

filaments. The square archaea contain purple membranes, which are used in phototrophy by haloarchaea; light absorption by thin sheets would be highly efficient, and especially by sheets oriented normal to the incoming light [18]. Buoyed up by gas vesicles, the square cells might, on reaching the water surface, come to rest parallel to the horizontal surface. Square cells that swim to the surface cannot do this because they are rotated by flagellar movement. Gas vesicles offer another advantage: in slowly growing cells they provide upward movement at less energetic cost than flagella [15]. These advantages could partly explain the paradox that these slowly growing square archaea can become dominant in hypersaline habitats [8]. Such habitats cover an area similar to that of freshwater [2] and square archaea may therefore be one of the more abundant organisms in nature.

With axenic cultures of the squares, many of the speculations discussed above can be verified: the turgor pressure, the identity of compatible solutes, the efficiency of light absorption, and their ability to form surface films. The thin square cells might also provide useful test-beds for experiments on division and polar development of cells [19]. But already the isolation has settled any argument that these squares are living square archaea.

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## Insights into the obligate methanotroph *Methylococcus capsulatus*

Donovan P. Kelly<sup>1</sup>, Christopher Anthony<sup>2</sup> and J. Colin Murrell<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, University of Warwick, Coventry, UK CV4 7AL

<sup>2</sup>School of Biological Sciences, University of Southampton, Southampton, UK SO16 7PX

**Completion of the genome sequence of *Methylococcus capsulatus* Bath is an important event in molecular microbiology, and an achievement for which the authors deserve congratulation. *M. capsulatus*, along with other methanotrophs, has been the subject of intense biochemical and molecular study because of its role in the global carbon cycle: the conversion of biogenic methane to carbon dioxide. The methane monooxygenase enzymes that are central to this process also have high biotechnological potential. Analysis of the genome**

**sequence will potentially accelerate elucidation of the regulation of methane-dependent metabolism in obligate methanotrophs, and help explain the cause of obligate methanotrophy, the phenomenon making most methanotrophs unable to grow on any substrates other than methane and a very small number of other one-carbon compounds.**

#### Introduction

Ward *et al.* [1] have produced a fairly comprehensive annotation of the 3113 open-reading frames (ORFs) that

Corresponding author: Kelly, D.P. (D.P.Kelly@warwick.ac.uk).

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are encoded by the 3.3 Mb genome of *Methylococcus capsulatus* Bath (MCA0001–MCA3113), enabling searches for genes of potential interest to be conducted. The identification of some coding sequences has also led to speculation surrounding the broader metabolic potential of *M. capsulatus* [1]. Although interesting, some of this speculation is overly enthusiastic, and we suggest caution in this approach. Analysis of the whole genome is beyond our scope here, therefore, we have identified topics of particular interest.

### Methane oxidation

The presence of a single set of genes has been confirmed for the soluble methane monooxygenase, sMMO (*mmoXYBZDC*), as has the duplication of *pmoA* and *pmoB* for the particulate MMO, with three copies of the *pmoC* gene. The nucleotide sequence of *pmoC* (MCA2855), which curiously is also described as ammonia monooxygenase, is identical to that of *pmoC1* (MCA1978), but differs somewhat from the *pmoC3* sequence – points not made by Ward *et al.* [1]. No new information on the *mmo* genes and their regulation is immediately apparent from the complete genome sequence. The suggestions that insights into copper regulation of the MMOs can be obtained directly from the genome, and that the genome is a potential source of new environmental probes for methanotrophs, are unfounded. Effective probes have already been in use for almost ten years, including those for *pmoA*, *mmoX* and *mxoF* [2–4].

### C<sub>1</sub>-compound assimilation

Assimilation of formaldehyde (derived from methane oxidation) by the ribulose monophosphate (RuMP) cycle is the predominant pathway for carbon fixation by *M. capsulatus*, although some minor carbon assimilation through the serine pathway and even the Calvin cycle are also possibilities. Evidence for all three systems exists and the genome sequence confirms the presence of relevant genes, including duplication of the hexulose 6-phosphate synthase gene. However, Ward *et al.* [1] possibly go too far in promoting the importance of serine in the methylotrophic metabolism of *M. capsulatus*.

The metabolic pathway scheme presented in the recent publication by Ward *et al.* [1] gives a good impression of the possible interconnection of pathways and of the complexity of metabolism, but the scheme does not make clear the relevance of some pathways. A 'biosynthetic' pathway must have endpoints, but the way the scheme is depicted and the mixing of biosynthetic and catabolic pathways produces a somewhat confusing maze. This is also illustrated by the authors' apparent misunderstanding of some aspects of it. For example, the RuMP pathway has some enzymes in common with the Calvin cycle and the pentose phosphate pathway for oxidation of glucose 6-phosphate. Also a disadvantage is how the complex reactions of the metabolic pathway scheme are summarized by Ward *et al.* [1]; for example, it is implied that erythrose 4-phosphate (C<sub>4</sub>) can be converted to sedoheptulose 7-phosphate (C<sub>7</sub>) without the necessary involvement of fructose 6-phosphate (C<sub>6</sub>) and the production of phosphoglyceraldehyde (C<sub>3</sub>). The paper by Ward *et al.* [1]

supposedly predicts 'flow of carbon from methanotrophy pathways into carbon fixation pathways and thence into glycolysis/gluconeogenesis and the TCA cycle'. This is somewhat misleading as the methylotrophic pathways following the initial oxidation of methane are the fixation pathways; gluconeogenesis in the classical sense is not required because glucose can be generated from the RuMP cycle, nor is there a need for a complete TCA cycle, and the 'serine cycle' has no end-product. A pathway is indicated for a step from acetyl CoA to oxaloacetate, but the only such pathway effecting this step is the glyoxylate bypass, which is not mentioned and is probably unlikely. The metabolic pathway scheme presented by Ward *et al.* [1] needs to be used in conjunction with the list of the encoded enzymes that have been identified to date (e.g. MCA3045 is a transaldolase equivalent – if this was mentioned then the implication of a single enzyme apparently converting a C<sub>4</sub> sugar to a C<sub>7</sub> sugar would have been avoided). Also, the pyruvate/phosphoenolpyruvate interconversion is shown to be reversibly catalyzed by pyruvate kinase, but the pyruvate kinase reaction is actually irreversible. There is a part of the scheme labelled 'glycine cleavage', but there do not appear to be any reactions that are relevant to this. There is however an odd suggestion of the addition of glycine to serine to give methylenetetrahydrofolate, apparently a result of a misplaced arrow from a glycine residue on the scheme diagram. In the discussion of gluconeogenesis there is a statement that a key enzyme of gluconeogenesis, fructose biphosphatase, is absent, although the reaction of fructose biphosphate to fructose 6-phosphate, catalyzed by MCA1251–2, is depicted clearly in the metabolic pathway scheme. In fact, MCA1251–2 encodes the pyrophosphate-dependent 6-phosphofructokinase, which has previously been assayed in *M. capsulatus* [5]; therefore, this reaction should be from fructose 6-phosphate to fructose biphosphate.

### The potential 'metabolic flexibility' of *M. capsulatus*

Ward *et al.* [1] suggest that the genome provides 'evidence suggesting the existence of previously unsuspected metabolic flexibility in *M. capsulatus*', including the possibility that 'a complete TCA-cycle might operate' and 'an ability to grow on sugars, oxidise chemolithotrophic hydrogen and sulfur, and live under reduced oxygen tension...'. All the physiological and biochemical evidence indicates that these organisms have very limited metabolic flexibility and the obligate nature of methanotrophy has been well established during the past 40 years [6–11]. Of course, it could be that some conditions might be found under which *M. capsulatus* would grow with other substrates. We consider the various claims made by the authors.

### The Krebs cycle

The genes for the E1 and E2 subunits of 2-oxoglutarate dehydrogenase do occur in the genome (MCA1952, MCA1953), but it is well established that no activity of this enzyme can be detected *in vivo*, and without it the Krebs cycle cannot function [6]. Conditions under which the enzyme might be expressed cannot currently be envisaged.

## Growth on sugars (and other multicarbon organic compounds)

Ward *et al.* [1] state that the small number of transport-related genes for organic nutrients detected in the *M. capsulatus* genome might be 'consistent with its autotrophic [sic] lifestyle'. More could have been made of the transporter genes that were found. Certainly, *M. capsulatus* assimilates only very small amounts of glucose or sucrose [12], but does exhibit general uptake systems for amino acids, as well as assimilating exogenous acetate [12–14]. Among the transporter genes found are an amino acid ABC transporter (MCA0840) as well as numerous uncharacterized ABC transporters and others specific for sugars (MCA1924, 1941, 1942, 1944), carbohydrates and C<sub>4</sub>-dicarboxylate (MCA1872). In the case of the obligate chemolithoautotroph *Nitrosomonas europaea*, with which *M. capsulatus* shares some biochemical properties, the complete genome [15] was used to identify an uptake system (a phosphoenolpyruvate-dependent sugar phosphotransferase) for fructose, leading to the demonstration that *N. europaea* could grow chemolithoorganotrophically on fructose with ammonia oxidation as the energy source [16]. A parallel ability in *M. capsulatus* could be sought using the genome sequence for guidance.

## Hydrogen chemolithotrophy

In the case of hydrogenases, the presence of appropriate genes is consistent with earlier observations [17] – that hydrogen can be oxidized as a supplementary energy source – but the suggestion that *M. capsulatus* might benefit from syntrophic partnerships with anaerobic hydrogen producers is a step too far in genome interpretation. The emphasis on growth under low oxygen tensions in this context is curious; *M. capsulatus* grows very well in the laboratory and in nature at relatively high temperatures (45°C) and oxygen tensions of less than half air-saturation [18], as do many other bacteria.

## Sulfur chemolithotrophy

Regarding the suggested ability to obtain energy from inorganic sulfur compound oxidation, and hence grow chemolithotrophically, the case is rather weak. The putative *soxC* gene for sulfite oxidase is not a surprising find: numerous heterotrophs incapable of sulfur-lithotrophy have this enzyme. The cluster of 'intracellular sulfur oxidation protein' genes (MCA1331–1334) does show around 50% polypeptide similarity to part of the *dsr* cluster of *Chromatium vinosum* (GenBank accession number U84760) but nucleotide sequence similarities are very low, and numerous similarities also exist to sequences of other non-chemolithotrophs (e.g. *Vibrio*, *Shigella* and *Yersinia*). The *dsr* cluster encodes (among other proteins) the dissimilatory siroheme sulfite reductase that is implicated in sulfite oxidation in some phototrophs and *Thiobacillus denitrificans*, but does not necessarily confer chemolithotrophic ability. The identification of MCA1987 and MCA1989 as genes for the sulfur compound-chelating proteins SoxY and SoxZ is similarly tenuous because the translated polypeptide sequences show only 39–46% similarity to database sequences for these proteins, and BLASTN analyses of the nucleotide sequences reveal high identity (95%) for only

### Box 1. The big questions

- What is the exact molecular mechanism by which copper regulates the expression of methane monooxygenases?
- Can the apparently 'silent' genes of central intermediary metabolism (e.g. for 2-oxoglutarate dehydrogenase) ever be expressed?
- Does the genome contain genes encoding transporters for specific sugars or amino acids, and if so can these be expressed at levels that might enable sugars or amino acids to be used as major carbon sources?

23 or 24 sequential nucleotides of the *Chlorobium limicola soxY* and *soxZ*, with comparable identities to short sequences from non-chemolithotrophs. The gene *soxB*, which encodes a potentially essential sulfur-oxidizing enzyme, is not reported. The question of any sulfur-lithotrophic potential is far from answered.

### Final comment

The availability of this genome presents great potential, assessed only rather superficially by Ward *et al.* [1]. It points the way to a great deal of 'work at the bench' to correlate what appears to be genomic potential with translation into physiological action in the real world (Box 1). The way is open for the use of marker-exchange mutagenesis [19–21], proteomics [22,23], and other techniques already in place for work on this organism. The genome sequence also encodes around 1000 hypothetical proteins [1], so the potential for further 'gene mining' is immense. This has already begun with the discovery of a new class of the cytochrome P450 superfamily in *M. capsulatus* [24]. Outstanding questions include: the further elucidation of the basis of obligate methanotrophy, the regulation of the MMO complexes, and the roles of the numerous inorganic and organic transporters identified in the genome.

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## Erratum

# Erratum: It's a cold world out there (but the prospects are hot)

[*Trends Microbiol.* 12 (2004), 532–534]

The Microbial Genomics article by Barbara Methé, Karen E. Nelson and Claire M. Fraser, which appeared in the December 2004 issue of *Trends in Microbiology*, was published with a sentence that had a reference citation missing. *Trends in Microbiology* would like to clarify this line through insertion of the reference.

On page 533 the following sentence 'In the study, the authors used a randomly selected group of bacterial and archaeal species for which complete genome sequences are available (growth temperatures ranged from 27°C to 100°C), however, no strict correlation between optimal growth temperatures and primary nucleotide composition

could be identified.' should read 'In the study, Rabus *et al.* [5] used a randomly selected group of bacterial and archaeal species for which complete genome sequences are available (growth temperatures ranged from 27°C to 100°C), however, no strict correlation between optimal growth temperatures and primary nucleotide composition could be identified.'

The journal would like to apologise for any confusion caused. DOI of original article [10.1016/j.tim.2004.10.007](https://doi.org/10.1016/j.tim.2004.10.007).

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