Diversity, prevalence, and expression of cyanase genes (cynS) in planktonic marine microorganisms

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Cyanate (OCN⁻) is an oxidation product of cyanide and a decomposition product of urea [1, 2]. It is considered as an organic nitrogen source for diverse prokaryotic and eukaryotic microbes in terrestrial and aquatic ecosystems [2–7] with concentrations in the nanomolar range [4, 7–9]. Cyanate is also formed intracellularly from urea and carbamoyl phosphate, making it part of the central nitrogen metabolism [10–12]. In spite of the central metabolic role of cyanate, it has received much less attention than other organic nitrogen compounds, particularly for marine environments. However, it has been found that cyanate is likely an essential N source for cyanobacteria in oligotrophic oceans [2, 6, 13, 14] and an alternate N substrate for marine nitrification and anammox [15–17].

Cyanate metabolism relies on the well-characterized enzyme cyanase, which catalyzes the reaction of cyanate with bicarbonate to produce ammonium and carbon dioxide [18]. The cyanase gene cynS has been identified in many terrestrial and aquatic species and was reported to play a significant role in the assimilation of exogenous cyanate and detoxification of internally generated cyanate [6, 13, 16, 19–28]. However, knowledge on the diversity and evolution of cynS in marine microbes is rather limited, including its prevalence in the oceanic system.

To address this knowledge gap, we retrieved 260 cynS homologs across the tree of life (Table S1) based on the full-length cDNA from the marine dinoflagellate *Alexandrium pacificum* (APcynS, 653 bp, GenBank accession number: MZ666876) (Fig. S1; Table S2) and its deduced amino acid sequence (APcyanase). This reference dataset was used to query marine metagenomes and metatranscriptomes using the Ocean Gene Atlas (OGA) [29] to explore the biogeography and in situ expression pattern of cynS (full methods were described in supplements).

An amino-acid alignment composed of 260 homologs revealed the presence of nine ultra-conserved residues (Fig. 1a), potentially responsible for the catalytic activity and structural stability. Six of them have not been documented before [25, 30–32]. Modeling of the 3D enzyme structure indicated the following subunit organization: a decameric holoenzyme with a core formed by five dimers [30, 31] (Fig. 1b, f; Fig. S2). Five active sites are located between dimers forming an inner ring (Fig. 1c, g), non-covalently bound with five oxalate di-anions (Fig. 1d, e, h, i; Table S3), indicating the possible binding sites of cyanate. In *E. coli*, there are four types of residue-oxalate interactions known for binding cyanate (Fig. 1j) [30]. However, only type 1 is present in modeled APcyanase (Fig. 1j), suggesting reduced plasticity in binding cyanate. Whether this structural variation translates into different binding affinities and therefore potentially physiological roles of the APcyanase remains to be seen.

Four major clades of cynases were identified based on their phylogenetic relationships (Fig. 1k, Fig. S3). Interestingly, horizontal...
gene transfer (HGT) of cynS contributed to the evolution of bacteria, archaea, and eukaryotes including microalgae (Bathycoccus, Micromonas) and metazoans, which provides evidence that HGT of cynS is more common than previously documented [3, 6, 13, 16, 27, 33–35].

To contextualize cyanate metabolism in the upper ocean from the surface (epipelagic) down to the intermediate depths of ca. 1000 m (mesopelagic (MES)), we analyzed the prevalence and expression of cynS in comparison to ureC, the gene encoding the urease subunit alpha. The urea cycle, unlike cyanate metabolism, is well studied in many marine microbes including the acquisition of urea as an organic nitrogen source. Homologs of both genes and their corresponding transcripts could be retrieved from almost all sampling stations of the OGA (Table S4), which suggests their overall prevalence in many marine microbes. However, the normalized gene activity of cynS and ureC differed depending on the size class, taxonomic group, and the water depth (Fig. 2; Fig. S4). Interestingly, the transcriptional activity of ureC appears to be much lower compared to cynS in the larger size fraction (0.8–2000 μm) mostly representing eukaryotic microbes and for both, surface (SRF) and deep chlorophyll maximum (DCM) (Fig. 2a, b, f, g). Pelagophytes, dinophytes, bacillariophytes, and fungi contributed the most cynS transcripts in the epipelagic ocean with pelagophyte transcripts dominating the surface layer (Fig. 2a, b). Transcripts for both genes in the smaller size fraction (0.22–3 μm) were mostly derived from prokaryotes (Figs. 2c–e, 2h). In the surface ocean, Synecococcus contributed most of the cynS transcripts in non-polar oceans whereas picoclorhophyte cynS transcripts were most dominant in the coastal Arctic (Fig. 2c). In contrast, proteobacteria together with unclassified microbes contributed most of the ureC transcripts in surface ocean metatranscriptomes regardless of geography (Fig. 2h). The taxonomic contributions of the ureC transcripts did not change much for the DCM and not even the MES zone although Gammaproteobacteria appear to have contributed more ureC transcripts in the MES compared to the epipelagic (Fig. 2i, j). By comparison, unclassified microbes together with Prochlorococcus contributed more cynS transcripts in the DCM (Fig. 2d). For the MES, most of the cynS transcripts were contributed by unclassified microbes, Nitrosopinae and Proteobacteria (Fig. 2e).

The abundance of cynS transcripts from most prokaryotic and eukaryotic pycholeptokon was negatively correlated (p < 0.05) with dissolved inorganic nitrogen concentrations (Fig. S5a, b; Table S5a, b). The latitudinal differences in the taxonomic contributions of cynS correlated negatively with temperature (p < 0.05), suggesting that cyanates are induced in picochlorophytes by low temperature. As no Arctic samples were available for the larger size fraction (0.8–2000 μm) representing mainly eukaryotes, it remains elusive if this group of organisms does have similar cynS expression patterns under polar conditions. Although much less cynS transcripts were detected in the MES, the contribution of Nitrospinae was more significant compared to the epipelagic (Fig. 2e). Members of the phylum Nitrospinae are known to be the most abundant nitrite-oxidizing bacteria (NOB) in the oceans with an important role in dark-ocean carbon fixation [3, 16]. Cyanate metabolism of NOBs is common and essential for the global nitrogen cycle, supplying ammonia oxidizers with ammonium, which is nitrified by this nitrifying consortium.
the deep chlorophyll maximum (DCM) and the mesopelagic (MES). Fig. 2

CynS Transcript Abundance

UreC Transcript Abundance

We observed a high abundance of transcripts from Nitrospinae in the MES zone (Fig. 2j) and positively correlated with depth and the concentration of nitrate (p < 0.05, Fig. S5b; Table S5b), suggesting that cyanate metabolism in Nitrospinae may facilitate marine nitrification. In contrast, CynS was not detected in marine ammonia-oxidizing archaea of the phylum Thaumarchaeota. However, the ureC transcript from this taxon was detected mainly in MES zone (Fig. 2l) and positively correlated with depth and the concentration of nitrate (p < 0.05, Fig. S5b, Table S6b). This corroborates previous findings as marine Thaumarchaeota genomes lack the canonical CynS gene but the organisms can utilize cyanate and urea to fuel nitrification [15]. The contents of all the retrieved unigenes from OGA have been summarized in Supplementary Tables S7–S14.

Fig. 2 Biogeographic distribution and taxonomic composition of CynS and ureC transcripts in the global ocean from the surface (SRF) to the deep chlorophyll maximum (DCM) and the mesopelagic (MES). CynS (a–e) and ureC (f–j) in eukaryote-enriched (0.8–2000 μm fraction) metatranscriptomes. Samples from different size fractions have been pooled in each station. No data are available for 0.8–2000 μm fraction in MES.

Abundance Level
(Percent of total RPKM per station)

1e-3
5e-4
1e-4
5e-5
1e-5

Sampling Layer

SRF: surface layer
DCM: deep chlorophyll maximum
MES: mesopelagic zone

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including NOBs [3]. In our study, CynS transcripts from Nitrospinae in the epipelagic layers were limited to only few stations in the Eastern Pacific and Arctic Ocean (Fig. 2c, d). However, more prevalent and abundant were these transcripts in the MES (Fig. 2e). The abundance of CynS transcripts from Nitrospinae was positively correlated with nitrate and nitrite (p < 0.05, Fig. S5b; Table S5b), suggesting that cyanate metabolism in Nitrospinae may facilitate marine nitrification. In contrast, CynS was not detected in marine ammonia-oxidizing archaea of the phylum Thaumarchaeota. However, the ureC transcript from this taxon was detected mainly in MES zone (Fig. 2l) and positively correlated with depth and the concentration of nitrate (p < 0.05, Fig. S5b, Table S6b). This corroborates previous findings as marine Thaumarchaeota genomes lack the canonical CynS gene but the organisms can utilize cyanate and urea to fuel nitrification [15]. The contents of all the retrieved unigenes from OGA have been summarized in Supplementary Tables S7–S14.

Taken together, CynS is a conserved gene ubiquitous across the tree of life, transferred frequently via HGT. Comparative analyses based on the prevalence and expression of CynS and ureC representing intertwined processes of organic N metabolism in marine microbes suggest that cyanate is at least as important as urea in the oceans. Cyanate likely supports the assimilation of organic N in photoautotrophs when inorganic N is scarce and it appears to contribute to remineralisation by the activity of nitrifying bacteria which produce nitrate in deeper layers of the oceans.
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