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Methionine synthase interreplacement in diatom cultures and communities: Implications for the persistence of B<sub>1</sub>, use by eukaryotic phytoplankton

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ABSTRACT: Three proteins related to vitamin B,, metabolism in diatoms were quantified via selected reaction monitoring mass spectrometry: B,2-dependent and B,2-independent methionine synthase (MetH, MetE) and a  $B_{12}$  acquisition protein (CBA1).  $B_{12}$ -mediated interreplacement of MetE and MetH metalloenzymes was observed in Phaeodactylum tricornutum where MetH abundance was highest (0.06 fmol µg" protein) under high B,, and MetE abundance increased to 3.25 fmol  $\mu g^{-1}$  protein under low  $B_{12}$  availability. Maximal MetE abundance was 60-fold greater than MetH, consistent with the expected ~ 50 - 100-fold larger turnover number for MetH. MetE expression resulted in 30-fold increase in nitrogen and 40-fold increase in zinc allocated to methionine synthase activity under low B<sub>12</sub>. CBA1 abundance was 6-fold higher under low-B<sub>12</sub> conditions and increased upon B,2 resupply to starved cultures. While biochemical pathways that supplant B., requirements exist and are utilized by organisms such as land plants, B., use persists in eukaryotic phytoplankton. This study suggests that retention of B., utilization by phytoplankton results in resource conservation under conditions of high B,2 availability. MetE and MetH abundances were also measured in diatom communities from McMurdo Sound, verifying that both these proteins are expressed in natural communities. These protein measurements are consistent with previous studies suggesting that B,2 availability influences Antarctic primary productivity. This study illuminates controls on expression of B<sub>12</sub>-related proteins, quantitatively assesses the metabolic consequences of B,, deprivation, and demonstrates that mass spectrometry - based protein measurements yield insight into the functioning of marine microbial communities.

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