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Cobalamin-independent Methionine Synthase Distribution and Influence on Vitamin B12 Growth Requirements in Marine Diatoms

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The requirement for cobalamin (vitamin B_{12}) in microalgae is primarily a function of the type of methionine synthase present within their gene repertoires. Our study validates this concept through analysis of the distribution of B_{12}-independent methionine synthase in ecologically relevant diatom genera, including the closely related bloom-forming genera *Pseudo-nitzschia* and *Fragilariopsis*. Growth and gene expression analysis of the vitamin B_{12}-requiring version of the methionine synthase enzyme, METH, and the B_{12}-independent version, METE, demonstrate that it is the presence of the *METE* gene which allows *Fragilariopsis cylindrus* to grow in the absence of B_{12}. *Pseudo-nitzschia granii*'s lack of a functional *METE* gene means that it cannot survive without the vitamin. Through phylogenetic analysis, we further substantiate a lack of obvious grouping in METE presence among diatom clades. In addition, we also show how this trend may have a biogeographical basis, particularly in regions such as the Southern Ocean where B_{12} concentrations may be consistently low. Our findings demonstrate the important role vitamins can play in diatom community dynamics within areas where vitamin supply may be variable and limiting.

Key words: Cobalamin; vitamin B_{12}; transcriptomics; methionine synthase; phylogenetics; biogeography.

Introduction

Vitamin B_{12}, or cobalamin, is a cobalt-containing organometallic molecule that is synthesized by certain bacteria, archaea, and cyanobacteria (Bertrand et al. 2007; Bonnet et al. 2010). Its biological importance lies predominantly in its role as a cofactor in one form of the methionine synthase enzyme (METH) that catalyzes the synthesis of the amino acid methionine and is present in all examined marine eukaryotic microalgae (Banerjee and Matthews 1990; Helliwell et al. 2011).

Many of these algal species are B_{12} auxotrophs, meaning they have an obligate requirement for B_{12} and likely only possess the *METH* gene (Croft et al. 2005). However, a cobalamin-independent methionine synthase (METE) that does not require vitamin B_{12} to create methionine also exists in plants, some bacteria, and some algae, including certain diatom species. There is a strong demonstrated correlation between an absence of a functional *METE* gene

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and B_{12} auxotrophy among examined microalgae (Helliwell et al. 2011).

Both forms of methionine synthase catalyze the conversion of homocysteine and methyltetrahydrofolate to tetrahydrofolate and methionine, although the exact pathway is less resolved for the METE enzyme (reviewed in Bertrand and Allen 2012). Despite functional similarity, METE and METH are unrelated both in sequence homology and protein folding structure (Pejchal and Ludwig 2005). In addition, METE is approximately 100-fold less catalytically efficient than METH, with significantly slower turnover times for the enzyme observed in *E. coli* (Gonzalez et al. 1992). This inefficiency results in 30-40 fold increases in the nitrogen and zinc requirements allocated to METE activity over what is used for METH (Bertrand et al. 2013).

Although the only currently known determinant of whether METH or METE is utilized is vitamin B_{12} availability, these genomic alterations can have ecosystem-scale implications. Until recently, research in High-Nitrate, Low-Chlorophyll (HNLC) regions of the world's oceans focused primarily on the role of iron limitation in sustaining low primary production. Iron fertilization experiments have shown that these regions are limited by bioavailable iron, and subsequent iron fertilization of HNLC waters creates diatom-dominated phytoplankton blooms (De Baar et al. 2005; Boyd et al. 2007) that have the potential to sequester large amounts of carbon in seafloor sediments (Smetacek et al. 2012).

More recent investigations in iron-limited regions of the ocean have shown that in some cases, by adding both vitamins and iron, growth of certain diatoms can be enhanced beyond that of iron enrichment alone. In particular, under certain environmental conditions vitamin B_{12} is capable of increasing growth with or without iron in HNLC regions (Bertrand et al. 2007; Koch et al. 2011; Panzeca et al. 2006). Vitamin B_{12} is also capable of affecting phytoplankton growth and community structure in coastal and estuarine waters (Gobler et al. 2007; Koch et al. 2011; Sañudo-Wilhelmy et al. 2006).

The biosynthesis of vitamin B_{12} by marine prokaryotes affects oceanic B_{12} distributions, as these organisms are the sole source of the vitamin to eukaryotic algae, including diatoms (Croft et al. 2005). Phytoplankton may acquire the vitamin from dissolved B_{12} released through excretion, cell lysis, and direct symbiotic interactions with bacteria and archaea (Bonnet et al. 2010; Droop 2007; Kazamia et al. 2012). Therefore, an important determinant of vitamin B_{12} distribution is the abundance and composition of marine prokaryotic communities. In addition, diatoms without *METE* may compete with other B_{12}-auxotrophic algae and bacteria when vitamin concentrations are low (Bertrand et al. 2015).

Prior to this study, the phylogenetic patterns among diatom species possessing *METE* were difficult to determine due to a scarcity of genomic information. Recent phylogenetic analysis of *METE* sequences among diverse algal groups indicates that multiple independent gene losses are likely the mechanism behind widespread but randomly distributed B_{12} auxotrophy (Helliwell et al. 2011). The discovery of *METE* unitary pseudogenes in multiple algal species corroborates this theory (Helliwell et al. 2013). Our study uses transcriptomic analysis to further investigate this theory, as well as the possibility of a biogeographical basis for *METE* retention in marine diatoms. Our primary objective was to investigate whether variations in B_{12}-related gene repertoires among ecologically important marine diatom species may determine B_{12} auxotrophy and thus affect large scale processes such as community composition and carbon sequestration potential within areas of known variable vitamin supply.

Results

Steady-state Growth Rates and Photosynthetic Efficiencies

Determining growth rate differences between non-AT (antibiotic-treated) and AT +/$-B_{12}$ treatments for *P. granii* and *Thalassiosira* sp. UNC1203 were not possible as these diatoms could not be maintained in exponential phase for more than 2-3 transfers in $-B_{12}$ medium, prohibiting growth rates of acclimated cultures from being obtained (Table 1). While *Chaetoceros* sp. UNC1202 $-B_{12}$ and AT $-B_{12}$ treatments did not cease growth completely, growth rates were significantly slower in both $-B_{12}$ treatments compared to $+B_{12}$ treatments (Mann-Whitney U Statistic = 3.5). Growth rates were also significantly different between *Chaetoceros* sp. UNC1202 $-B_{12}$ and AT $-B_{12}$ treatments ($p = 2.5 \times 10^{-5}$). *Skeletonema* sp. UNC1201 exhibited significantly slower growth between $+B_{12}$ and $-B_{12}$ treatments, and growth ceased in AT $-B_{12}$ treatments ($p = 3.10 \times 10^{-6}$). There were no significant differences between B_{12} treatments for *F. cylindrus* and *F. kerguelensis* for both non-AT and AT cultures.