BIOCHEMISTRY

A microbe that eats ethane under the sea

A microorganism that consumes ethane in the absence of environmental oxygen has been discovered. In the depths of the sea, this microbe, which oxidizes ethane, partners with another that reduces sulfate to sulfide. SEE LETTER P.108

STEPHEN W. RAGSDALE

Plumes of natural gas emanate from the bottom of the ocean. These hydrocarbon emissions comprise around 90% methane, with ethane making up most of the rest. Such deep-sea sources are responsible for 5% of the methane, a greenhouse gas, present in Earth's atmosphere[1]. The amount of hydrocarbon released from the deep sea into the atmosphere is substantially reduced by microorganisms that capture and use these gases as their sole source of cellular carbon and energy. Chen *et al.*[2] describe on page 108 the culmination of a ten-year scientific mission to identify and characterize the microorganisms and reactions that are responsible for ethane consumption at hydrocarbon seeps in the deep sea.

The microbes that metabolize the small alkane molecules methane, propane and butane to carbon dioxide in the absence of environmental oxygen (a process called anaerobic oxidation) have been identified, but those that use ethane have been elusive. Although the oxidation of alkanes by enzymes known as oxygenases in the presence of environmental oxygen (aerobic oxidation) is a thermodynamically favourable reaction, and thus likely to happen, the anaerobic process is thermodynamically unfavourable. Therefore, anaerobic alkane oxidation can occur only if it is coupled to an extremely thermodynamically favourable reduction reaction.

Chen *et al.* show that the anaerobic oxidation of ethane involves a mutually beneficial relationship, called syntrophy, between two microorganisms (Fig. 1). A newly identified microbe, the archaeon '*Candidatus* Argoarchaeum ethanivorans', oxidizes ethane to CO_2. Like all known anaerobic microbes that oxidize methane, propane and butane, *Ca.* A. ethanivorans is classified as an anaerobic methanotrophic (ANME) archaeon. Chen *et al.* show that the oxidation of ethane to CO_2 requires another biochemical reaction, the reduction of sulfate to sulfide. The genome of *Ca.* A. ethanivorans lacks sulfate-reducing enzymes, but an analysis of the microorganisms that live in the same microbial culture as *Ca.* A. ethanivorans revealed the presence of two bacterial strains from the genus *Desulfosarcina* that reduce sulfate to sulfide.

There is active debate concerning the nature of the syntrophic relationship between alkane-oxidizing archaea and sulfate-reducing bacteria. Does it involve the exchange of metabolites such as hydrogen molecules, small organic molecules or sulfur-containing molecules between the partner species? Or does it involve the direct interspecies transfer of electrons? Evidence for the direct transfer of electrons is supported by the observation that alkane-oxidizing microbes contain genes that encode cytochrome enzymes with multiple haem groups, which catalyse reduction and oxidation reactions[3–5]; such reactions involve the transfer of electrons between molecules. Furthermore, transmission electron microscopy has revealed the presence of filament-like nanowire connections between methane- or butane-oxidizing microbes and sulfate-reducing microbes. These filaments are thought to be needed for interspecies electron transfer[3,4]. Nevertheless, contrasting evidence also shows that ANME archaea involved in marine methane oxidation produce a sulfur-containing molecule that is transferred to a sulfate-reducing partner microorganism[6].

Chen *et al.* think that, for ethane oxidation, the transfer of a sulfur-containing metabolite is the process most likely to underlie the interaction between these two types of microorganism. This is because *Ca.* A. ethanivorans produces a lot of sulfur during growth, lacks the nanowire structures that have been observed in methane-oxidizing microbes, and grows as single cells rather than as a biofilm (a structure in which microbial cells stick to each other). All of these features could facilitate interspecies electron transfer. But Chen *et al.* also observed that the genomes of the sulfate-reducing bacteria they studied encode multi-haem cytochromes and the components of nanowire connections similar to those that are found in cultures of alkane-oxidizing and sulfate-reducing microbes grown together[3]. So, the jury is still out on how this interspecies coupling occurs.

What is the biochemical pathway for

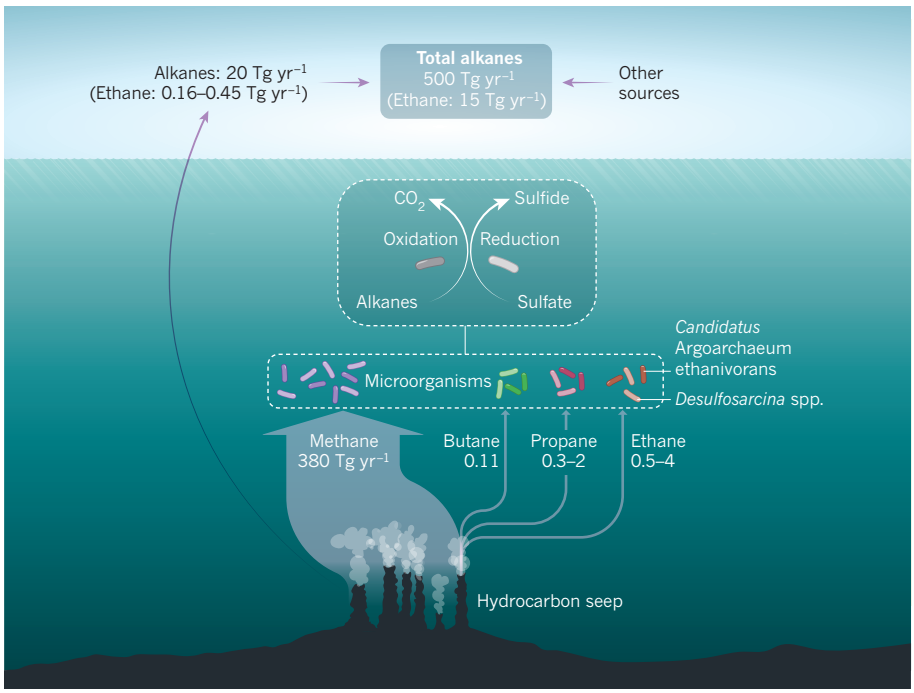

Figure 1 | The fate of deep-sea hydrocarbons. The plumes of gas that are produced at the ocean bottom are composed mainly of methane, the smallest alkane molecule, but also contain smaller amounts of other alkanes, including ethane, propane and butane (quantities shown in teragrams per year; 1 Tg = 10^{12} grams)[1,10–13]. Deep-sea microorganisms consume a large proportion of these gases, and only a small proportion ends up in the atmosphere, where it contributes to the greenhouse effect. The microbial oxidation of alkanes into carbon dioxide in the absence of oxygen (anaerobic oxidation) requires a complementary reaction comprising the reduction of sulfate to sulfide. These two reactions are carried out by distinct alkane-oxidizing (deep-colour symbols) and sulfate-reducing (pale symbols) microorganisms. Other microorganisms perform aerobic oxidation nearer the sea's surface (not shown). Chen *et al.*[2] identified the archaeon '*Candidatus* Argoarchaeum ethanivorans' as the first known microbe to oxidize ethane anaerobically. They also identified two strains of bacterium from the genus *Desulfosarcina* that reduce sulfate to sulfide and enable ethane oxidation to occur.

transforming ethane into CO_2? Chen and colleagues demonstrate unambiguously that the genome of *Ca.* A. ethanivorans contains three genes that encode the subunits of a previously unknown enzyme, which we call here ethyl-coenzyme M reductase (ECR). They then identify the protein sequence of ECR using mass spectrometry. ECR is closely related to an enzyme called methyl-coenzyme M reductase (MCR), which is present in microorganisms that oxidize or generate methane. Chen *et al.* also identify the final product of the reaction catalysed by ECR, ethyl-coenzyme M.

Chen *et al.* modelled the 3D structures of the *Ca.* A. ethanivorans ECR and the butane-metabolizing enzyme (butyl-coenzyme M reductase) of '*Candidatus* Syntrophoarchaeum'[4], and compared them with the known structure of the MCR of *Methanothermobacter marburgensis*[7]. On the basis of this structure comparison, as well as an alignment of the sequences of all known MCR-related proteins, they conclude that ECR and the other enzymes that metabolize non-methane hydrocarbon gases form a distinct cluster within an overarching group of enzymes called the alkyl-coenzyme M reductase (ACR) family, which catalyse the anaerobic oxidation of alkanes.

By analogy with the well-studied enzymatic mechanism of MCR[8], it is likely that ECR initiates anaerobic ethane oxidation by transforming ethane into an ethyl radical molecule, which is very reactive. It will be exciting when researchers are able to generate sufficient amounts of ECR to determine its enzymatic and biophysical properties. For example, how does it selectively catalyse the oxidation of ethane when the natural gas plumes where *Ca.* A. ethanivorans grows are so rich in methane? The authors' analysis did not reveal any obvious distinguishing features of the binding pockets of ACR enzymes, including ECR, that could explain their preference for metabolizing specific alkanes. However, the structures that the authors compared represent the inactive states of ACRs. It is to be hoped that the crystal structures of the active states of this enzyme family will soon be determined to help clarify this and other questions about their mechanism.

Chen *et al.* propose a plausible route from ECR-generated ethyl-coenzyme M to CO_2. In this model, ethyl-coenzyme M is transformed into acetyl-coenzyme A, a molecule involved in many other metabolic processes, which is then oxidized through a mechanism called the reverse Wood–Ljungdahl pathway[9]. The authors identified proteins that are involved in this pathway using genomic and proteomic methods. However, the conversion of ethyl-coenzyme M to acetyl-coenzyme A still requires experimental validation. A similar gap in knowledge also exists for other non-methane oxidation pathways, including anaerobic butane oxidation by *Ca.* Syntrophoarchaeum[4].

The next logical steps are to resolve the controversy regarding the nature of the communication between the newly identified ethane-oxidizing microorganism and its sulfate-reducing partner, and to build the metabolic bridge between ethyl-coenzyme M and acetyl-coenzyme A. ■

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ECOLOGY

Coral symbiosis is a three-player game

DNA analysis and microscopy reveal a third organism in the symbiosis that forms coral. The finding underscores the functional and evolutionary complexity of the symbiotic relationships that support many ecosystems. SEE LETTER P.103

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Symbiosis is deceptively easy to define: two or more organisms live together in a long-term association. Coral, the partnership between an animal from the Anthozoa group and a microbial alga called *Symbiodinium*, is an archetypal model of symbiosis. The anthozoan provides a home for the alga, which uses photosynthesis to produce sugars that are given to the animal as 'rent'[1]. These stable and highly productive two-player symbioses build the enormous reefs that shape marine ecosystems. On page 103, Kwong *et al.*[2] challenge this simple binary model of coral symbiosis by identifying a third player in the association.

Microorganisms, by definition, are tiny, and as such are difficult to isolate, grow and study. The vast extent of microbial diversity has been recognized only in the past two decades, after the application of molecular-biology techniques to the field[3]. The majority of newly discovered microbial groups are cryptic lineages that greatly outnumber the 'known' diversity of life[4], and are recognized only as DNA sequences stored in databases. We know little about what these microbes look like, how their cells function or what they do in an ecosystem. The challenge, therefore, is to map the DNA sequences that identify these microbes to physical cells, and to uncover the biology of such organisms. This is not an easy task.

Two such types of mystery DNA sequence, called ARL-V (apicomplexan-related lineage-V) and type-N, have been consistently found in samples from coral ecosystems[5]. Phylogenetic trees that map how the organisms containing ARL-V and type-N DNA are related to known microbes suggest that these organisms belong to the Apicomplexa. This group includes parasites that infect terrestrial animals, such as the *Plasmodium* species that cause malaria, so understanding more about the provenance and evolution of the microbes these sequences represent is of broad interest.

Many apicomplexan parasites live in the dark, but they contain the vestige of a plastid[6], a DNA-containing structure found in plant and algal cells that is required for photosynthesis. The evolutionary origin of plastids in the Apicomplexa is poorly understood. Apicomplexan plastids are non-photosynthetic, but they have retained some biochemical pathways that are found alongside the light-processing pathways in photosynthetic plastids. Many of these pathways are potential targets of antimalarial drugs. Photosynthetic relatives of the Apicomplexa have also been discovered in marine environments[7]. But how do the elusive microbes that contain ARL-V and type-N DNA fit into this picture, and what can they tell us about coral ecosystems and the evolutionary history of the Apicomplexa?

"The challenge now is to identify the role of the extra parties in corals, lichens and many other symbioses."

Kwong and colleagues were intrigued by the apparent association of the