alkyl-CoM intermediate), and ASS enzymes in alkane-oxidizing bacteria.

(MCR) complex, which performs the final step of methane production in all methanogens.

Candidatus Methanoliparia species also carry the genes that encode an enzymatic complex called the alkyl-coenzyme M reductase (ACR) complex, which was reported in the past few years to mediate the first step in the degradation of short-chain alkanes — forming alkyl-coenzyme M (alkyl-CoM) — in non-methanogenic archaea[11–13]. Members of *Ca.* Methanoliparia are the first microorganisms reported to have both an MCR complex and an ACR complex, an observation that initially led to the proposition that they might be capable of methanogenesis using multi-carbon alkanes[5,10].

Following the same principle as for short-chain alkanes, the activity of the ACR complex on long-chain alkanes should also lead to the formation of alkyl-CoM. In cultures enriched with *Ca.* Methanoliparia and fed hexadecane (a long-chain alkane containing 16 carbons), Zhou *et al.* detected the presence of hexadecyl-CoM, confirming that the ACR complex is involved in the first step of the degradation of long-chain alkanes. The authors made similar observations for the long-chain hydrocarbons *n*-hexadecylbenzene and *n*-hexadecylcyclohexane: addition of such molecules to the culture led to the formation of hexadecyl benzene-CoM and hexadecyl cyclohexane-CoM. The genes encoding MCR and ACR complexes in *Ca.* Methanoliparia were among the most highly expressed in the culture, supporting the proposal that these microorganisms couple hydrocarbon degradation with methane production. Taken all together, these findings greatly expand the range of substrates that are known to be used by the ACR complex, and by methanogens in general.

Could it be that short-chain alkanes are also targets of *Ca.* Methanoliparia ACR, as previously described in non-methanogenic archaea? Apparently not, because no alkyl-CoM or methane was produced in response to the addition to the culture of alkanes containing two to eight carbons. This suggests that some ACR complexes might use short-chain hydrocarbons, whereas others use long-chain hydrocarbons. Now that cultures of *Ca.* Methanoliparia are available, it will be exciting to resolve the 3D structure of their ACR complex and to compare it with that of evolutionarily related MCR and ACR complexes that can catalyse the oxidation of methane and short-chain alkanes, respectively[13,14]. Such investigation will probably reveal the molecular mechanisms that underlie the activity of these enzymes, including how they distinguish between hydrocarbons depending on length.

As is the case for other archaea that use the ACR complex to initiate the breakdown of hydrocarbons, such as *Candidatus* Syntrophoarchaeum[11], several steps in the process of hydrocarbon degradation in *Ca.* Methanoliparia are unknown (Fig. 1). Zhou and colleagues suggest that one of these steps might involve a type of enzyme called a long-chain fatty-acid CoA ligase. Intriguingly, although the authors' study focused on the most enriched *Ca.* Methanoliparia species (*Ca.* Methanoliparum thermophilum), which contains both ACR and MCR complexes, another *Ca.* Methanoliparia species (*Candidatus* Methanoliparum whitmanii) whose genome was sequenced by the authors lacks the genes that encode the ACR complex, but does carry genes encoding long-chain fatty-acid CoA ligases. Thus, the diversity of methanogenic metabolic pathways in this class of archaea could be even greater than that proposed by the authors, because some species might perform methanogenic degradation of long-chain fatty acids directly, instead of starting from hydrocarbons.

Future studies of *Ca.* Methanoliparia members will probably uncover intriguing new molecular mechanisms of methanogenesis and reveal even more of the microorganisms' already impressive range of substrates. *Candidatus* Methanoliparia also represent a useful model with which to investigate the evolutionary and environmental factors that favour either the division of labour between several species or the 'do-it-yourself' approach of this class. Investigating these microbes further will also provide a better understanding of the role of microorganisms in the fate of petroleum in oil reservoirs deep underground.

Finally, the findings shed light on a previously unappreciated source of methane production. It will be crucial to consider the contribution of this process to methane emissions and, in turn, to global warming.

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