Mini-review

Adaptive mechanisms of nitrogen and carbon assimilatory pathways in the marine cyanobacteria Prochlorococcus

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Abstract

Prochlorococcus is an abundant marine cyanobacterium responsible for a significant part of global primary production. There exist various ecotypes adapted to conditions found along the water column, showing that largely modified photosynthetic apparatus efficiently harvest the light energy penetrating into their habitats. In view of the recent availability of three Prochlorococcus genomes, we review here additional adaptive changes observed in nitrogen and carbon metabolism.

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1. Introduction

The intertropical oceans comprise large regions subjected to strong oligotrophic conditions. For many years, these areas were considered virtually devoid of living organisms due to their poor nutrient supply. Thus, the discovery of abundant populations of unicellular cyanobacteria thriving in these waters, namely Synechococcus in 1979 [40] and Prochlorococcus in 1988 [4], marked a turning point in the field of marine ecology. Since then, Prochlorococcus has proven to be a fascinating organism capable of growth over an irradiance range of four orders, from the ocean surface down to more than 200 m in depth [27]. Two possible explanations were proposed for this performance: either an outstanding plasticity of its photosynthetic apparatus, or the occurrence of different ecotypes adapted to the specific conditions (including, but not restricted to, the availability of light energy) found at different depths along the water column. The large body of knowledge achieved on the photosynthetic apparatus of Prochlorococcus, recently reviewed [26,38], has shown that indeed a certain degree of acclimation occurs for each studied strain; but the key to the optimal utilization of such a wide range of irradiances is the presence of evolutionally adapted photosynthetic systems, largely differing from those of other cyanobacteria, and also differing between high and low light-adapted Prochlorococcus ecotypes. These findings were reinforced and completed by comparative analysis of three recently sequenced Prochlorococcus genomes [6,18,31] corresponding to strains representative of ecotypes adapted to life near the ocean surface (MED4) or deeper within the water column (MIT9313 and SS120).

Although the most noticeable parameter subjected to gradient along the water column is irradiance, many other changing factors exist, such as dissolved oxygen, pressure, temperature, salinity, and the concentration and molecular form of different nutrients (nitrogen, phosphate, etc.). Given the large genetic diversity of Prochlorococcus, one could reasonably expect that these gradients also induced modifications in other important metabolic pathways. The aim of this review is to analyze the current state of the art on nitrogen and carbon assimilation, and its possible importance in the ecological success of Prochlorococcus.
2. The utilization of nitrogen by Prochlorococcus

The cyanobacteria are one of the oldest living groups on Earth [33]. They are considered to include the ancestor of chloroplasts from higher plants [39]; furthermore, it is believed that the appearance of oxygen in the atmosphere was due to a photosynthetic cyanobacterial metabolism millions of years ago. Hence, in this view, our planet has been shaped in a significant way by cyanobacteria. During the evolution of these organisms, a highly diverse radiation of the genus allowed them to colonize virtually any imaginable niche in all kinds of environments, from deserts to oceans. The cyanobacteria have long been considered capable of utilizing very different forms of nitrogen [10], from the most oxidized (including molecular nitrogen, taken directly from air, or nitrate) to the most reduced (such as ammonium), thus providing remarkable metabolic flexibility for coping with changes in the environment. Interestingly, molecular nitrogen and nitrate are the most abundant nitrogen sources in nature; but both require high energetic expense in order to reduce them prior to assimilation into biological molecules, as amino acids or nucleic acids [2]. On the contrary, ammonium and other reduced forms of nitrogen are readily assimilated by cyanobacteria, with minimum metabolic effort [9,12].

Thus, one could expect the deployment of a regulatory system of the assimilatory pathways for fine modulation of the assimilated nitrogen molecule, so as to optimize the available metabolic energy. In other words, when several nitrogen sources are available for cells, it is paramount to first select the reduced ones (amino acids, urea, ammonium). A requirement of this model is the development of a regulatory system for detecting when reduced nitrogen is available. This is indeed the case in most cyanobacteria, where global nitrogen regulation is based on the transcriptional repressor protein NtcA [17]. When ammonium is available, the expression of all enzymes and transporters involved in inorganic nitrogen assimilation (nitrate and nitrite transporters, nitrate reductase, nitrite reductase, etc.) is repressed by NtcA. Only upon exhaustion of ammonium are the pathways for utilizing other nitrogen sources activated.

Yet some of the main common assumptions summarized above are deeply challenged by recent advances in our knowledge of nitrogen metabolism of the marine cyanobacterium Prochlorococcus (Fig. 1). One of them is the benefit obtained from the ability to assimilate several nitrogen sources: in fact, some Prochlorococcus strains are restricted to ammonium (and possibly amino acid) assimilation, such as SS120 [6]. The second is the advantage of having fine regulatory systems to optimize nitrogen assimilation: some studies performed in vivo on Prochlorococcus [19,25] showed that, in spite of possessing the genes involved in standard nitrogen/carbon regulation in cyanobacteria (namely, ntcA and glnB, encoding, respectively, the NtcA and PII proteins), none of them seem to work as previously described. Furthermore, the regulation of enzymes such as glutamine synthetase [7,8,15] and isocitrate dehydrogenase (A. López-Lozano, J. Diez and J.M. García-Fernández, unpublished) is clearly different from that of their counterparts in other cyanobacteria.

Although a majority of marine microorganisms are non-culturable (including, until very recently, the most abundant bacterial group on Earth, the SAR11 clade [28]), Prochlorococcus can be grown in culture after long efforts [3,4]. However, these cultures are not easy to maintain and show periodic lag stages which remain unexplained to date [7]. If we take into account the fact that the storage of Prochlorococcus frozen samples has only very recently been described, it is clear why early studies on Prochlorococcus metabolism were scarce. In the case of nitrogen nutrition, no clear conclusion has been reached until very recently. This is particularly the case for nitrate, a nitrogen source assimilated by all studied cyanobacterial genus to date. Because of this, it was somehow assumed that Prochlorococcus could use it, although no specific studies on this issue were published until 2000. Around that time, several reports [7,25,29] showed that the only available axenic strain, PCC 9511, was unable to grow on nitrate; furthermore, six other strains of Prochlorococcus seemed to lack nitrate reductase [20], and the gene encoding this enzyme (narB) was also lacking in the first sequenced Prochlorococcus genome, from the strain MED4.

As illustrated by the lack of nitrate assimilation, the utilization of other inorganic nitrogen sources seems to differ from that of typical cyanobacteria, showing variations depending on the considered strain. For example, although some low irradiance-adapted strains were shown to grow on nitrite [22], most could not utilize it for growth. Similar situations have been observed for organic nitrogen sources such as urea: although it was initially believed that Prochlorococcus could assimilate it, and thus it was consequently used in culture media for many strains, recent studies show that strain SS120 lacks urease [6], the enzyme catalyzing the production of ammonium from urea.

All this information has raised the hypothesis of diversification of nitrogen assimilatory pathways in Prochlorococcus, as previously observed in other metabolic processes such as the photosynthetic apparatus. The comparative analysis of four marine cyanobacterial genomes published later [6,24,30], and physiological studies currently underway (O. Rangel, J. Diez and J.M. García-Fernández), seem to reinforce this hypothesis.

The emerging picture from these analyses suggests that Prochlorococcus abundance is mainly based on the occurrence in nature of a range of ecotypes evolved to thrive at specific depths of the water column [23], with a number of biological adaptations to conditions found at such depths. Examples include light harvesting [26,38], or specific metabolic routes such as nitrogen [7,8,12,15,20,22], phosphorus, or carbon metabolism. The physiological explanation behind the diversity of ecotypes is closely connected to another key feature of Prochlorococcus: its extremely small size [27], close to the theoretical minimum for a photo-
synthetic organism. This provides at least three outstanding advantages: a low shading between cells (so that light harvesting is not hampered by the proximity of *Prochlorococcus* cells), a high surface/volume ratio (for better uptake of the limited nutrients typical of oligotrophic environments), and finally, lower energetic expenses (thus allowing growth in regions with very low light availability). The small size seems to be paramount for *Prochlorococcus*, as different mechanisms have been described to allow compaction of the cell, i.e., the reduction in genome size [35], or utilization of antenna complexes significantly less voluminous than the phycobilisomes found in most cyanobacteria [13].

The reduction in the genome to nearly minimal size for an oxyphototrophic organism induced an increase in the percent of coding regions as compared to other cyanobacteria, but also the deletion of dispensable genes [6,31]. The evolutive logic for removing these genes lies in strictly maintaining the set of genes necessary for optimal performance at the depth at which that particular ecotype is living. With regard to nitrogen assimilation, this would mean that when specific nitrogen sources are usually absent (i.e., nitrate in the upper ocean), the genes directly involved in its utilization (from transporters to enzymes) can be safely deleted without significant impact on the fitness of the ecotype for its habitat. Furthermore, this economizes the energy required for the replication of these genes and for the biosynthesis of their corresponding mRNAs and proteins.

This hypothesis explains the occurrence of *Prochlorococcus* strains restricted to the use of ammonium [6], while others assimilate urea, ammonium and nitrite. It is interesting to note that annotation of the genomes suggested hypothetical utilization of amino acids and even oligopeptides [31], although this has not been demonstrated in vivo to date. The array of utilizable nitrogen sources for each ecotype should meet the best balance for optimizing the ratio of available energy vs. required energy for nitrogen assimilation [12]. Since light availability is obviously higher near the ocean surface, it could be expected that ecotypes inhabiting those regions would retain a wider choice of nitrogen sources than ecotypes living further down the water column.
However, their metabolic abilities do not seem to support this hypothesis, as for instance, MIT9313 can assimilate nitrite, urea and ammonium, SS120 only ammonium, and MED4 cyanate, urea and ammonium. Comparative analysis of the genomes of more strains will help to complete the puzzle so as to better understand factors determining the size and genome complement of each ecotype.

A second field in which evolution seems to be playing a role is that of regulatory networks for controlling the utilization of nitrogen and coordinating it with that of carbon [12]. The first physiological studies on regulation of enzymes involved in nitrogen assimilation in Prochlorococcus focused on glutamine synthetase [7,8,15]. The regulation of this enzyme has been extensively studied in Escherichia coli and also in several cyanobacteria, particularly Synechocystis PCC 6803 [9]. The response of GS to some key environmental changes (such as nitrogen limitation, or darkness) is fairly standard in most photosynthetic organisms studied, from cyanobacteria to higher plants: lack of nitrogen induces an upregulation of the enzyme, while darkness promotes a progressive decrease in its activity and expression. Both are reasonable responses for organisms living in changing habitats; when nitrogen concentration goes down, the GS activity increases to assimilate as much nitrogen as possible. On the contrary, darkness stops photosynthesis and thus production of carbon skeletons and energy; consequently, it makes sense to also stop the assimilation of nitrogen.

But in the case of Prochlorococcus, the premise behind this interpretation is not valid: if we look at a specific ecotype from a given depth, we can consider that Prochlorococcus cells live in a fairly constant environment, mostly in very oligotrophic regions of the oceans. Thus the coherent physiological responses in this case are in opposition to those described above: if the nitrogen concentration is permanently low (and the light conditions are not subjected to strong changes in the case of strains living at depths), it would be too costly to maintain fine regulatory mechanisms which depend on both parameters. This is indeed the observed response in vivo: GS activity/expression did not change significantly either under nitrogen starvation or darkness [7,8], in sharp contrast with GSs from other cyanobacteria, algae or higher plants. However, the overall structure and kinetic parameters of the enzyme are conserved [8], probably due to its key position in the nitrogen assimilatory pathway.

Interestingly, analogous situations seem to occur in the regulation of other important enzymes such as isocitrate dehydrogenase. Initial experiments on the behavior of this enzyme showed it to be similarly unresponsive to nitrogen starvation or darkness (A. López-Lozano, J. Diez and J.M. García-Fernández, unpublished), thus suggesting that streamlined control may be the rule and not the exception in the regulatory networks of Prochlorococcus.

These physiological examples of simplified regulation of enzymes normally subjected to fine controls fit nicely with the very low number of signal transduction and transcription factors found in Prochlorococcus genomes [31], in comparison with other freshwater counterparts. However, two key players controlling nitrogen gene expression in cyanobacteria have been conserved: the global nitrogen regulator ntcA [17], and the PII protein [37], involved in nitrogen/carbon coordination. Yet their function in Prochlorococcus remains to be understood. It has been shown that in strain PCC 9511 both genes are translated, but they do not carry out standard regulation, i.e., PII is apparently non-phosphorylated [25], thus indicating that standard functionality (based on the interconversion between forms with different levels of phosphorylation) is probably not occurring. Similarly, a number of ntcA binding sites have been found upstream from many nitrogen-related genes (including ntcA itself, glnA, amtl, etc.) in the Prochlorococcus genomes [31]. However, under nitrogen limitation, the ntcA gene was upregulated—as described for most cyanobacteria—but the expression of amtl was unchanged—contrary to most cyanobacteria [19]. If we add the unusual GS regulation under similar conditions, it is clear that global nitrogen regulation in Prochlorococcus is atypical.

Further work is required to understand the behavior of those regulator proteins in Prochlorococcus, and the reason why they have been conserved, although with a modified role with regard to other cyanobacteria.

Interestingly, the pathway-specific nitrate assimilation transcriptional activator ntcB [1] is found in all available cyanobacterial genomes (including some marine species such as Trichodesmium), but not in the unicellular marine Synechococcus and Prochlorococcus, although other LysR family transcription factors have been retained. It would be interesting to study whether the progressive loss of the nitrate/nitrite assimilatory pathway in the evolution of Synechococcus/Prochlorococcus is somehow related to the lack of ntcB in this phylogenetic cluster. In fact, since ntcB has been proposed to be involved in rapid adaptation to the changing availability of nitrate/nitrite [1], its absence in a group of organisms characterized by progressive loss of inorganic nitrogen assimilation abilities is not surprising, probably due to the unchanging nature and/or concentration of the available nitrogen sources. It is noteworthy, however, that most marine Synechococcus isolated thus far are able to assimilate both inorganic nitrogen sources [32], indicating that the presence of ntcB is non required in these cyanobacteria for appropriate utilization of nitrate.

3. Carbon vs nitrogen metabolism in Prochlorococcus: adaptive mechanisms

In marine photosynthetic organisms of global importance, such as Prochlorococcus and Synechococcus, the relevance of carbon metabolism is obvious, due to their key participation in the processes of carbon fixation and biomass production. However, very little is known about carbon metabolism in these cyanobacteria. Moreover, the possibil-
ity of heterotrophy in *Prochlorococcus* has been the subject of much speculation based either on preliminary laboratory studies [11] or on genomic data [31]. However, it has been rarely studied in spite of its potential importance for the ecological fitness of *Prochlorococcus*. A main drawback to this kind of study is that *Prochlorococcus* cultures are readily contaminated when organic carbon sources are added, leading to rapid disappearance of photosynthetic cells from the culture by takeover of the contaminating bacteria, thus preventing the obtaining of conclusive evidence. Hence we will summarize what can be inferred from *Prochlorococcus* genomes, although this will require physiological confirmation by in vivo studies.

The first interesting observation involves the presence of hypothetical sugar transporters in the sequenced genomes [6,31]. However, since it is currently accepted that *Prochlorococcus* is subjected to recent processes of speciation by genome reduction, those transporters could either be fully functional, or simply the remnants of currently disappearing heterotrophic pathways from *Prochlorococcus* ancestors. Interestingly, the annotation of these genomes showed no pathway for complete oxidation of glucose [31]. This is due, among other things, to the presence in cyanobacteria (including *Prochlorococcus*) of an incomplete Krebs cycle [31], preventing full oxidation of glucose. However, the possible occurrence of alternative routes, including some unknown genes, enabling the utilization of carbon skeletons, cannot be discarded. Indeed, there is increasing evidence that many annotated genomes miss key genes [5,16], suggesting that a more refined annotation based both on in vivo and in silico studies is needed to complement the current picture.

In addition, the absence of a pathway enabling complete oxidation of sugars does not preclude their partial utilization for obtaining of energy. This possibility would be particularly interesting for ecotypes inhabiting environments with very low light and oxygen concentrations, as previously suggested [14,31]. Under these conditions of strong energy limitation, any possibility for using sugars could confer selective advantages [34]. In this view, the presence in the SS120 genome of gene *ldhA*, encoding D-lactate dehydrogenase but absent in MIT9313 and MED4, is remarkable. This enzyme allows recovery of NAD$^+$ produced by glycolysis, while transforming pyruvate to lactate (Fig. 2); in the absence of lactate dehydrogenase it would be impossible for cells to recover that NAD$^+$, thus preventing the utilization of sugar. If this hypothesis holds true, this would indicate an anaerobic-like fermentation of glucose in *Prochlorococcus* SS120, leading to minor (but significant: 2 molecules of ATP per glucose metabolized) energy input for cells living in the lower part of the water column, with very low light available [14]. Therefore, while it appears that *Prochlorococcus* genomes lack complete heterotrophic pathways, as reported [31], the partial oxidation outlined above could confer the necessary energy for survival in the lower part of the water column, where oxygen and light irradiance may not be sufficient to support normal autotrophic growth [14].

Whatever the case, comparison of carbon metabolism pathways in genomic databases provides interesting information indicating that in these routes as well, *Prochlorococcus* underwent an adaptive process leading to the disappearance of specific genes during evolution. For instance, analysis of the tricarboxylic acid cycle pathway in the genome of the model freshwater cyanobacterium *Synechocystis* PCC 6803 vs available genomes from unicellular marine cyanobacteria (*Prochlorococcus* MED4, MIT9313 and SS120 and *Synechococcus* WH8102) reveals that three genes have been removed: those encoding malate dehydrogenase and succinyl coA-synthetase (in the four marine genomes) and succinate dehydrogenase (in MED4). The presence of the latter in both low-light-adapted *Prochlorococcus* genomes (MIT9313 and SS120) suggests that it confers some metabolic advantages upon organisms living at lower depths and thus receiving comparatively low energy input from light.

In order to gain further insights into the evolutionary history of carbon and nitrogen metabolism in *Prochlorococcus*, we utilized the Kyoto Encyclopedia on Genes and Genomes (KEGG, http://www.genome.ad.jp/kegg/pathway.html) to perform comparative analyses of specific pathways among the available cyanobacteria, utilizing *Synechocystis* PCC 6803 as the organism of reference, given its status as a widely utilized model in the literature; some results are summarized in Table 1. For each studied pathway, we outlined the genes found in PCC 6803 that are missing in at least one of the *Prochlorococcus* genomes. Interestingly, we observed that the percent of genes lost in the marine cluster ranged from 10 to 27% for carbon pathways, while in the two
studied cases of nitrogen pathways (nitrogen metabolism and glutamate metabolism, as described in the KEGG) it increased to 78 and 42%, respectively. This fact certainly points to the central position of carbon metabolic pathways in cell metabolism (thus making many genes essential for the cell); on the other hand, it becomes clear that evolution is also involved in such core routes. Moreover, the higher rate of gene loss in nitrogen pathways strongly suggests that these may be subjected to further reduction in order to keep functional only the central route (i.e., ammonium assimilation) so as to save energy, allowing the conservation (or even the acquisition [31]) in specific strains of genes encoding transporters and enzymes involved in assimilation of nitrogen sources available at the depth inhabited by those strains (i.e., nitrite for MIT9313, or cyanate for MED4).

A more detailed analysis of pathways shown in Table 1 provides additional hints on their speciation processes during evolution of the Synechococcus/Prochlorococcus cluster. Among genes lost in at least one genome from this cluster, all those which belong to carbon metabolism pathways (carbon fixation, pentose phosphate, citrate cycle and glycolysis/gluconeogenesis) are missing in all Synechocystis and Prochlorococcus genomes, strongly suggesting that they disappeared upon early divergence of the cluster in cyanobacterial radiation. This is the case for genes encoding malate dehydrogenase, glucose 1-phosphate dehydrogenase, 6-phosphofructokinase, succinyl CoA synthetase, and NAD- and NADP-dependent alcohol dehydrogenases. There exists a single exception to this rule: the gene encoding succinate dehydrogenase, which is missing in MED4 but is found in the genomes of WH8102, MIT9313 and SS120.

Interestingly, the NADP-dependent glyceraldehyde-3-phosphate dehydrogenase encoding gene appears in the genomes of Prochlorococcus, but not in those from other cyanobacteria such as Thermosynechococcus elongatus, Anabaena PCC 7120, Synechocystis PCC 6803 or Synechococcus PCC 6301, or even the marine Synechococcus WH8102. Furthermore, the gene encoding deoxyribokinase appears in the four marine genomes, but not in those of Synechococcus or other freshwater cyanobacteria. The physiological significance of these facts remains to be studied.

In contrast to the situation described above for the carbon pathways, when we look at the nitrogen-related genes lost in at least one marine genome (Table 1), most of them have been selectively removed or not, depending on the strain. This refers to genes encoding carbonic anhydrase, asparaginase and nitrate reductase (all of them conserved only in WH8102), nitrite reductase (conserved in WH8102 and MIT9313), glutamate dehydrogenase and glutamate decarboxylase (conserved only in MIT9313) and cyanase (conserved in WH8102 and MED4). In these pathways, there are only four genes missing in all studied marine genomes: those encoding carbamyl kinase, glutaminase, 1-pyruvino-5-carboxylate dehydrogenase, and succinic semialdehyde dehydrogenase.

### 4. Concluding remarks

Although the comparative analysis described in the preceding paragraphs is based on databases which need further refined annotation, we can reach several interesting conclusions:

First, in the evolution of the marine Synechococcus/Prochlorococcus cluster, both carbon and nitrogen metabolism pathways have been significantly modified, probably for better adaptation to the specific conditions of their habitat.

Second, the nitrogen-related pathways have been extensively modified to retain only the central ammonium assimilation route and some complementary pathways enabling utilization of nitrogen sources available at the depth at which the cyanobacterium lives (i.e., nitrite, urea, cyanate, amino acids, oligopeptides). The criterion for which these additional nitrogen sources have been selected seems to be the right balance between nitrogen availability and the necessary energy for performing its assimilation. This would explain why nitrite is assimilated by some Prochlorococcus strains living at depth (as MIT9313), but not nitrate, in spite of its abundance in the lower parts of the water column. The higher energetic cost of nitrate assimilation would prevent Prochlorococcus from utilizing it, since at the depths at which this nitrogen source is abundant, the available energy from light is extremely limited (less than 0.1% of the total irradiance penetrating at the surface).

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**Table 1**

Loss of genes in different metabolic pathways of the genomes of Prochlorococcus compared to that of Synechocystis PCC 6803 according to information available at the Kyoto Encyclopedia of Genes and Genomes website.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Genes in Synechocystis PCC 6803</th>
<th>Lost genes in Prochlorococcus</th>
<th>Lost genes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon fixation</td>
<td>18</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Pentose phosphate</td>
<td>18</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Glycolysis/gluconeogenesis</td>
<td>22</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Citrate cycle</td>
<td>11</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>Glutamate metabolism</td>
<td>19</td>
<td>8</td>
<td>42</td>
</tr>
<tr>
<td>Nitrogen metabolism</td>
<td>14</td>
<td>11</td>
<td>79</td>
</tr>
</tbody>
</table>

1 As defined by the KEGG.
2 Number of genes lost in at least one of the Prochlorococcus genomes.
Third, in Prochlorococcus speciation processes leading to the appearance of ecotypes adapted to the different conditions along the water column, nitrogen pathway modifications seem to play a more important role than those observed in carbon-related pathways, since for the latter, most observations (i.e., loss of genes) are common to all the genomes of marine Synechococcus and Prochlorococcus.

Fourth, contrary to the longstanding view of metabolic pathways and genomes as relatively stable models, recent advances in comparative genomics are showing that, in much the same way as many other biological structures, both of them should be considered as dynamic systems subjected to fine modulation in evolution. Thus, the “reference metabolic pathway” concept is useful as a theoretical framework for physiological studies, but it should not be considered as representative of actual organisms, as evidenced by the wide diversification of Synechococcus/Prochlorococcus ecotypes discussed in this paper. Recent reports on viruses affecting both Synechococcus and Prochlorococcus [21,36] point to their prominent role as mechanisms for transferring genetic material between the two phylogenetic groups. This should lead us to consider the whole pool of Synechococcus and Prochlorococcus genotypes as a continuum subjected to frequent gene transfer and reorganization, leading to the very wide genetic diversity observed in this cluster.

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References


