Two distinct pools of B_{12} analogs reveal community interdependencies in the ocean

Katherine R. Heal[a], Wei Qin[b], Francois Ribalet[a], Anthony D. Bertagnolli[b,1], Willow Coyote-Maestas[a,2], Laura R. Hmelo[a], James W. Moffett[c,d], Allan H. Devol[a], E. Virginia Armbrust[a], David A. Stahl[b], and Anitra E. Ingalls[a,3]

[a]School of Oceanography, University of Washington, Seattle, WA 98195; [b]Department of Civil and Environmental Engineering, University of Washington, Seattle, WA 98195; [c]Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0894; and [d]Department of Civil and Environmental Engineering, University of Southern California, Los Angeles, CA 90089-0894

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Organisms within all domains of life require the cofactor cobalamin (vitamin B_{12}), which is produced only by a subset of bacteria and archaea. On the basis of genomic analyses, cobalamin biosynthesis in marine systems has been inferred in three main groups: select heterotrophic Proteobacteria, chemoautotrophic Thaumarchaeota, and photoautotrophic Cyanobacteria. Culture work demonstrates that many Cyanobacteria do not synthesize cobalamin but rather produce pseudocobalamin, challenging the connection between the occurrence of cobalamin biosynthesis genes and production of the compound in marine ecosystems. Here we show that cobalamin and pseudocobalamin coexist in the surface ocean, have distinct microbial sources, and support different enzymatic demands. Even in the presence of cobalamin, Cyanobacteria synthesize pseudocobalamin—likely reflecting their retention of an oxygen-independent pathway to produce pseudocobalamin, which is used as a cofactor in their specialized methionine synthase (MetH). This contrasts a model diatom, *Thalassiosira pseudonana*, which transported pseudocobalamin into the cell but was unable to use pseudocobalamin in its homolog of MetH. Our genomic and culture analyses showed that marine Thaumarchaeota and select heterotrophic bacteria produce cobalamin. This indicates that cobalamin in the surface ocean is a result of de novo synthesis by heterotrophic bacteria or via modification of closely related compounds like cyanobacterially produced pseudocobalamin. Deeper in the water column, our study implicates Thaumarchaeota as major producers of cobalamin based on genomic potential, cobalamin cell quotas, and abundance. Together, these findings establish the distinctive roles played by abundant prokaryotes in cobalamin-based microbial interdependencies that sustain community structure and function in the ocean.

B_{12} | cobalamin | Thaumarchaeota | pseudocobalamin | Cyanobacteria

Cobalamin (vitamin B_{12}) is synthesized by a select subset of bacteria and archaea, yet organisms across all domains of life require it (1–3). In the surface ocean, cobalamin auxotrophs (including most eukaryotic algae) (3) obtain the vitamin through direct interactions with cobalamin producers (3) or breakdown of cobalamin-containing cells (4, 5). Interdependencies between marine cobalamin producers and consumers are critical in surface waters where primary productivity can be limited by the availability of cobalamin and the compound is short-lived (1, 6, 7). The exchange of cobalamin in return for organic compounds is hypothesized to underpin mutualistic interactions between heterotrophic bacteria and autotrophic algae (3, 6, 8, 9). The apparent pervasiveness of cobalamin biosynthesis genes in chemoautotrophic Thaumarchaeota and photoautotrophic Cyanobacteria genomes (1, 10, 11) raises the question of whether cobalamin production by these autotrophs may underlie additional, unexplored microbial interactions.

Cobalamin is a complex molecule with a central cobalt-containing corrin ring, an α ligand of 5,6-dimethylbenzimidizole (DMB), and a β ligand of either OH-, CN-, Me-, or Ado- (12) (Fig. 1). Previous studies have shown that instead of producing cobalamin, Cyanobacteria produce pseudocobalamin (11, 13, 14), an analog of cobalamin in which adenine substitutes for DMB as the α ligand (12) (Fig. 1). Production of pseudocobalamin in a natural marine environment has not been shown, nor have reasons for the production of this compound in place of cobalamin been elucidated.

To explore the pervasiveness of cobalamin and pseudocobalamin supply and demand in marine systems, we determined the standing stocks of these compounds in microbial communities from surface waters across the North Pacific Ocean using liquid chromatography mass spectrometry (LC-MS). Our LC-MS method (15) quantifies cobalamin and pseudocobalamin with different β ligands. We found that in the surface ocean, pseudocobalamin and cobalamin concentrations associated with organisms and detritus captured on a 0.2-μm filter (particulate fraction) were often of equal magnitude (Fig. 2*B*). Pseudocobalamin had peak concentrations within the euphotic zone at each station and was not detected below the euphotic zone. In contrast, cobalamin was measurable throughout the sampled waters and maintained similar or higher concentration from the lower euphotic zone to our deepest samples (Fig. 2*A* and Fig. S1).

The overlapping spatial distribution of cobalamin and pseudocobalamin suggests that these cofactors are produced in each other's presence, likely with different sources and sinks. To investigate correlations between Cyanobacteria and pseudocobalamin abundance, we compared observations of Cyanobacteria carbon inferred from

Significance

Cobalamin (vitamin B_{12})-dependent organisms span all domains of life, making procurement of the vitamin from the few prokaryotic producers an essential function in organismal interactions. Yet not all key producers of cobalamin have been identified in the ocean. We show that in the marine environment, select heterotrophic bacteria and Thaumarchaeota produce cobalamin, while Cyanobacteria, the most abundant phytoplankton on earth, supply and use pseudocobalamin. These chemically distinct cofactors support different members of the microbial community because they are not interchangeable as cofactors in enzymes. Our findings identify key organisms supporting cobalamin-based interdependencies that underpin primary production and microbial interactions in the ocean.

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Data deposition: The sequences reported in this paper have been deposited in the European Nucleotide Archive [accession no. PRJEB10943 (ERP012248)] (project title "Variable Influence of light on the activity of Thaumarchaea")].

[1]Present address: School of Biology, Georgia Institue of Technology, Atlanta, GA 30332.

[2]Present address: Department of Biochemistry, Biophysics and Molecular Biology, University of Minnesota, Minneapolis, MN 55455.

[3]To whom correspondence should be addressed. Email: aingalls@uw.edu.

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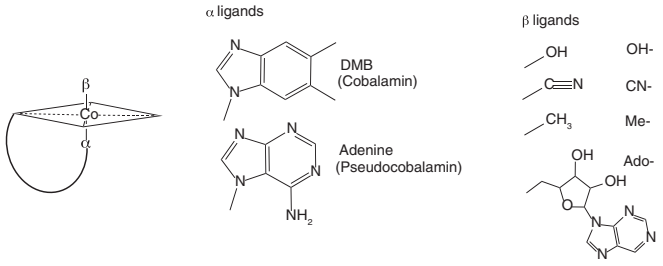

Fig. 1. General form of cobalamin analogs. Shown is a schematic of the conserved corrin ring with various upper (β) and lower (α) ligands. Structures of cobalamin analogs monitored in this study (eight total) are shown.

flow cytometry with pseudocobalamin measurements taken at the same depth. In cases where we had both continuous measurements (by SeaFlow) (16) and discreet flow cytometry data, we used the discreet measurements, as collection of these samples was closely coupled in time and space to pseudocobalamin sampling ($n = 16$ for discreet, $n = 4$ for continuous). Pseudocobalamin concentrations are statistically correlated with carbon from *Synechococcus* and *Prochlorococcus* ($R^2 = 0.71$, $P < 0.001$), both in the surface and into the subsurface ocean (Fig. 2*C*), suggesting a primarily cyanobacterial source. No significant correlation existed between Cyanobacteria carbon and cobalamin concentrations (Fig. S2).

To identify the major producers of cobalamin and pseudocobalamin in the environment, we investigated representative marine isolates and then expanded our search into available genomes that encompass the phylogenetic diversity at our study site. As expected (1, 8), two strains of marine Alphaproteobacteria with cobalamin synthesis genes (*Sulfitobacter* sp. SA11 and *Ruegeria pomeroyi* DSS-3) produced cobalamin, whereas the gammaproteobacterium *Vibrio fischerii* ES114 (which lacks cobalamin biosynthesis genes) did not (Table S1). Four pure strains of marine chemoautotrophic Thaumarchaeota (*Nitrosopumilus* spp. SCM1, HCE1, HCA1, and PS0) also produced cobalamin (Table S1), confirming earlier suggestions based on the presence of cobalamin biosynthesis genes in Thaumarchaeota genomes (10). Like other Cyanobacteria (11, 13, 14), four axenic strains of marine Cyanobacteria (*Prochlorococcus* MED4 and MIT9313 and *Synechococcus* WH8102 and WH7803) produced pseudocobalamin (Table S1). In all of the cobalamin or pseudocobalamin producers, we detected compounds with β ligands Me-, Ado-, and OH- but not CN- (Table S1).

The observed cell quotas of cobalamin or pseudocobalamin per cellular carbon varied greatly among producers (Table S1). Laboratory cultures of Alphaproteobacteria and *Procholorococcus* strains had lower amounts of cobalamin or pseudocobalamin (less than 1,200 nmol cobalamin analog per mole carbon) than *Synechococcus*

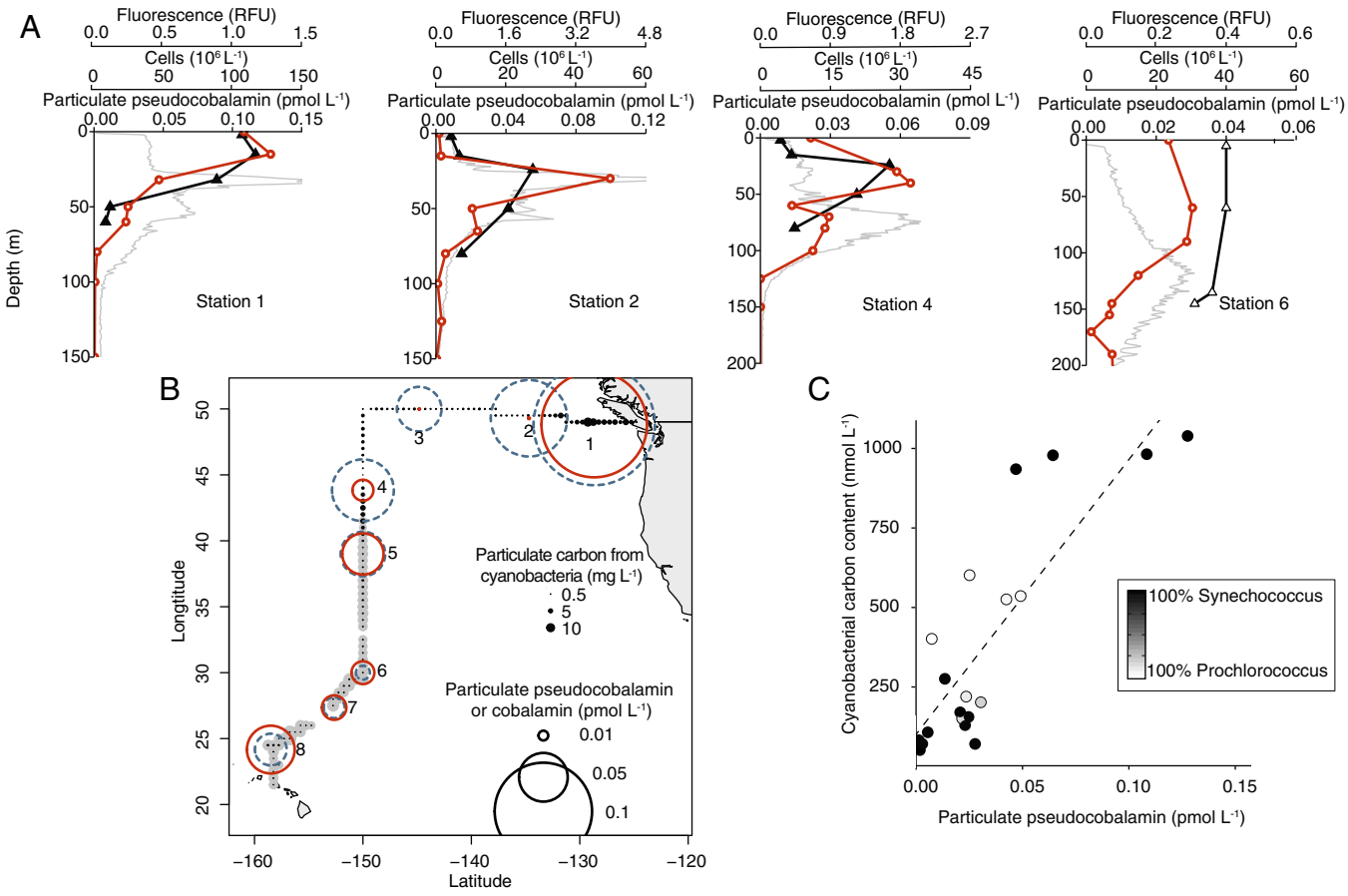

Fig. 2. Pseudocobalmin and Cyanobacteria cooccurrence. (*A*) Depth profiles of particulate pseudocobalamin (orange circles), in situ chlorophyll a (gray), and cell abundance of *Synechococcus* (closed triangles) or *Prochlorococcus* (open triangles), whichever was the dominant Cyanobacteria at each station. (*B*) Location of sampling with surface concentration of particulate cobalamin (dashed blue circles) and pseudocobalamin (orange circles). Closed circles are cyanobacterial (*Synechococcus*, black; *Prochlorococcus*, gray) carbon content calculated from cell abundance and size estimates (at 5 m via SeaFlow) (16). (*C*) Correlation between environmental pseudocobalamin and calculated Cyanobacteria carbon content ($n = 20$, $R^2 = 0.71$, $P < 0.001$). Cobalamin and pseudocobalamin concentrations are summed values of the detected β ligands for these compounds (Ado-, Me-, and OH- for cobalamin; Me- and OH- for pseudocobalamin); contributions of each beta ligand are provided in Dataset S2. Pseudocobalamin concentrations are presented in cobalamin equivalents (see *SI Materials and Methods*).

and Thaumarchaeota isolates (1,480–11,600 nmol cobalamin analog per mole carbon). Published values (17) for sea ice bacterial isolates estimated using a bioassay were highly variable (0.6–6,800 nmol cobalamin analog per mole carbon). In our environmental samples, we observed an average stoichiometry of 87 nmol pseudocobalamin per mole cyanobacterial carbon, lower than the cyanobacterial isolates (Fig. 2*C*). This finding suggests that the cellular stoichiometry of pseudocobalamin is variable and possibly influenced by environmental factors like nutrient availability and growth rate.

To expand the breadth of our survey beyond laboratory isolates, we inspected publically available whole genome sequences from bacteria and Thaumarchaeota for evidence of cobalamin biosynthesis. This analysis expands on previous work (18) while focusing on the phylogenetic groups present at our study site. We analyzed full genomes from the Integrated Microbial Genomes (IMG) database (https://img.jgi.doe.gov) from phylogenetic groups that encompassed >99.9% of the Bacterial 16S rRNA gene sequences from our environmental samples to develop a systematic inference of cobalamin synthesis capacity (3,410 genomes). Alpha- and Gammaproteobacteria are hypothesized to be major marine cobalamin producers (1, 10), and 94% of the surveyed genomes from these groups that contain genes necessary for corrin biosynthesis (i.e., *cbiA*/*cobB*, *cbiH*/*cobJ*) (2) also have genes for DMB synthesis and activation (*bluB*, *cobT*) (2, 19, 20) (Fig. 3), consistent with the synthesis of cobalamin and the results from our representative cultures. All of the eight available high-quality Thaumarchaeota genomes in the IMG database code for corrin and DMB biosynthesis genes (Fig. 3). Most of the lower quality, incomplete genomes available follow this same pattern (17/19, Dataset S1). No Thaumarchaeota genomes possess the *cobT* gene and thus must activate DMB through a different pathway. Of the 255 cyanobacterial whole genomes, 247 possessed genes for the synthesis of the corrin ring, but only one genome possessed an annotated *bluB* or *cobT* gene (Fig. 3), suggesting the vast majority of Cyanobacteria are unable to produce DMB, in agreement with a recent study that examined a subset of the available Cyanobacteria genomes (11).

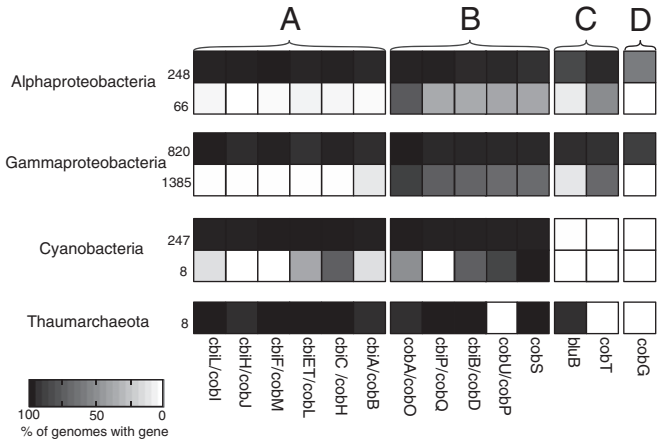

Fig. 3. Presence or absence of annotated cobalamin biosynthesis genes in full-genome representatives. Four major groups of microbes contributing cobalamin biosynthesis genes in marine surface waters (10) are shown; each group is split into potential cobalamin producers (top row) and non-cobalamin producers (bottom row), with the number of genomes in each group. All of the high-quality Thaumarchaeota genomes are potential cobalamin producers. The shade of each box indicates the percent of genomes with that gene. Genes are grouped as follows: (*A*) corrin ring biosynthesis genes that are common to both O_2-dependent and -independent pathways in bacteria and archaea, (*B*) final synthesis and repair genes, (*C*) genes for DMB synthesis and activation, and (*D*) O_2-dependent *cobG* in the O_2-dependent corrin ring biosynthesis pathway. In Archaea, *cobY* is used in place of *cobU*/*cobP* (10, 55). For the list of genomes in each group, see Dataset S1, summarized in Table S1.

All of the 49 *Prochlorococcus* genomes have genes for corrin synthesis without genes for DMB synthesis or activation, and our analysis demonstrated that *Prochlorococcus* MED4, with its highly streamlined genome (21), has maintained these genes to synthesize pseudocobalamin. We propose this originates from an ancient specialization to the production and use of pseudocobalamin in lieu of cobalamin among Cyanobacteria. Biosynthesis of the corrin ring can occur via two separate pathways: an O_2-dependent or an O_2-independent pathway (2, 18). DMB synthesis can also occur via two separate routes, the O_2-dependent BluB (19) or the O_2-independent and O_2-sensitive Bza pathway (22). Rhodobacters have the O_2-dependent corrin ring and DMB pathways (11), whereas Thaumarchaeota likely possess the O_2-independent pathway for the corrin ring and the O_2-dependent DMB pathway (10). In some bacteria, pseudocobalamin can be produced if DMB synthesis is impaired; this is due to the natural presence of adenine in cells and enzyme substrate promiscuity that allows adenine to substitute for DMB in some organisms' CobT (22–27). Cyanobacteria use the O_2-independent pathway for corrin ring synthesis and neither pathway for DMB synthesis (11, 18) (Fig. 3). The use and production of cobalamin as a cofactor predates oxygenic photosynthesis (28, 29). Possessing an O_2-independent mode for producing a cobalamin analog that is not impaired by O_2 may have been necessary for the success of oxygenic photosynthetic Cyanobacteria that were largely responsible for the rise of O_2 on earth and likely endured variable O_2 concentrations over time (30).

Cyanobacteria use pseudocobalamin as a cofactor in two enzymes: methionine synthase (MetH) and class II ribonucleotide reductase (NrdJ). The 3D structure of MetH contains three β pleated sheets and two α helices that form a pocket for the DMB ligand of cobalamin in *Escherichia coli* (31, 32). Cyanobacterial MetH is predicted to form the same pocket (13). However, conserved amino acids within this pocket in the cyanobacterial MetH differ from sequences of organisms known to use cobalamin (Figs. S3 and S4), suggesting a structure that preferentially binds pseudocobalamin in place of cobalamin as experimentally demonstrated in the Cyanobacteria *Arthrospira* (13). Many Cyanobacteria also code for O_2-independent NrdJ (33), which has limited sequence similarity to noncyanobacterial NrdJ (34, 35). Similar to pseudocobalamin biosynthesis in Cyanobacteria, NrdJ is both O_2-independent and O_2-insensitive and has been hypothesized as an important bridge between the pre- and postoxygenated oceans (36). The continued maintenance of both the biosynthetic pathway and pseudocobalamin-dependent enzymes throughout the diverse Cyanobacteria phylum (from *Arthrospira* and *Synechocystis* to the highly streamlined *Prochlorococcus*) suggests the production of pseudocobalamin is an ancient relic that persists in the oxic marine environment today.

For many eukaryotic algae, pseudocobalamin supports lower growth yields than cobalamin (11, 37, 38). We examined the underlying cause of this reduced growth by supplying the model diatom *Thalassiosira pseudonana* (CCMP 1335) with pseudocobalamin and tracking it into the cells. Like others, we found that growth of *T. pseudonana* is limited at 1 pM cobalamin (39), and the addition of pseudocobalamin (at 200 pM) is unable to overcome this limitation (11, 38). We observed that *T. pseudonana* takes up the inactive OH-pseudocobalamin from their growth media and converts it into the cofactor form Ado-pseudocobalamin yet is unable to recover to cobalamin-replete growth rates (Fig. 4, Fig. S5, and Table S2). The role of Ado-cobalamin in diatoms is unclear, although *T. pseudonana* does code for Ado-cobalamin–dependent methylmalonyl-CoA mutase (MCM) and actively transcribes a putative adenosylcobalamin transferase (which converts OH-cobalamin to Ado-cobalamin) under cobalamin limitation (39). Previous studies suggest that when diatoms are starved for cobalamin, low Me-cobalamin (required for MetH activity) deprives cells of S-adenosylmethionine (SAM) (7, 39). We found that when pseudocobalamin is supplied to cobalamin-limited *T. pseudonana*, they contain significantly less SAM per cell than cobalamin-replete conditions (Fig. 4, Fig. S5, and Table S2). The depressed levels of SAM and lack of detectable Me-pseudocobalamin within cells suggest that *T. pseudonana*

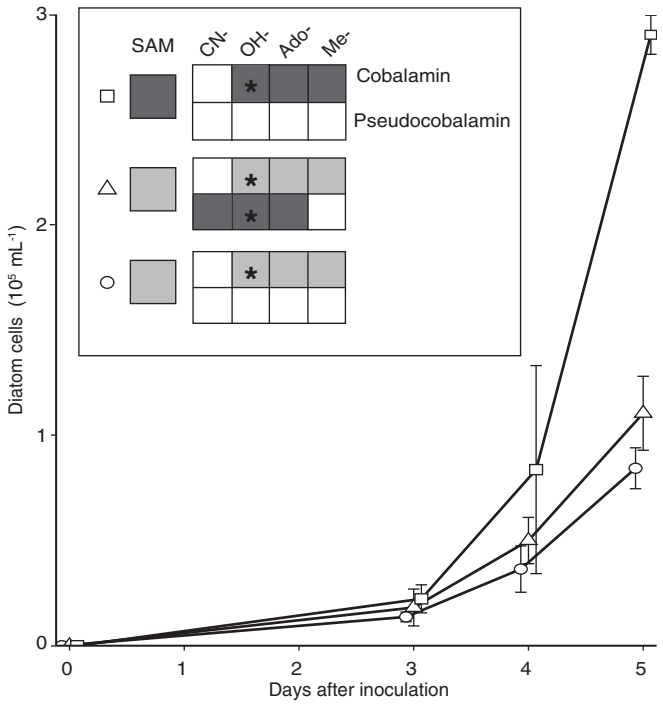

Fig. 4. Growth of the diatom *T. pseudonana* with pseudocobalamin. (*A*) Growth of diatoms under Control (1 pM cobalamin, circle), +Pseudocobalamin (1 pM cobalamin with 200 pM pseudocobalamin, triangle), and +Cobalamin (200 pM cobalamin, square) treatments over time; error bars, SD from three replicates. *Inset* shows the final SAM, cobalamin, and pseudocobalamin cellular contents compared with Control treatment on a per cell basis: not detected (white), similar range to control (light gray), or statistically higher than control (Student's unpaired *t* test, $P < 0.05$, dark gray). Black stars indicate what forms of cobalamin or pseudocobalamin were added to each treatment. See Fig. S5 for absolute values of cobalamins and SAM.

are unable to efficiently use pseudocobalamin in their MetH. This same phenomenon has been demonstrated physiologically in marine cobalamin-dependent bacteria: Cobalamin or methionine stimulates growth, but pseudocobalamin does not (40). These combined findings demonstrate that marine Cyanobacteria achieve both independence and a competitive advantage by producing and requiring only pseudocobalamin; they meet their own need for their preferred cofactor while also not directly supplying cobalamin to other photoautotrophs.

Corrin synthesis genes in oligotrophic surface water metagenomes are dominated by pseudocobalamin-producing Cyanobacteria (10), suggesting that de novo production of true cobalamin in these regions is limited to a small subset of heterotrophic bacteria, including clades like Rhodobacterales that are commonly associated with eukaryotic algae (9). Cobalamin auxotrophs may foster close physical or chemical relationships with these cobalamin producers, offsetting the expense of cobalamin biosynthesis to secure a consistent cobalamin source. Alternatively, organisms may employ techniques to use closely related compounds like pseudocobalamin. Many organisms without the biosynthetic capacity for de novo production of cobalamin have salvage or remodeling strategies for reactivating cobalamin analogs (11, 20, 41). A recent study has shown that some eukaryotic algae have the genetic capacity to make cobalamin when supplied with pseudocobalamin and DMB, although this phenomenon may be limited by low DMB concentrations in natural seawater (11), and DMB biosynthesis seems to be limited to bacteria and archaea. Of the 3,408 phylogenetically representative prokaryote genomes we surveyed, we found 73 genomes that did not have the biosynthetic pathway for the corrin moiety of cobalamin but did have genetic capacity for cobalamin repair as well as synthesis and activation of DMB, which has no known role in cells beyond α ligand of cobalamin. These organisms include several heterotrophic bacteria known to occur in marine environments such as *Methylophaga* and *Marinobacter* (Dataset S1). Previous work has suggested that ligand-bound cobalt in low latitudes may be cobalamin degradation or precursor compounds, which are present at concentrations much higher than has been observed for dissolved cobalamin (1, 15, 42). In these environments, organisms capable of salvaging and repairing cobalamin degradation products or precursors could be major suppliers of cobalamin to the environment and de novo biosynthesis may take a minor role. The prevalence of cobalamin salvage pathway transcripts in marine systems (6) suggests that synthesizing DMB or remodeling cobalamin is a common strategy that plays an important role connecting the production of pseudocobalamin to the growth of cobalamin auxotrophs in the low latitude oceans where Cyanobacteria are abundant.

We inspected genomes of marine prokaryotes to identity likely cobalamin producers in our study site. We combined bacterial 16S rRNA gene sequence-derived phylogenetic data with current knowledge of corrin biosynthesis genes (including our analysis)

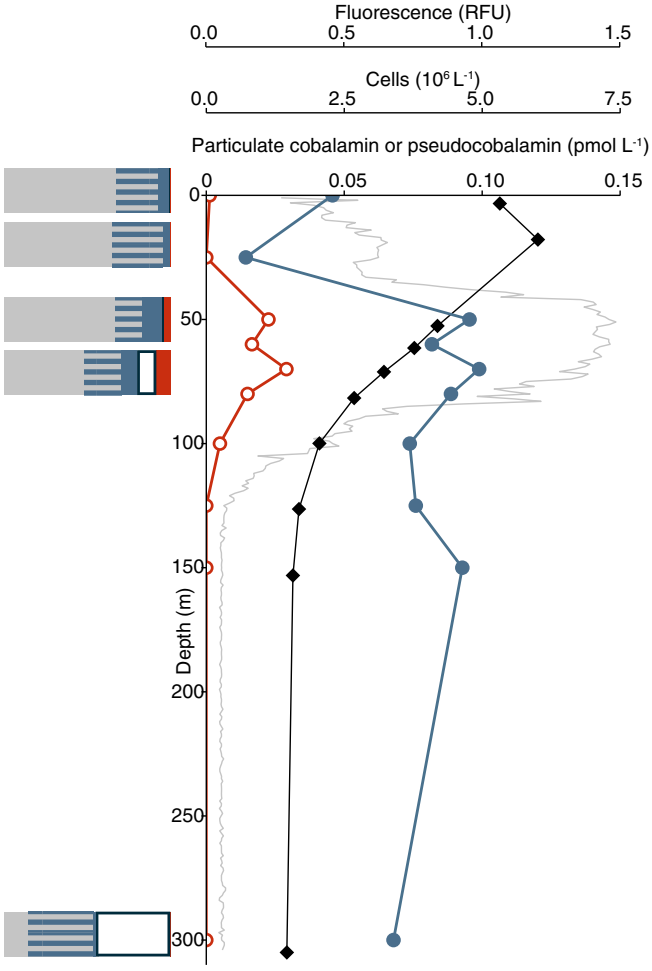

Fig. 5. Pseudocobalamin and cobalamin producers at station 3. Shown are particulate cobalamin (closed blue circles), pseudocobalamin (orange open circles), prokaryote cell abundance (black diamonds), and in situ fluorescence (gray). Bar graphs show prokaryote population with the following predicted cobalamin strategies: unlikely producers (gray), bacteria with unknown cobalamin biosynthesis capacity (gray/blue striped), bacteria that are likely to produce cobalamin (blue), Thaumarchaeota (likely to produce cobalamin, white), and Cyanobacteria (likely to produce pseudocobalamin, orange) at the corresponding depths. Cobalamin and pseudocobalamin concentrations are as in Fig. 2.

across different phylogenetic groups to infer the cobalamin biosynthetic capacity of organisms in the microbial communities at our study site as likely, unknown, or unlikely (Table S3). We quantified Thaumarchaeota by quantitative PCR (qPCR) (43, 44) and calculated their contribution to the microbial population by comparing this value to direct counts of prokaryotic cells determined for each sample. Our analysis at five targeted locations in the North Pacific suggested that Thaumarchaeota represent 30–80% of prokaryotes having likely or unknown genetic capacity to synthesize cobalamin in the lower euphotic zone and deeper (Fig. 5 and Fig. S1). High cobalamin contents on a per carbon basis in cultured Thaumarchaeota implicate them as a major source of cobalamin in deeper waters (Table S1).

Like *Prochlorococcus*, marine Thaumarchaeota have maintained several genes committed to the biosynthesis of cobalamin in their small genomes. Detection of MCM, NrdJ, BluB, and cobalamin biosynthesis proteins in the proteome of an oceanic thaumarchaeote with a highly streamlined genome, "*Candidatus* Nitrosopelagicus brevis," implies that cobalamin is actively produced and used in these microbes (45). In Thaumarchaeota, the cobalamin-dependent MCM catalyzes a key step in their exceptionally energy-efficient pathway for carbon fixation (46–48). The scarcity of dissolved cobalamin in the water column (often <1 pM as assessed by bioassay) (1) and enzymatic demands like MCM may have necessitated that Thaumarchaeota retain the ability to synthesize cobalamin. Thaumarchaeota likely play a critical role in the microbial loop in the lower euphotic zone and deeper by providing an essential nutrient to cobalamin auxotrophs. In turn, the auxotrophs provide substrates that promote Thaumarchaeota growth (49, 50)—most critically the ammonia required by the ammonia-oxidizing Thaumarchaeota (46).

Although Thaumarchaeota and select heterotrophic bacteria synthesize cobalamin, undoubtedly benefiting from being their own source of their preferred cofactor, the production of dissimilar cobalamin analogs by marine Cyanobacteria is likely a result of their distinct ecological niches, enzymatic demands, and interactions with other cobalamin-dependent organisms. Producers of cobalamin and related compounds thus play distinct roles in cobalamin-based microbial interdependencies that sustain primary productivity and shape marine community structure.

Materials and Methods

Environmental samples for cobalamin and pseudocobalamin, phytoplankton abundance, archaeal gene quantification, prokaryotic cell abundance, and DNA for 16S rRNA sequencing were collected in August 2013 along the historical transect Line P to ocean station papa (our station 3), then following 150 W to the south into the North Pacific subtropical gyre sampling from the surface down to a maximum depth of 300 m, as shown in Fig. 2. Culture and environmental samples were analyzed for cobalamins, pseudocobalamins, and SAM using an organic solvent extraction (51) paired with LC-MS (15), both modified as described in *SI Materials and Methods* (Figs. S6 and S7 and Table S4). Phytoplankton abundance was monitored continuously using SeaFlow (16), in addition to discreet samples taken at depth and analyzed by flow cytometry.

We investigated 11 axenic strains of marine prokaryotes for demonstrable evidence of cobalamin or pseudocobalamin production: four strains of *Nitrosopumilus* spp. (SCM1, HCA1, HCE1, and PS0), two strains of *Prochlorococcus* (MED4 and MIT9313), two strains of *Synechococcus* (WH7803 and WH8102), *Sulfitobacter* sp. SA11 (52), *R. pomeroyi* DSS-3, and *V. fischerii* ES114. We used the model diatom *T. pseudonana* to investigate the fate of pseudocobalamin in eukaryotic algae by culturing it under three conditions: cobalamin limited, cobalamin replete, and cobalamin limited with pseudocobalamin. To compare cobalamin- and pseudocobalamin-binding sites in MetH, we gathered MetH amino acid sequences from organisms known to use true cobalamin or pseudocobalamin as their cofactor. We then aligned the sequences and used a known crystal structure (32, 53) to visualize the binding pocket.

We used publically available full genomes from the IMG database that phylogenetically represent organisms at our study site and searched for cobalamin biosynthesis genes in these genomes (genomes and functions we used are listed in Dataset S1). We quantified Thaumarchaeota via qPCR and performed direct cell counts to quantify total prokaryotes as previously described (43, 44, 54). DNA for 16S rRNA sequencing was extracted, amplified, and sequenced as described in *SI Materials and Methods*. Operational taxonomic units (OTUs) were called using a 97% nucleotide identify threshold, and taxonomic inference was based on the SILVA rRNA gene database (https://www.arb-silva.de). This yielded phylogenetic information we combined with the current knowledge of cobalamin-biosynthesis capacity (from the literature and our whole genome analysis) to estimate the contribution of Thaumarchaeota to the prokaryotic community with the potential for cobalamin biosynthesis capacity at our sampling sites. Further details on all aspects of the methods are given in *SI Materials and Methods*. Environmental data are supplied in Dataset S2.

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