

## COMMENT



# To methanotrophy and beyond! New insight into functional and ecological roles for copper chelators

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In their study published in this issue, Kang and colleagues have addressed a very important question: whether copper chelators secreted by aerobic methanotrophs, which play a major role in controlling methane accumulation in the atmosphere, might serve as “public goods”, akin to siderophores and other secreted compounds [1], with important environmental implications. These would include a potential competition for copper among different types of methanotrophs, whether producing such public goods or not, and also competition between methanotrophs and other organisms requiring copper for vital functions, such as ammonia oxidizers, thus effecting biogeochemical cycling of both carbon and nitrogen. The methanotrophs require high supplies of copper as an essential cofactor of the main enzyme in the aerobic methanotrophy: particulate methane monooxygenase [2]. It has been nearly two decades since methanobactin (MB) was discovered to serve as a copper chelator (a “chalkophore”, χαλκός is Greek for copper) to supply this enzyme [3]. Such copper chelating compounds, of which very few are chemically characterized, are small peptides that are similar to siderophores, which are well-studied iron-binding peptides [2]. In alphaproteobacterial methanotrophs of the *Methylocystaceae* family, two classes of MBs have been characterized, with somewhat different properties and varying affinities for copper [2]. Known MBs are secreted into the environment where they bind/solubilize/reduce copper and then transport it into cells, via TonB type transporters that are encoded as parts of the gene clusters involved in MB precursor synthesis and modification [2]. These chelators seem rather promiscuous, potentially binding a variety of metals beside copper, including iron and gold, but their synthesis nevertheless is strictly regulated by the availability of copper; genes for their synthesis are only expressed under copper-limited conditions [2]. Interestingly, due to their promiscuity, MBs have been implicated in playing a role in binding and transporting methylmercury ( $\text{CH}_3\text{Hg}^+$ ), a potent neurotoxin, thus potentially playing a role in its detoxification in certain environmental niches [4]. The mechanism for  $\text{CH}_3\text{Hg}^+$  degradation by methanotrophs remains elusive, but methanol dehydrogenase, an important enzyme for methanotrophy, may be involved [4].

Despite their important role for methanotrophs, MBs are not the only mechanism used to secure copper for the essential reaction of methane oxidation. Several methane oxidizers employ a protein named MopE/MopE\*, so far only characterized in *Methylococcus capsulatus*, which is a gammaproteobacterial methanotroph. This protein also has high affinity for copper and efficiently transports it

into the cell [5], thus, it is another type of chalkophore. At this time, the mechanism for its transport has not been established experimentally. In addition, poorly characterized secreted substances have been reported for several other gammaproteobacterial methanotrophs, distinct from MBs, representing additional type(s) of chalkophores. While the machinery for MB synthesis and secretion has not been recognized in those corresponding genomes [6], the mechanism for synthesis of these poorly characterized chalkophores remains completely unknown.

In their study, Kang and colleagues focused on the role of MBs in copper acquisition. Three different methanotrophs were studied in these experiments, none encoding recognizable MB synthesis/modification functions, in addition to testing two purified MBs from different MB classes. While not encoding MBs, all three methanotrophs encoded homologs for the MB transport function, suggesting a potential for MB acquisition as a public good. Because an MB-specific antibody test was unreliable,  $\text{CH}_3\text{Hg}^+$  degradation capability has been used as a proxy (i.e., when an organism is able to acquire a MB,  $\text{CH}_3\text{Hg}^+$  would be transported into the cell and demethylated [1]).

Remarkably, two of the methanotrophs (*Methylocystis* sp. Rockwell and *Methylomicrobium album* BG8) responded to the externally supplied MBs, indicating that some methanotrophs can benefit from the public goods in terms of metal supply, whereas the third (*Methylococcus capsulatus* Bath) had no such response. It appears though that *M. capsulatus* has at least two mechanisms to acquire copper, via MopE/MopE\* or via an alternative chalkophore, which might be similar to the one partially characterized in this study and thus rather different from known MBs. Thus, perhaps *M. capsulatus* does not need to “steal” MBs because (a) it may be already sufficiently equipped to secure the supply of copper, and (b) it can employ an alternative soluble methane monooxygenase that does not require copper [2]. In contrast, *M. album* appears to be able to steal MBs from alphaproteobacterial methanotrophs even if it also produces an alternative chalkophore [1]. For *Methylocystis* sp. Rockwell, stealing appears to be the only way to get ahead with copper acquisition.

Although this study only involved three methanotrophs, none encoding known genes for the synthesis of MBs, the results already present a complex system that expands our understanding of methanotroph ecology. The authors demonstrate that methanotrophs can employ secreted MBs as public goods, even though they may have their own means to bind and transport copper. This implies a complex balance in the relevant abundance

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and function of different types of methanotrophs inhabiting the same environmental niche. These results also question whether other interactions exist among different types of methanotrophs that allow the ones with no means of acquiring copper to coexist with the ones that have multiple means. In addition, the study further highlights the involvement of MBs in environmental degradation of  $\text{CH}_3\text{Hg}^+$  and, thus, an important role for methanotrophs beyond methanotrophy.

Intriguingly, gene clusters for MB biosynthesis have been identified in the genomes of non-methanotrophic microorganisms [7], but their roles have not yet been studied. One may speculate that MBs in non-methanotrophs are simply used to acquire copper for enzymes other than methane monooxygenase. However, a different scenario could be imagined, of MBs being used as a “gift” for methanotrophs, in exchange for some other valuable compound or function. For example, microorganisms with no ability to demethylate toxic  $\text{CH}_3\text{Hg}^+$  could benefit from a collaboration with methanotrophs. In another scenario, MBs, as well as other secreted chalkophores, could serve as signals that prompt methanotrophs to excrete carbon, which they are known to do in other circumstances [8]. Two other aspects of potential complex communal interactions should be mentioned, between the methanotrophs and the nitrifiers [9] and methanotrophs and the denitrifiers, via competition for copper [10], in which copper chelators may be playing a significant role. This highlights yet another potential function of methanotrophs beyond methanotrophy, in the global nitrogen cycle.

Although Kang and colleagues [1] report a significant advance in understanding the role of MBs in both copper supply for the essential function in methane oxidation as well as in  $\text{CH}_3\text{Hg}^+$  degradation, many questions remain unanswered. How many different types of MBs exist? Why do known gammaproteobacterial methanotrophs produce no MBs but, instead, produce other chalkophores? The chemistry and properties of the latter need to be further studied, including their mode of biosynthesis. Furthermore, the role of methanol dehydrogenase in  $\text{CH}_3\text{Hg}^+$  demethylation needs to be demonstrated, or an alternative mechanism needs to be discovered. Even though a quorum sensing mechanism has been demonstrated for some methanotrophs, so far, it has not been connected to chalkophore production. Thus, regulation of their synthesis may be unlike regulation of siderophore synthesis. The most interesting question is whether MBs and other chalkophores participate in community function and the underlying mechanisms for that influence.

Although aerobic methanotrophs have been studied extensively for over 100 years [6], they continue to be enigmatic, full of surprises, and a continual source of puzzles to solve.

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## AUTHOR CONTRIBUTIONS

L.C. conceived the manuscript and wrote the text.

## COMPETING INTERESTS

The author declares no competing interests.

## ADDITIONAL INFORMATION

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