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Streamlined Regulation and Gene Loss as Adaptive Mechanisms in *Prochlorococcus* for Optimized Nitrogen Utilization in Oligotrophic Environments

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INTRODUCTION

Cyanobacteria are one of the oldest groups of organisms on Earth (69). They have colonized almost every available niche for the last 3.5 billion years, demonstrating an outstanding ability for adaptation to extremely different habitats. Thus, it was somehow not surprising to discover abundant cyanobacteria (27) (namely, *Synechococcus* [78] and *Prochlorococcus* [6]) thriving in environments with a very poor nutrient supply, such as the vast intertropical gyres of the oceans (4, 28, 83), which were previously considered to be almost empty of living cells. However, their ecological importance was truly unexpected, since it is currently accepted that about half of the global primary production occurs in the oceans (81) and that marine cyanobacteria contribute two-thirds of it (16, 35). Since the discovery of *Prochlorococcus* in 1988 (6), its major importance in the ecology of the oceans has become evident, leading to a large number of studies (59) and to the sequencing of the genomes of five representative ecotypes, MED4, MIT9313, MIT9312, and NATL2A (http://www.jgi.doe.gov/JGI_microbial/html/index.html) and SS120 (http://www.sb-roscoff.fr/Phyto/ProSS120/), making *Prochlorococcus* one of the most-studied microorganisms from a genomic point of view (8, 24, 65).

The striking ecological success of *Prochlorococcus* has been the subject of many studies, focused on some of the most intriguing abilities of this organism, e.g., to live at depths of up to >200 m. This means that *Prochlorococcus* has to cope with the natural gradients of different parameters occurring along the water column, including light irradiance, which decreases almost 4 orders of magnitude from the ocean surface to the end of the euphotic zone. These conditions have induced the occurrence of a number of peculiarities and a remarkable diversity in the photosynthetic apparatus of *Prochlorococcus* (for recent reviews, see references 58 and 73), enabling these microorganisms to efficiently harvest very low levels of light energy. These are some of the keys to the ubiquity and abundance of *Prochlorococcus* in the oceans (57). However, there also exist other less obvious, but not less important, gradients that could provoke similar adaptive modifications in metabolic pathways, including gradients of temperature, pressure, UV light penetration, or nutrient availability. Nitrogen is one of the key environmental factors in the ocean (29, 83), and nitrogen gradients have been proposed as one of the main forces driving the evolution of *Prochlorococcus* (36, 47, 73). This review focuses on the adaptive features of the nitrogen assimilatory pathway of *Prochlorococcus*, with special emphasis on a comparative analysis with other cyanobacteria (in particular, the coexistent marine cyanobacterium *Synechococcus*) (67, 78).

ADAPTATION TOWARD THE ASSIMILATION OF SELECTED NITROGEN SOURCES AND SIMPLIFICATION OF THE REGULATORY NETWORKS

Cyanobacteria can use a wide variety of nitrogen sources available in nature, including molecular nitrogen, nitrate, nitrite, ammonium, urea, and some amino acids, such as arginine or glutamine (12). The oxidation state of these molecules has direct consequences for the energy required for their assimilation, as the most oxidized forms of nitrogen (molecular nitrogen or nitrate) are rather expensive to utilize (Table 1). The enormous amount of ATP necessary for the breakdown of molecular nitrogen (16 ATPs per N_2) explains why nitrogenase is far from ubiquitous in nature and, particularly, its absence in *Prochlorococcus*. Also, a comparison between the full reduction of nitrate to glutamate versus ammonium to glutamate (Table 1) shows that one ATP is necessary in both cases but that nitrate reduction requires fivefold more electrons than ammonium reduction (i.e., 10 electrons for nitrate reduction to glutamate versus 2 electrons for ammonium reduction to glutamate), making nitrate reduction much more expensive in bioenergetic terms. We can make a simple calculation, based on the estimations proposed by Losada et al. (37). Let us

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TABLE 1. Bioenergetics of nitrogen source assimilation

Enzyme(s)	Gene(s)	Step	No. of electrons	No. of ATP molecules
Nitrogenase	*nif* cluster	$N_2 \rightarrow NH_4^+$	8	16
Nitrate reductase	*narB*	$NO_3^- \rightarrow NO_2^-$	2	0
Nitrite reductase	*nirA*	$NO_2^- \rightarrow NH_3^+$	6	0
Glutamine synthetase/ glutamate synthase	*glnA*/*gltB*	$NH_3^+ \rightarrow$ Glu	2	1

assume that all reducing power from cells is consumed by carbon and nitrogen reduction. If we consider that the C/N ratio in nutrient-replete *Prochlorococcus* cells is ca. 5 to 10 (2, 21) and that reduction of the carbon atom from CO_2 to (CH_2O) requires four electrons (37) while the reduction of the nitrogen atom from NO_3^- to glutamate requires 10 electrons (37), then a hypothetical *Prochlorococcus* strain utilizing nitrate would expend 20 to 33% of the total reducing power in nitrogen assimilation. If we then consider that *Prochlorococcus* switches to utilizing only ammonium as a nitrogen source, the reducing power expense in ammonium would be decreased by a factor of 5, i.e., to 4 to 6.6%, liberating the remaining (18 to 26.4%) for other physiological needs. This rough estimation shows the importance of preferential utilization of ammonium when it is available in the environment. Furthermore, if we take into account that nitrate is most abundant at greater depths, where there is a strong energy limitation due to low light penetration, it is clear that avoiding the assimilation of nitrate could be essential to optimize energy utilization in order to fulfill all metabolic requirements.

Consequently, ammonium is the preferred nitrogen source for most cyanobacteria. Furthermore, the regulatory mechanisms of nitrogen assimilation in these microorganisms are usually built upon the basis of detecting the presence or absence of ammonium in the environment. This complex regulatory network includes a transcriptional factor, the global nitrogen regulator, NtcA (22), and the signal transduction protein P_{II} (72), which coordinates the metabolism of nitrogen and carbon in the cell.

Although some cyanobacteria are able to fix molecular nitrogen, the central nitrogen assimilatory pathway, which is common for most of these microorganisms, is composed of the nitrate reductase (Nar), nitrite reductase (Nir), and glutamine synthetase/glutamate synthase cycle (GS/GOGAT) (Fig. 1A).

Some unicellular marine cyanobacteria (assigned to the genera *Synechococcus* [45] and *Synechocystis* [84]) perform nitrogen fixation in the oceans. However, no *Prochlorococcus* isolate has been shown to utilize molecular nitrogen to date, in good agreement with the lack of *nif* genes (involved in nitrogen fixation) in the studied *Prochlorococcus* genomes (8, 65). This suggests that the evolutionary constraints that affect the genome size (70) and the low light energy available at depth in the oceans (14) prevented *Prochlorococcus* from utilizing this ubiquitous (4), but very expensive, nitrogen source.

The first unusual and probably most surprising traits of nitrogen assimilation in all studied strains of *Prochlorococcus* are their inability to utilize nitrate (9, 36, 47, 56, 63) and the fact that only some low-light-adapted isolates grow on nitrite (47), while most coexistent *Synechococcus* strains can assimilate both nitrogen sources (7, 13, 47, 67). Physiological studies have shown the lack of nitrate reductase in both high- and low-irradiance-adapted *Prochlorococcus* strains (36). Furthermore, in nonaxenic *Prochlorococcus* cultures transferred to media containing nitrate as the sole nitrogen source, it has been observed that no reduced nitrate (either nitrite or ammonium) was transferred from the heterotrophic contaminant bacteria to *Prochlorococcus* (36). Genomic analysis of strains MED4 (equivalent to PCC 9511 [63]), MIT9313, and SS120 confirmed the absence of several genes required for nitrate assimilation (8, 47, 65). This is an apparent ecological contradiction, as nitrate is considered to be the main nitrogen source at depth in the oceans (4), where *Prochlorococcus* is abundant. In the lower parts of the euphotic zone, however, the available light is very limited, and amino-acid-like molecules could provide less expensive reduced nitrogen forms to *Prochlorococcus* (20). Recent observations showing the higher rate of organic nitrogen compound uptake by *Prochlorococcus* than by *Synechococcus* (85) fit nicely with this hypothesis. On the other hand, the evolutionary pressure probably induced a fine equilibrium between nutrient and energy requirements. Since the genome of *Prochlorococcus* is subjected to a process of compaction with deletion of nonessential genes (24, 70), the lack of nitrate utilization could provide selective advantages in ocean niches where there are more convenient nitrogen sources available.

Oceanographic studies in the field could, however, widen this picture: since its discovery, it was noted that maximal abundances of *Prochlorococcus* occurred slightly above the nitracline (6, 50, 75). Besides, addition of nitrate stimulated *Prochlorococcus* cell cycling in the Mediterranean Sea (75), and nitrogen enrichment provoked an increase in *Prochlorococcus* abundance in the North Atlantic (18). Moreover, there are preliminary studies suggesting the possible occurrence of some *Prochlorococcus* isolates utilizing nitrate (47, 82). Therefore, the existence of specific *Prochlorococcus* ecotypes that are capable of nitrate assimilation seems rather probable. This would be consistent with the currently accepted model of *Prochlorococcus* as a recent organism in evolutionary terms, derived from *Synechococcus*-like ancestors adapted to iron-depleted areas of the oceans (31, 73) in a progressive process of genome reduction (24, 70), including the described losses of phycoerythrin (23, 24, 62) and nitrate/nitrite reductases (8, 36, 47, 65) in specific strains. Whatever the case, *Prochlorococcus* remains to date the only described genus of cyanobacteria in which all of the studied strains are unable to grow on nitrate as the sole nitrogen source (36, 47).

The utilization of organic nitrogen compounds by *Prochlorococcus* has been hypothesized (36) to explain the lack of nitrate assimilation at depths where nitrate is abundant. Only very recently, however, flow cytometry uptake studies in the field with radiolabeled methionine provided the proof of amino acid uptake by *Prochlorococcus* (85). Nevertheless, although this observation is consistent with the presence of putative amino acid transporters in the genomes of these microorganisms (8, 65), preliminary results obtained with *Prochlorococcus* in laboratory cultures suggest that differential amino acid utilization could also occur between different ecotypes (O. Rangel, J. Diez, and J. M. García-Fernández, unpublished results). For example, strain PCC 9511 does not utilize arginine, glutamine, or glutamate for growth, and addition of glycine, proline, glutamine, arginine, tryptophan, or methio-

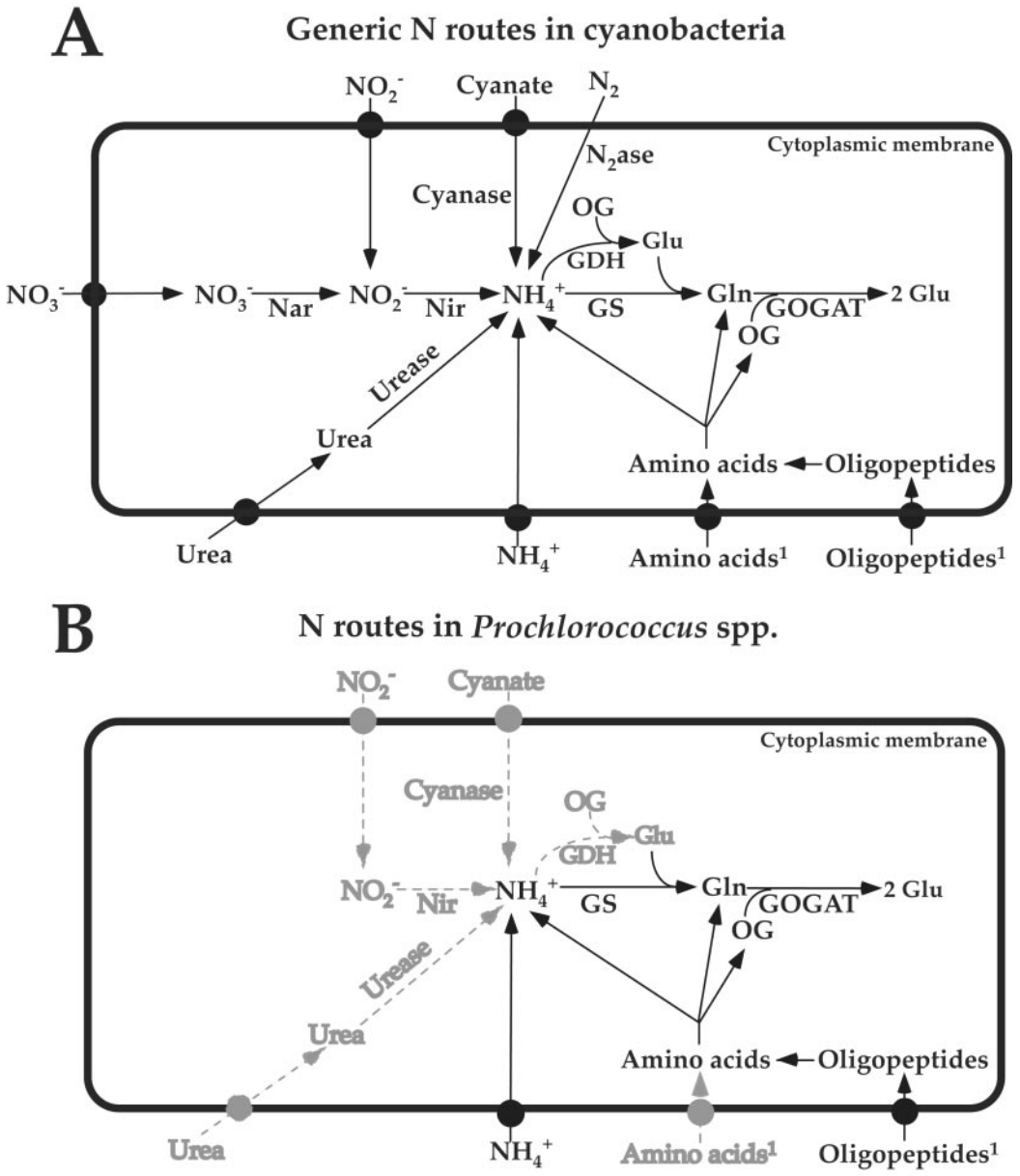

FIG. 1. Comparison between the generic nitrogen assimilation routes in cyanobacteria (A) and the modifications observed in different isolates of *Prochlorococcus* (B) (12). Black circles represent specific transporters. Gray indicates nitrogen sources or pathways not utilized by all *Prochlorococcus* strains. Superscript 1 indicates that amino acid uptake by *Prochlorococcus* has been shown in the field (85), but otherwise utilization of amino acids, oligopeptides, and cyanate is unknown, although it is strongly suggested by genomic analysis.

nine has no beneficial effect on cell growth (63). Interestingly, the rate uptake of organic nitrogen compounds in the field by *Prochlorococcus* is 10-fold higher than that by marine *Synechococcus* (85), possibly explaining the dominance of *Prochlorococcus* in oligotrophic oceans. That study also showed that one-third of the amino acid pool was consumed by *Prochlorococcus*, which could obtain as much as 10% of its total nitrogen requirements solely from dissolved amino acids (85). Little is known about the actual molecular structure of the total organic matter found at depth in the oceans, but recent methods allowed the demonstration of nonselective preservation of organic matter in sinking organic particles at depth (20). For example, in water samples from the equatorial Pacific and the Arabian Sea, where high *Prochlorococcus* abundances have been reported (57), the fraction attributed to amino acid-like molecules varied from 11 to 15% of the total organic matter (20). These results further support the hypothesis of organic nitrogen molecules as important nitrogen sources at depth in oligotrophic oceans and provide a possible explanation for the occurrence of low-irradiance *Prochlorococcus* ecotypes that are unable to utilize either nitrate or nitrite, such as strains SS120 and MIT 9211 (36, 47).

Ammonium is the only nitrogen source so far reported to be utilizable by all *Prochlorococcus* isolates (36, 47, 63). The central position of this nitrogen source in the N assimilatory pathways in cyanobacteria (Fig. 1) explains the pivotal role of

glutamine synthetase (GS) in the regulation of nitrogen assimilation in these photosynthetic organisms. Accordingly, the gene *glnA*, encoding GS, is found in all available genomes (8, 10, 65). Interestingly, the amino acid sequence, isoelectric point, molecular size, and kinetic parameters of GS from *Prochlorococcus* have been shown to be very similar to those of the enzymes from other cyanobacteria, either freshwater or marine strains (10), indicating very slight modifications of the properties of this enzyme during evolution. Regulation of GS by oxidative modification in *Prochlorococcus* has also been shown to occur (17), in a process that induces the inactivation and subsequent degradation of the enzyme. Catalase and peroxidase, but not superoxide dismutase, effectively protected GS against inactivation, suggesting the mediation of hydrogen peroxide. This regulatory mechanism is common to other enteric bacteria (32), cyanobacteria (40, 41), green algae (25), and higher plants (53). It has been proposed to be involved in the response to oxidative stress produced by specific conditions in the metabolism (53).

However, when the physiological regulation of this enzyme in the axenic strain *Prochlorococcus* sp. strain PCC 9511 was studied in detail (9, 10), very unusual regulatory features were found (Fig. 2A and B): GS was not upregulated under conditions of nitrogen starvation (9, 10) and was not inactivated in darkness (9), although both are fairly standard responses observed in other cyanobacteria (11, 12). Preliminary results on the expression of *glnA* in the same strain confirm these observations (S. El Alaoui, A. López-Lozano, J. Diez, and J. M. García-Fernández, unpublished data). These differences cannot be explained on the basis of structural changes of the enzyme (10) but may rely on modifications of the regulatory networks of nitrogen assimilation. The advantages of having a simplified regulatory system of regulation for a key enzyme like GS might be rooted in the natural environment where *Prochlorococcus* is most abundant: large oligotrophic intertropical areas of the oceans, without abrupt changes in the kind or concentration of nitrogen sources at a given depth. Under these conditions, the maintenance of a sophisticated and costly regulatory machinery to finely tune the assimilation of ammonium could simply represent an unjustified energetic expense for an organism like *Prochlorococcus*. In this view, the evolution could have favored an inexpensive, streamlined regulatory network, leading to the observed lack of response of GS to changes in key parameters (such as lack of light and/or nitrogen) whose detection is crucial to other cyanobacteria inhabiting more changing environments. This hypothesis is also supported by the similarly simple behavior of other nitrogen-related regulatory proteins (P_{II} [56] or NtcA [33]) (Fig. 2C and D), enzymes (urease [55]), or transporters (the ammonium transporter encoded by *amt1* [33]) (Fig. 2D).

The ammonium transporter *amt1* is highly expressed in *Prochlorococcus* strain PCC 9511 cultures either growing on ammonium or subjected to nitrogen limitation, and only severe nitrogen starvation leads to a slight decrease in its expression (33) (Fig. 2D). This is another most unusual trait in the regulation of nitrogen assimilation in *Prochlorococcus*, in sharp contrast with that in other cyanobacteria (46) and other organisms (33). It points to a permanently high capacity for ammonium uptake, thus reinforcing the importance of this nitrogen source for this microorganism. It is noteworthy that the expression of *amt1* is not under the control of NtcA (33), in contrast to the situation described for *Synechococcus* sp. strain PCC 7942 (76).

In much the same way as nitrite, urea is utilized by some (55, 63) but not all *Prochlorococcus* isolates (e.g., SS120 lacks the *ure* cluster [8]), indicating the relevance of this nitrogen source in some marine environments, where its concentration varies between 0.1 and 1 μM and it is the dominant component among the dissolved organic nitrogen compounds (1). The measured urease activities were fairly constant irrespective of the nitrogen source supplied to *Prochlorococcus* strain PCC 9511. This apparent lack of regulation, similar to that observed for GS, led to hypothesize that NtcA was not involved in the control of urease biosynthesis in *Prochlorococcus*, in spite of the presence of putative NtcA binding sites located next to the *ure* genes (encoding the urease structural and accessory molecules) (55). These genes are organized in two overlapping, opposite clusters in *Prochlorococcus* strain PCC 9511 (55) and *Synechococcus* strain WH 7805 (7), in contrast to the case for most freshwater cyanobacteria, where these genes are scattered throughout the genome (55). Additional differences were observed in the quaternary structure (two heterotrimers) and in the molecular mass of the enzyme (168 kDa), which is the lowest among the described ureases (55). The physiological significance of these observations remains to be further investigated.

The signal transduction protein P_{II} of *Prochlorococcus* strain PCC 9511 is encoded by a gene (*glnB*) that is very similar to its orthologs in cyanobacteria, with a putative NtcA binding site in its promoter regions (56). Moreover, the deduced amino acid sequence shows typical cyanobacterial signatures (56). The *Prochlorococcus* P_{II} is therefore of the cyanobacterial type, but, interestingly, it forms a separate subclade with other oceanic strains within the *glnB* cyanobacterial radiation (Fig. 3). In cyanobacteria, the P_{II} protein, whose main function is to coordinate nitrogen and carbon metabolism, is posttranslationally modified by phosphorylation in response to the nitrogen and carbon status of the cells (72). In *Prochlorococcus*, however, the P_{II} protein is not phosphorylated under any of the tested conditions (56) (Fig. 2C). In agreement with this observation, the *pphA* gene, encoding the PP2C-like phosphatase that dephosphorylates P_{II} in *Synechocystis* strain PCC 6803 (26), is absent in the genome of *Prochlorococcus* strain MED4 (65). P_{II} could act, nevertheless, as a sensor of the intracellular concentration of 2-oxoglutarate. As proposed for *Synechocystis* strain PCC 6803 (56), both molecules could indeed form a complex involved in the direct or indirect inhibition of a high-affinity inorganic carbon transport system, in a manner independent of the P_{II} phosphorylation state. Since the genome of MED4 lacks orthologs of the genes involved in C_i acquisition in other cyanobacteria, it has been proposed that the CynABD transport system might be utilized for the transport of both bicarbonate and cyanate in these *Prochlorococcus* strains (56), but this remains to be experimentally demonstrated.

The role of the global regulator NtcA (22) in *Prochlorococcus* is rather uncertain (33). In several cyanobacteria, the expression of the genes encoding nitrate reductase, nitrite reductase, glutamine synthetase, and the transporters of nitrate, nitrite, and ammonium, as well as that of *ntcA* itself, is downregulated in the presence of ammonium and induced under

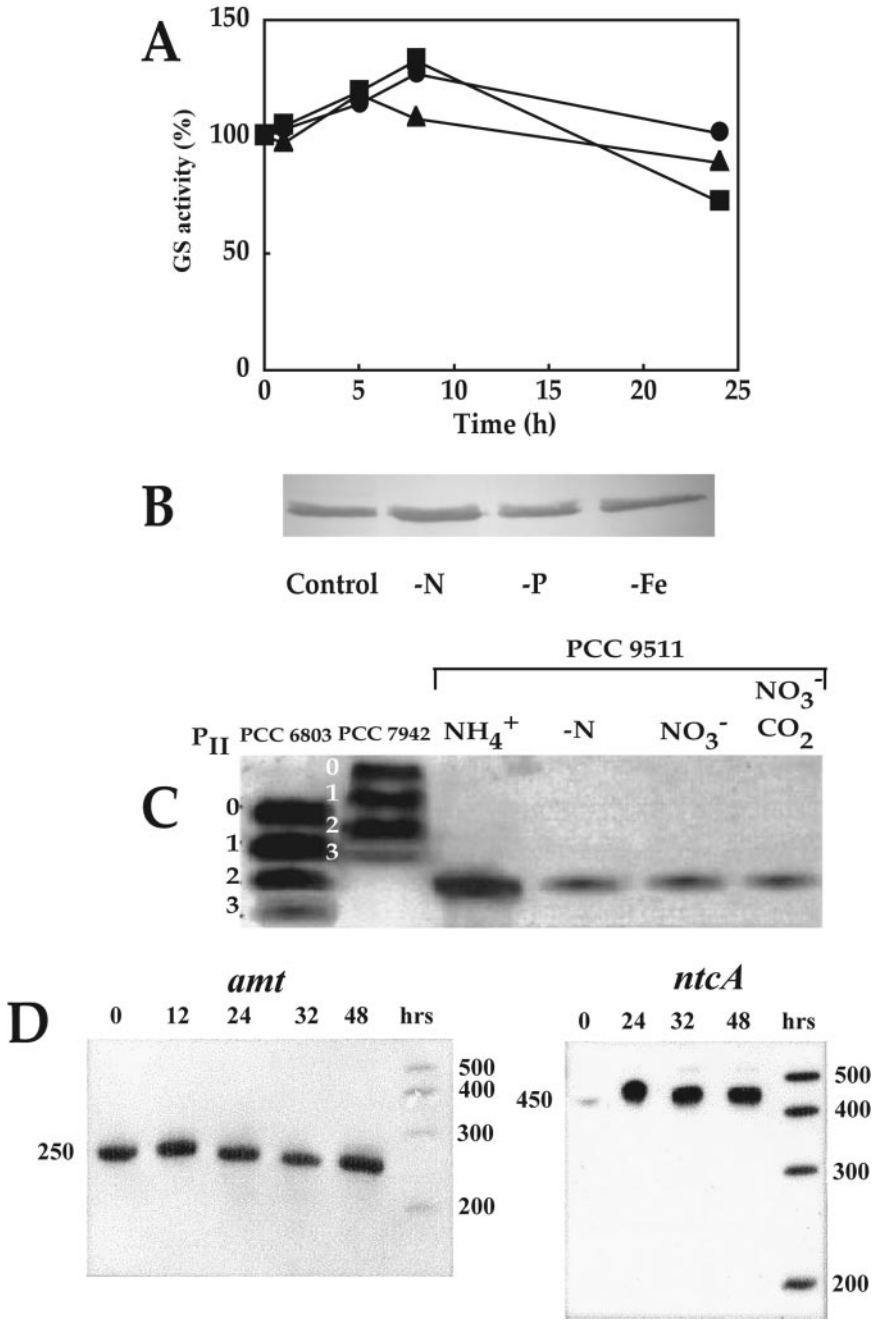

FIG. 2. Regulatory responses of nitrogen metabolism in *Prochlorococcus*. (A) Time course of glutamine synthetase activities in *Prochlorococcus* strain PCC 9511. Squares, control (illuminated culture growing on ammonium); circles, culture subjected to darkness; triangles, cultures subjected to nitrogen starvation. Data are taken from reference 9. (B) Western blotting of *Prochlorococcus* strain PCC 9511 extracts from cells subjected for 312 h to standard conditions (control) or to starvation in nitrogen, phosphorus, or iron, using antibodies against GS from *Synechocystis* strain PCC 6803. Reprinted from reference 10 with permission. (C) Western blotting of *Prochlorococcus* strain PCC 9511 extracts subjected to different N and C conditions, using P_{II} antibodies against P_{II} from *Synechococcus* strain PCC 7942. The levels of phosphorylation (groups designated 0 to 3) are indicated on the left, using cyanobacterial strains showing this kind of modification. Reprinted from reference 56 with permission. (D) Expression of the ammonium transporter *amt1* and the transcriptional regulator *ntcA* in *Prochlorococcus* strain PCC 9511 cultures subjected to nitrogen starvation, as determined by RNase protection assay. The times after transfer to no nitrogen medium are indicated. Reprinted from reference 33 with permission.

conditions of nitrogen stress (22, 34, 38, 39). However, in *Prochlorococcus* strain PCC 9511, nitrogen limitation induces the expression of *ntcA* but not that of *amt1* (33) (Fig. 2D). Moreover, the expression of GS is not induced under conditions of nitrogen starvation in the same strain (9, 10). In *Prochlorococcus* strain PCC 9511, the upstream region of *ntcA*, in contrast to that of *amt1*, contains a typical −10 box (33), usually found near the NtcA binding site. Although these results confirm that *amt1* might not be under NtcA control in this strain, further work is required to convincingly establish a

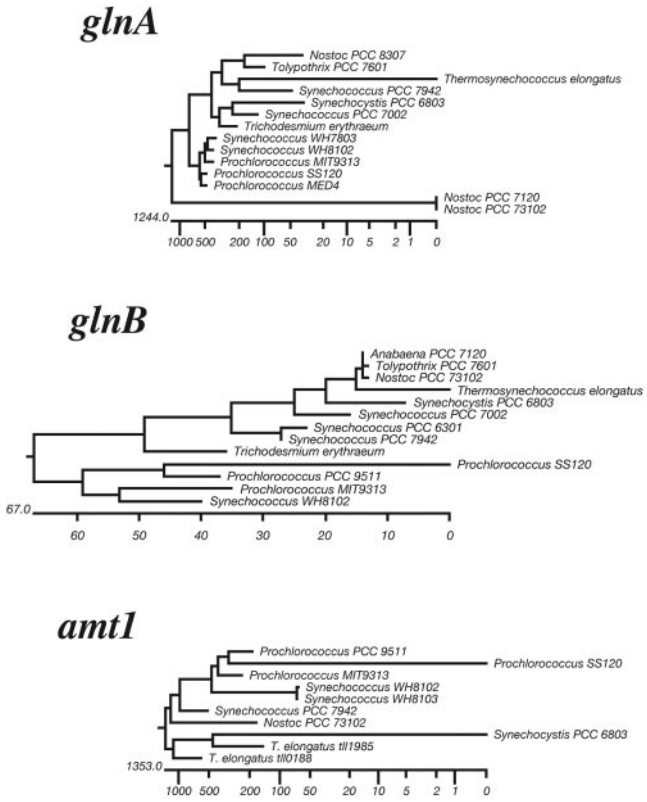

FIG. 3. Phylogeny of nitrogen metabolism-related genes in marine cyanobacteria. Phylogenetic trees obtained by Jotun-Hein alignment (MegAlign 4.0 software, Lasergene package) of the available amino acid sequences from glutamine synthetase (encoded by *glnA*), the signal transducer P_{II} (encoded by *glnB*), and the high-affinity ammonium transporter (encoded by *amt1*) from marine and freshwater cyanobacteria are shown. Note that *Prochlorococcus* strains MED4 and PCC 9511 correspond to the same genotype, with the only difference being that PCC 9511 is axenic.

correlation between the lack of a −10 box and the occurrence of an unusual regulatory behavior for a given gene.

The reviewed literature on nitrogen assimilation in *Prochlorococcus* clearly suggests a simplification of the regulatory networks. This is reflected in different observations, such as the fairly constant concentration and activity of GS (9, 10), the constitutive expression of the ammonium transporter (33), the lack of phosphorylation of the P_{II} protein (56), and the apparent lack of nitrogen control by NtcA (33). This is in good agreement with the small number of response regulators and histidine kinases observed in the *Prochlorococcus* genomes (8, 42, 65, 68). Since this is also observed in marine *Synechococcus* (54, 68), it is tempting to speculate that microorganisms require far less complicated systems of detection and transduction of environmental signals and metabolic control when they live in marine environments than when they live in other habitats, such as rivers, lakes, or soils. The rather constant conditions observed in the oligotrophic gyres of intertropical oceans could have provoked a progressive loss of these kinds of genes, in order to produce streamlined regulatory mechanisms for better adaptation. In addition, this could contribute to the reported compactness of the *Prochlorococcus* genomes (70).

The traditional dilemma of physiological acclimation versus evolutionary adaptation is illuminated on the basis of the current knowledge on the large degree of genetic diversity in several representative isolates of *Prochlorococcus*. Different studies on the photosynthetic apparatus showed that any *Prochlorococcus* isolate seems to be able to grow under a large range of irradiances (48, 60), thus supporting the importance of physiological acclimation. However, there exist largely divergent light-harvesting adaptations within the genus *Prochlorococcus* (since, for example, there are eight *pcb* genes in the SS120 strain [8, 14] with specific expression patterns [3, 15], versus only one in MED4, and three *psbA* genes in MIT 9313 versus one in MED4 or SS120 [8, 24]). On the other hand, adaptation with gene loss seems to be the rule in the field of nitrogen assimilation. Indeed, depending on the ecotype (49) considered, the utilizable sources are rather restricted, going from oxidized sources such as nitrite in some low-light-adapted strains (47) to reduced forms such as urea (55), ammonium (9, 36), or amino acids (63, 85). Physiological acclimation is therefore more advantageous in some biological aspects, while in others evolutionary adaptation is more beneficial. Hence, it seems that evolution drove the genomes of *Prochlorococcus* strains in different, sometimes opposite, directions, such as gene loss, gene multiplication, and extensive genome reorganizations (8, 65). The outcome of all of these processes is the current pool of *Prochlorococcus* genotypes, where biological selection induced an extremely efficient, yet remarkably simplified, combination of maximum economy by removing genes that were not strictly essential (thus saving the energy required for their replication, transcription, and translation) and considerable expense by multiplying those coding for proteins of paramount importance under conditions of strong limitations (3, 14, 15).

DIVERSITY AND PHYLOGENY OF NITROGEN ASSIMILATION PATHWAYS IN THE GENOMES OF *PROCHLOROCOCCUS* STRAINS

Comparative analysis of two *Prochlorococcus* genomes provided interesting insights into the photosynthetic apparatus of these cyanobacteria (24). The current availability of three *Prochlorococcus* genomes (8, 65), including two from low-irradiance-adapted ecotypes (MIT9313 and SS120) and an additional genome from the closely related marine *Synechococcus* clade (54), now permits a detailed analysis of the nitrogen assimilation strategies utilized by *Prochlorococcus* at different water depths, which correspond to different conditions of nitrogen supply. Table 2 summarizes some of the most interesting features from this comparison; Fig. 1 outlines the comparison between the generic N routes in cyanobacteria and those observed in *Prochlorococcus*. The central entry point for nitrogen assimilation in *Prochlorococcus* is ammonium, since no nitrogenase-related or nitrate reductase genes exist in *Prochlorococcus*, thus eliminating the possibility of assimilation of the two most abundant nitrogen sources in nature. Moreover, only one of the low-light-adapted ecotypes (MIT9313) possesses the gene *nirA*, encoding nitrite reductase. The genes encoding the ammonium transporter and the glutamine synthetase/glutamate synthase are found in all three genomes. The three strains also share the presence of genes encoding oligopeptide transporters, and amino acid transporters are found in the

TABLE 2. Presence of nitrogen metabolism-related genes in the genomes of *Prochlorococcus* strains MED4, MIT9313, and SS120 and *Synechococcus* strain WH8102

Gene(s)	Protein(s)	Presence[a] in strain:			
		MED4	MIT9313	SS120	WH8102
nif cluster	Nitrogenase and cofactors	−	−	−	−
narB	Nitrate reductase	−	−	−	+
nirA	Nitrite reductase	−	+	−	+
glnA	Glutamine synthetase	+	+	+	+
gdhA	Glutamate dehydrogenase	−	+	−	−
glsF	Fd-glutamate synthase	+	+	+	+
ure cluster	Urease and cofactors	+	+	−	+
amt1	Ammonium transporter	+	+	+	+
	Amino acid transporters	−	+	+	+
	Oligopeptide transporters	+	+	+	+
cynABD	Cyanate transporter	+	−	−	+
cynS	Cyanate lyase	+	−	−	+

[a] +, present; −, absent.

low-light-adapted strains (SS120 and MIT9313) but not in the high-light-adapted strain (MED4) (8, 65). Utilization of amino acids (either derived from oligopeptides or directly imported as single molecules into the cell) is therefore a common feature in *Prochlorococcus*. Given that amino acids are molecules containing reduced nitrogen and carbon skeletons, their utilization could provide an important advantage (51, 52) under conditions of oligotrophy and very low energy input from light at depth.

A remarkable outcome from comparative genome analysis is the presence in *Synechococcus* strain WH8102 and *Prochlorococcus* strain MED4 (but not in SS120 or MIT9313) of genes coding for cyanate ABC transporters (*cynABD*) and cyanate lyase (*cynS*) (8, 54, 56, 65). Furthermore, cyanate utilization has been reported for *Synechococcus* strain WH8102 (54). Initial results strongly suggest that cultures of *Prochlorococcus* strain MED4, in contrast to those of SS120, can grow by utilizing cyanate as the sole nitrogen source (Rangel et al., unpublished data). This points to the utilization of a potentially important nitrogen source, since cyanate is a degradation product of urea (19), which in turn is an excretion product of different organisms (77), reaching concentrations of 0.1 to 1 μM in the ocean (1, 4, 43).

The presence of the gene *gdhA*, encoding glutamate dehydrogenase, in strain MIT9313 but not in MED4, SS120, or even *Synechococcus* strain WH8102 is intriguing. This enzyme catalyzes an alternate route for ammonium incorporation directly into oxoglutarate, to produce glutamate (Fig. 1). Nevertheless, due to the higher Michaelis constant for ammonium of this enzyme with respect to that of glutamine synthetase, the main route for ammonium assimilation in cyanobacteria is that composed by GS/GOGAT (11, 12, 44). Preliminary investigations on *Prochlorococcus* showed that this activity is detected at relatively high levels in crude extracts from the MIT9313 strain and not in those from MED4 or SS120 (Rangel et al., unpublished data). It has been proposed that glutamate dehydrogenase confers selective advantages to *Synechocystis* strain PCC 6803 under nonexponential growth conditions (5). Whether this is also the case in *Prochlorococcus* strain MIT9313 remains to be studied.

Phylogenetic studies on different key genes from the nitrogen assimilation pathway, such as those shown in Fig. 3 for *glnA*, *glnB*, and *amt1* (similar trees have been constructed for other genes from this pathway [not shown]), are in good agreement with the current model of the evolution of *Prochlorococcus* within the cyanobacterial radiation: they show a rather recent speciation, leading to the appearance of a cluster corresponding to marine cyanobacteria, in which *Prochlorococcus* is closely related to marine *Synechococcus* isolates (30, 64, 74, 80). This suggests that the genes involved in nitrogen assimilation in *Prochlorococcus* have not been the result of lateral gene transfer (71), as has been observed for other important genes (8, 24, 65), but have evolved from their counterparts in ancestral cyanobacteria. There is, however, at least one exception to this rule: the proteobacterial-like nitrite transporter found in the genome of MIT9313 (65). Therefore one can speculate that the genome of a common ancestor of *Prochlorococcus* and marine *Synechococcus* strains contained a set of genes involved in nitrogen assimilation that was sufficient to allow fine metabolic tuning in order to optimize nitrogen assimilation in oligotrophic environments. This tuning would involve either modification of the regulatory networks or removal of unessential genes, without a major import of foreign components, leading to the appearance of specific genotypes for the different niches inhabited by *Prochlorococcus* and marine *Synechococcus*.

CONCLUDING REMARKS

The discovery of *Prochlorococcus* has led to a profound reconsideration of established models in marine ecology, from the composition of the marine phytoplankton to its contribution to the global primary production (59). While initial observations explained its ecological success mainly on the basis of photosynthetic adaptations to efficiently colonize most of the photic zone of the oceans (3, 14, 48, 58, 60, 61), it is now becoming clear that other major metabolic changes contribute significantly to such success, as evidenced in the present review on the nitrogen assimilation pathways in *Prochlorococcus*. Further work is required to understand the importance of these changes for *Prochlorococcus* fitness in oligotrophic regions of the oceans and their specific relationship with the niche differentiation described for the different *Prochlorococcus* ecotypes (49, 79, 80). (For further information online, see the genome databases available at http://www.jgi.doe.gov/JGI_microbial/html/ for *Prochlorococcus* strains MED4, MIT9313, MIT9312, and NATL2A and *Synechococcus* strain WH8102 and at http://www.sb-roscoff.fr/Phyto/ for *Prochlorococcus* strain SS120 and *Synechococcus* strain WH7803. [Note that sequencing and/or annotation of the genomes from *Prochlorococcus* strains MIT9312 and NATL2A and *Synechococcus* strain WH7803 is in progress, and consequently their genome databases are still not available at the corresponding websites.] Websites for research projects concerning *Prochlorococcus* are http://www.sb-roscoff.fr/PROMOLEC/ [the European project "*Prochlorococcus* Molecular Ecology"] and http://arep.med.harvard.edu/DOEGTL/ [the U.S. project "Microbial Ecology, Proteogenomics and Computational Optima"].)

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