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Research Letter

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Distinct features of C/N balance regulation in *Prochlorococcus* sp. strain MIT9313

María Agustina Domínguez-Martín, Antonio López-Lozano, Oriol Alberto Rangel-Zúñiga, Jesús Díez and José Manuel García-Fernández*,†

Departamento de Bioquímica y Biología Molecular, Campus Universitario de Excelencia ceiA3, Universidad de Córdoba

*Corresponding author: Departamento de Bioquímica y Biología Molecular, Edificio Severo Ochoa, Planta 1, Campus de Rabanales, Universidad de Córdoba, 14071 Córdoba, Spain. Tel/Fax: +34957211075; E-mail: jmgarcia@uco.es

One sentence summary: The marine cyanobacterium *Prochlorococcus* sp. strain MIT9313 shows differential features in the regulation of genes involved in C/N metabolism, probably related to the ancient origin of this strain.

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†José Manuel García-Fernández, http://orcid.org/0000-0003-2983-1214

ABSTRACT

The abundance and significant contribution to global primary production of the marine cyanobacterium *Prochlorococcus* have made it one of the main models in marine ecology. Several conditions known to cause strong effects on the regulation of N-related enzymes in other cyanobacteria lacked such effect in *Prochlorococcus*. *Prochlorococcus* sp. strain MIT9313 is one of the most early-branching strains among the members of this genus. In order to further understand the C/N control system in this cyanobacterium, we studied the effect of the absence of three key elements in the ocean, namely N, P and Fe, as well as the effect of inhibitors of the N assimilation or photosynthesis on the N metabolism of this strain. Furthermore, we focused our work in the effect of ageing, as the age of cultures has clear effects on the regulation of some enzymes in *Prochlorococcus*. To reach this goal, expression of the main three regulators involved in N assimilation in cyanobacteria, namely *ntcA*, *glnB* and *pipX*, as well as that of *icd* (encoding for isocitrate dehydrogenase) were analysed. Our results show that the control of the main proteins involved in the C/N balance in strain MIT9313 differs from other model *Prochlorococcus* strains.

Keywords: *Prochlorococcus*; nitrogen metabolism; C/N balance sensing; transcriptional regulation; starvation; adaptive mechanisms

INTRODUCTION

Prochlorococcus is the most abundant photosynthetic organism on Earth (Partensky, Blanchot and Vaulot 1999; Biller *et al.* 2015); together with marine *Synechococcus*, it is responsible for a very significant part of the global primary production (Partensky, Hess and Vaulot 1999; Scanlan *et al.* 2009). The ecological relevance of marine picocyanobacteria is even more significant in the light of global warming, since oceanic phytoplankton populations are an important carbon sink (Landschützer *et al.* 2015), and recent studies suggest that the effects of climate change will increase their populations (Flombaum *et al.* 2013). Furthermore, improving the understanding of the phytoplankton blooms has been considered the next frontier in order to get climate projections right (Tollefson 2016). Recent long-term studies addressed the factors controlling the appearance of cyanobacterial blooms in the ocean, pointing to temperature as one of the main elements (Hunter-Cervera *et al.* 2016). However, and despite the large number of studies on marine picocyanobacteria, there are still many aspects insufficiently known. Among them, the

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metabolism of carbon and nitrogen and their regulation is one of the most important fields, since it links photosynthesis (allowing the incorporation of atmospheric CO_2) to the utilisation of nitrogen. N is one of the main elements of life, since it constitutes an essential part of proteins and nucleic acids. Because N is scarcely available in the oligotrophic intertropical oceans inhabited by *Prochlorococcus*, there are large regions where N limitation imposes harsh conditions for this cyanobacterium to thrive (Graziano *et al.* 1996).

Our team has studied the regulation of several enzymes involved in the N and C metabolisms in *Prochlorococcus*, namely glutamine synthetase (El Alaoui *et al.* 2001, 2003; Gómez-Baena *et al.* 2001; Gómez-Baena *et al.* 2006, 2015), isocitrate dehydrogenase (McDonagh *et al.* 2012; Domínguez-Martín *et al.* 2014) and glutamate dehydrogenase (Rangel *et al.* 2009). Besides, we have carried out a gene expression study focused on genes encoding enzymes and regulatory proteins that control the C/N balance in the low-light *Prochlorococcus* strain SS120 (López-Lozano *et al.* 2009). One of our main conclusions was that this control system seems to have been streamlined in *Prochlorococcus*, probably due to an evolutionary trend in order to save energy (García-Fernández and Diez 2004; García-Fernández, Tandeau de Marsac and Diez 2004). This fits with the results observed by other authors using different approaches, such as global transcriptomic (Tolonen *et al.* 2006) or proteomic studies (Grzymski and Dussaq 2011; Read *et al.* 2017).

In order to further understand the C/N control system in *Prochlorococcus*, we present a study in *Prochlorococcus* sp. strain MIT9313, representative of the LLIV ecotype. This is one of the most early-branching strains among the cultured members of the *Prochlorococcus* genus (Biller *et al.* 2015). In global transcriptomic studies addressing nitrogen availability in *Prochlorococcus* sp. strains MED4 and MIT9313, it was shown that both strains integrate N and C metabolism in profoundly different ways (Tolonen *et al.* 2006). More recently, a comparative RNA-seq transcriptomics study between the same strains further illustrated those differences, particularly in the case of non-coding RNAs (Voigt *et al.* 2014). The aim of this work is to further understand the differential features in the regulation of the C/N balance in *Prochlorococcus* sp. MIT9313. To this goal, we studied the expression of *icd*, the gene encoding the key enzyme isocitrate dehydrogenase, and main regulatory genes of C/N metabolism in cyanobacteria: the master nitrogen regulator *ntcA* (Luque, Flores and Herrero 1994), and the genes encoding proteins that interact with NtcA, *pipX* and *glnB*, encoding the coactivator of NtcA, PipX (Espinosa et al. 2006) and the regulatory P_{II} protein (Forchhammer and Tandeau de Marsac 1994), respectively. We performed a series of experiments to determine gene expression by semiquantitative RT-PCR, under conditions representative of limitations of *Prochlorococcus* populations in nature; i.e. starvation for N, P and Fe; inhibition of the glutamine synthetase (GS) and glutamate synthase (GOGAT) enzymes; inhibition of the photosynthetic electron transport; and ageing of the cultures. In this work, we describe and discuss the results with respect to those observed in other cyanobacterial strains.

MATERIALS AND METHODS

Growth of cultures of *Prochlorococcus*

Prochlorococcus marinus sp. strain MIT9313 (LL-adapted) was grown as previously described (El Alaoui *et al.* 2001). Methionine sulfoximine (MSX), azaserine, 3-(3–4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) were dissolved and added to cultures as previously described (El Alaoui *et al.* 2001).

Table 1. Primers used for quantitative real-time RT-PCR reactions.

Primer	Sequence 5′- 3′	Gene
RT-FMI	CAACATGCTCTTCGGCTAAA	*icd*
RT-RMI	AGTTCATAGCCCCAGTCACG	
RT-FP2M	CGACGACGACAAGGTGGA	*glnB*
RT-RP2M	CTTGCTGTCACGCTCGCC	
RT-FTCA	GCGTGGGGTTACTTCTGC	*ntcA*
RT-RTCA	CGATGGACTGATGTGAGAGG	
RT-FPXM	TTACGCCACGCTCTATGC	*pipX*
RT-RPXM	AACAACTGCTGCCAACTCTC	
FRPM	CTATCTAGGACCGCCGTTAC	*rnpB*
RRPM	GAGAGTGCCACAGAAAAACA	

Cell collection

Cell collection and the preparation of culture media devoid of key elements for experiments addressing nutrient starvation are described in López-Lozano *et al.* (2009).

RNA isolation

RNA was isolated from 500 mL culture samples, using the TRIsure RNA Isolation Reagent (Bioline) following the method described previously by our team (Domínguez-Martín, Díez and García-Fernández 2016).

Real-time quantitative RT-PCR analysis of gene expression

cDNA synthesis from RNA samples was carried out with the iScript™ cDNA Synthesis kit from *Quanta* as recommended by the manufacturers (Domínguez-Martín *et al.* 2014). Specific primers for *icd, ntcA, glnB and pipX* genes (encoding isocitrate dehydrogenase, NtcA, P_{II} and PipX, respectively) were designed as previously described (Domínguez-Martín, Díez and García-Fernández 2016). Specificity of the qRT-PCR reactions was checked by agarose gel electrophoresis. The sequences of the primers used are listed in Table 1.

qRT-PCR semiquantitative gene expression determinations were carried out as previously described (Domínguez-Martín *et al.* 2014), according to the Pfaffl method (Pfaffl 2001), on an iCycler IQ multicolor real-time PCR detection system (Bio-Rad). The melting point of PCR products was used to confirm the absence of false amplifications. Triplicate determinations from at least three independent biological samples subjected to identical culture conditions were used for calculations. Results were endogenously normalised to *rnpB* expression (encoding RNase P), using the $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001), since this gene did not change under our experimental conditions.

The results shown in all figures correspond to relative expression, so that each value represents the changes under a specific condition with respect to the same culture under control conditions.

Prochlorococcus genomic sequences

Genomic sequences were retrieved from the Joint Genome Institute (http://genome.jgi-psf.org) and Cyorf (http://cyano.genome.ad.jp/) databases.

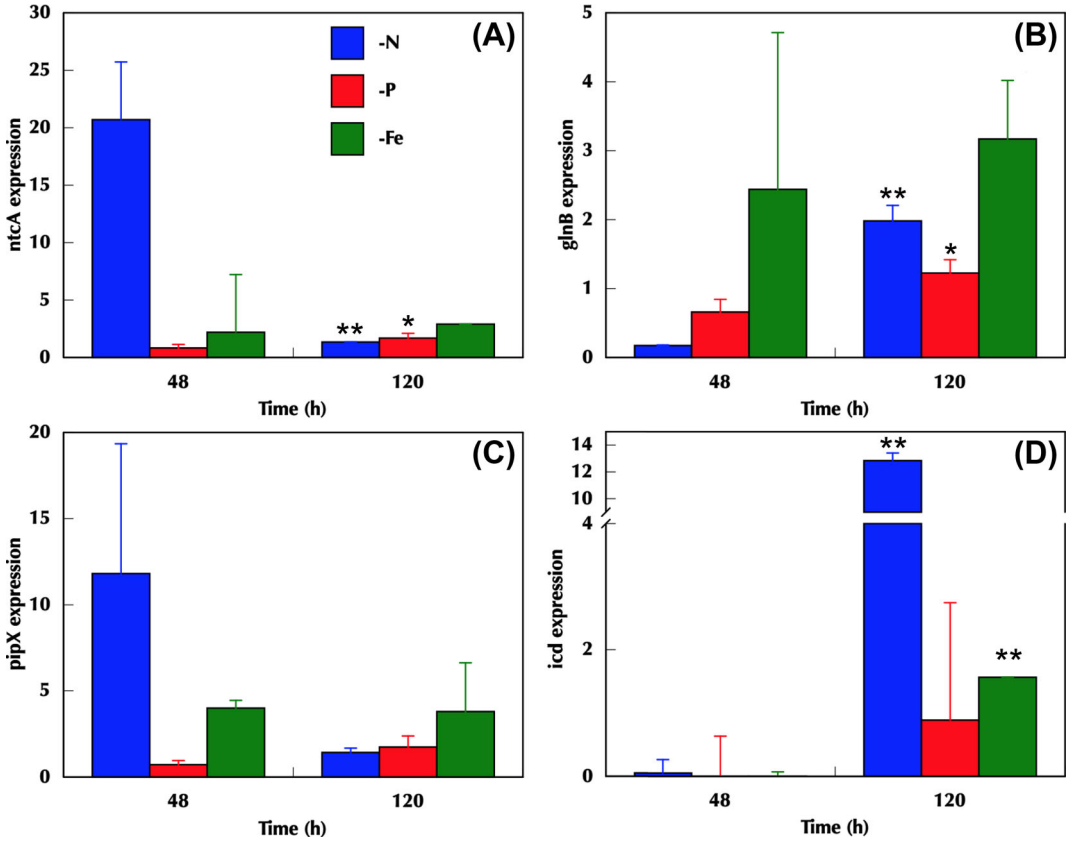

Figure 1. Effect of key elements starvation on gene expression in *Prochlorococcus* sp. strain MIT9313. Cells were subjected to nitrogen (blue), phosphorus (red) or iron (green) starvation for 5 days, keeping an aliquot growing under standard conditions as control. Samples were obtained at 48 and 120 h to determine gene expression by qRT-PCR. The expression levels represent the response of starved cells with respect to the control, for each sample. A, *ntcA* expression. B, *glnB* expression. C, *pipX* expression. D, *icd* expression. Error bars represent the standard deviation.

Statistical analysis

Results were obtained by using at least three different samples, and error bars show the standard deviation. Statistical analysis was done using the Student's *t*-test, as previously described (Domínguez-Martín *et al.* 2014). Assessment of data significance is indicated with asterisks: * indicates $P \leq 0.05$; ** indicates $P \leq 0.01$.

RESULTS AND DISCUSSION

Effect of key elements starvation

In previous studies on the regulation of nitrogen-related enzymes, we observed that several conditions known to cause strong effects in other cyanobacteria lacked such effect in *Prochlorococcus* (El Alaoui *et al.* 2001, 2003; Domínguez-Martín *et al.* 2014); thus, we were interested to check whether this might be due to a general streamlining on regulatory networks or to some specific response of these N-related enzymes. We decided to study the effect of the absence of three key elements in the ocean, namely N, P and Fe, which are often in limiting concentration in oligotrophic areas, on the N metabolism of *Prochlorococcus* sp. MIT9313.

Prochlorococcus MIT9313 cells were transferred to media without any added key element (either N, P or Fe), and were kept under such conditions for 5 days, sampling at 48 and 120 h, in order to test whether prolonged starvation effects were different with respect to those observed after 2 days starvation. Aliquots of the same cultures were kept under standard conditions.

Nitrogen

Figure 1 shows the relative expression changes observed for the *ntcA*, *glnB*, *pipX* and *icd* genes under N starvation, with respect to cells growing under standard conditions (standard PCR-S11 media, which includes 400 μM $(NH_4)_2SO_4$).

ntcA showed a strong increase (over 20-fold) at 48 h, which however disappeared almost completely at 120 h ($P = 0.0026$). *pipX* expression showed a similar pattern, although the induction at 48 h was less remarkable (ca. 12-fold). However, *glnB* and *icd* changed in the opposite way: their expression showed little change at 48 h, while it was strongly upregulated after 120 h ($P = 0.0002$ and $P = 0.0001$, respectively). This behaviour is consistent with previous reports in *Prochlorococcus* sp. MIT9313 (Tolonen *et al.* 2006) that showed upregulation of *ntcA* under nitrogen starvation, while *glnB* was slightly downregulated after 48 h. Our results also fit with previous studies in freshwater cyanobacterial strains, where *ntcA* (Luque, Flores and Herrero 1994) and *pipX* (Valladares *et al.* 2011) have been shown to increase upon nitrogen starvation.

The study of the promoters of *ntcA*, *glnB* and *pipX* associated with reported transcriptional start sites (TSS) upstream of the coding sequence of these regulatory genes in *Prochlorococcus* sp. MIT9313 (Voigt *et al.* 2014) showed the presence of a

putative NtcA-binding site in all of them (Fig. S1, Supporting Information). Although the NtcA-binding sites of these genes presented some variations with respect to the consensus palindromic sequence $GTAN_8TAC$ described for cyanobacteria (Luque, Flores and Herrero 1994), they still conserved the most necessary nucleotides required for this binding (Picossi, Flores and Herrero 2014). NtcA-activated promoters also show a canonical sigma-70 *Escherichia coli*-like -10 box (TAN_3T) that is found in either the *ntcA* or the *pipX* promoter, while that of *glnB* presents an incomplete version of this box in MIT9313 strain. Interestingly, these three regulatory genes also possess potential antisense RNA (aTSS) (Voigt *et al.* 2014) associated with NtcA-binding sites with either activating or repressing function (Fig. S1, Supporting Information). NtcA acts as a repressor when the NtcA-binding site overlaps the RNA polymerase-binding site (García-Domínguez, Reyes and Florencio 2000); while *ntcA*, *glnB* and *pipX* show repressive NtcA-binding sites for all their reported aTSS, only *glnB* and *pipX* present additional activator sites linked to –10 non-canonical boxes that could lead the expression of these aTSS. Hence, the balance between the expression of gene coding RNA (gTSS) and aTSS in *glnB* and *pipX*, likely controlled by the differential affinity of both NtcA and RNA polymerase for their corresponding binding sites, would explain the differences observed in the expression of those regulatory genes with respect to that of *ntcA* under nitrogen starvation, despite being modulated by the same transcription factor. This would require further experimental confirmation by northern blot by using specific probes.

A significant change was observed when we compared the gene expression results obtained with the *Prochlorococcus* sp. strain MIT9313 with those reported previously by our team on *Prochlorococcus* sp. strain SS120 (López-Lozano *et al.* 2009): with that strain, we could see a decrease in *glnB* and *ntcA* expression under N starvation. At the same time (48 h), we observed a stronger decrease in *glnB* expression in MIT9313, followed by an increase of roughly 2-fold at 120 h (Fig. 1B). Besides, we observed an increase in *ntcA* expression in MIT9313 (Fig. 1A), in contrast to the decrease observed in SS120 (López-Lozano *et al.* 2009). This suggests that, even comparing two low-light adapted strains as SS120 and MIT9313, there exists some differences regarding key regulator proteins as P_{II} and NtcA. The distinct evolutive stories of these strains might help to explain the observed differences (see later).

A possible reason for these differences might be that, unlike SS120, *Prochlorococcus* sp. strain MIT9313 seems to be capable of using glutamate, since it possesses the *gdh* gene, encoding glutamate dehydrogenase (Rangel *et al.* 2009). The downregulation effect on *gdh* expression and its activity when MIT9313 cells were subjected to N starvation suggest that this enzyme in *Prochlorococcus* is involved in the assimilation of amino acids, and is not performing direct ammonium assimilation to produce glutamate (Rangel *et al.* 2009). This might be useful to assimilate glutamate released to culture media in aged cultures (Rangel *et al.* 2009), thus providing an alternate source of nitrogen to *Prochlorococcus* sp. strain MIT9313, which might be unavailable for the strain SS120.

Phosphorus

Unlike the results observed under N limitation, P starvation induced the long-term upregulation of all studied genes (*ntcA*, *glnB*, *pipX* and *icd*). However, the changes were much less remarkable compared to those observed under N starvation (Fig. 1). This suggests that those effects are not mediated by the same regulatory mechanisms as those provoked by N starvation. Interestingly, no clear effect was observed in samples obtained 48 h after the beginning of P starvation. Since *ntcA*/*pipX* showed opposite regulatory patterns to *glnB* under N starvation, the results observed under P starvation might suggest that they are caused by a general stress response, leading to the upregulation of a number of genes. This is in good agreement with global transcriptomic studies focused on the effects of P limitation in *Prochlorococcus* sp. MIT9313 (Martiny, Coleman and Chisholm 2006). However, no specific changes for the genes studied in our paper were reported by Martiny and coworkers. In transcriptomic studies addressing P limitation in *Prochlorococcus* sp. MED4, no specific changes for the genes here studied were reported either (Reistetter *et al.* 2013).

Iron

Fe starvation induced a ca. 4-fold increase in the *glnB* and *pipX* expression, and slightly lower in the case of *ntcA*. Interestingly, this increase was roughly similar at both times of sampling (48 and 120 h), in contrast with the results obtained for both N and P starvation. There was a significant upregulation in *icd* expression after 120 h ($P = 0.001$). Our results might suggest that *Prochlorococcus* can quickly adapt to Fe starvation by increasing the expression of certain genes, keeping them upregulated as long as the starvation persists. This hypothesis, in turn, fits with previous studies suggesting that *Prochlorococcus* is an organism adapted to grow in environments with low iron concentrations, which has developed specific adaptation mechanisms (i.e. the utilisation of *pcb* genes as antenna, whose phylogeny is very related to *isiA*, a gene expressed under Fe starvation in other cyanobacteria; Bibby *et al.* 2003).

Effect of ageing

Previous studies of our team showed that age of cultures had clear effects on the regulation of some enzymes in *Prochlorococcus* as glutamine synthetase (Gómez-Baena *et al.* 2006) or glutamate dehydrogenase (Rangel *et al.* 2009). Hence, we studied whether this effect might be due to changes in the expression of regulatory genes, and consequently the expression of other enzyme-coding genes. Figure 2 shows the expression of *ntcA*, *glnB*, *pipX* and *icd* over the course of 11 days. We observed a progressive increase in the expression of the three regulatory genes: *ntcA* and *pipX*, with the latter showing the strongest upregulation (more than 15-fold increase; $P = 0.0022$), followed by *icd* (ca. 12-fold; $P = 0.0001$) and *ntcA* (ca. 10-fold); *glnB* expression showed a less marked increase (ca. 3-fold). This suggests that the consumption of nitrogen by *Prochlorococcus* cells might lead to a reduced availability of this element in aged cultures, thus inducing the expression of the regulatory genes here studied. Those changes, in turn, would induce the *icd* upregulation (Fig. 2).

These results are consistent with the evolution observed for *gdhA* expression in *Prochlorococcus* sp. strain MIT9313: it increased over 15-fold after 7 days of cell growth (Rangel *et al.* 2009). Interestingly, the glutamate dehydrogenase enzyme concentration did not significantly change in that period, suggesting that the increase in gene expression was caused by a faster turnover of the protein. Furthermore, we demonstrated that isocitrate dehydrogenase is subjected to oxidative modification (Gómez-Baena *et al.* 2006; McDonagh *et al.* 2012), and this process has been shown to be age-dependent in the case of glutamine synthetase (Gómez-Baena *et al.* 2006). We propose that ageing induces oxidative degradation in isocitrate dehydrogenase

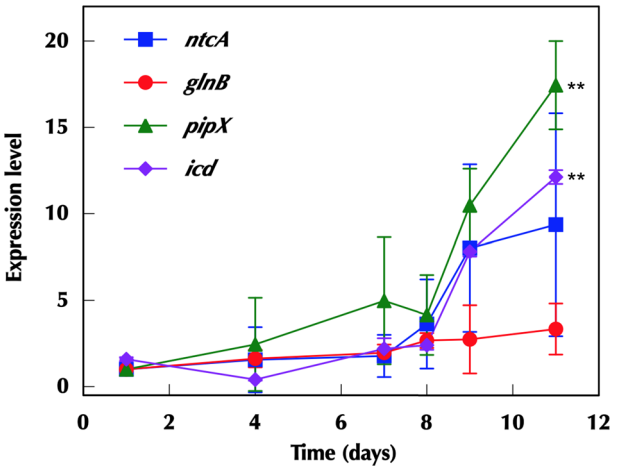

Figure 2. Effect of ageing on gene expression in *Prochlorococcus* sp. strain MIT9313. Cells were grown under standard conditions for 12 days and samples were obtained at the indicated times to determine gene expression by qRT-PCR. The expression levels represent the response of cells at the indicated times with respect to the values determined at the beginning of the experiment. The expression levels correspond to *ntcA* (blue), *glnB* (red), *pipX* (green) and *icd* (purple). Error bars represent the standard deviation.

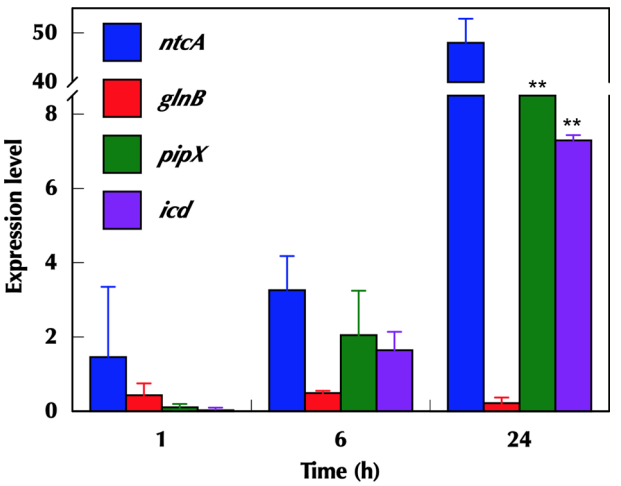

Figure 3. Effect of MSX addition on gene expression in *Prochlorococcus* sp. strain MIT9313. MSX (100 μM) was added to cultures at 0 time, keeping an aliquot growing under standard conditions without addition. Samples were obtained at the indicated times to determine gene expression by qRT-PCR. The expression levels represent the response of MSX-treated cells with respect to the control, for each sample. The expression levels correspond to *ntcA* (blue), *glnB* (red), *pipX* (green) and *icd* (purple). Error bars represent the standard deviation.

from *Prochlorococcus* MIT9313 cells, accelerating the turnover of this protein. This is compensated by an increased *icd* expression to overcome degradation of the enzyme under such conditions.

Effect of inhibitors of N assimilation

MSX and azaserine are specific inhibitors of GS and GOGAT, respectively (Pace and McDermott 1952; Oaks, Sivasankar and Goodfellow 1998), which have been used in physiological studies on the role of both enzymes and the regulatory system of nitrogen metabolism in cyanobacteria.

Addition of 100 μM MSX induced a strong upregulation of *ntcA* (ca. 50-fold), detectable 24 h after its addition to *Prochlorococcus* sp. MIT9313 cultures (Fig. 3); *pipX* and *icd* were also upregulated, but with a less pronounced effect (ca. 9-fold and 7-fold increase in expression, respectively), although in both cases it was significant ($P = 0.0001$ for both conditions 24 h after addition of inhibitor). The pattern was different in *glnB*, whose expression decreased at 24 h, after a small increase at 6 h.

Interestingly, the response in the case of *ntcA* in *Prochlorococcus* sp. MIT9313 is opposite to that observed in another low-light adapted *Prochlorococcus* strain: its expression decreased 0.5-fold 24 h after MSX addition in SS120 (López-Lozano *et al.* 2009) vs ca. 50-fold increase in MIT9313 (Fig. 3). This fact is particularly striking, since *ntcA* encodes the master nitrogen regulator, and its function seems to have been conserved in the whole cyanobacterial radiation. That two *Prochlorococcus* strains adapted to grow at depth show such a strong difference in a basic regulatory mechanism is a good example of the diversification experimented by the *Prochlorococcus* genus.

The response of *icd* after MSX addition was also different between both strains: it showed a comparable increase but with a very different timing, it increased very quickly in SS120 (6-fold after 1 h) but more slowly in MIT9313 (7-fold after 24 h; Fig. 3), despite the huge upregulation of *ntcA* in this strain.

When we analysed the effects of 100 μM azaserine to *Prochlorococcus* sp. MIT9313 cultures (Fig. 4), we observed an increase in the expression of *ntcA*, *glnB* and *pipX* 5 h after addition of the inhibitor; however, this effect disappeared after 24 h for the three genes, showing a significant decrease in the case of *glnB* ($P = 0.002$). Interestingly, *icd* expression did increase after 24 h. These changes were similar to those observed for the same genes in *Prochlorococcus* SS120 (López-Lozano *et al.* 2009).

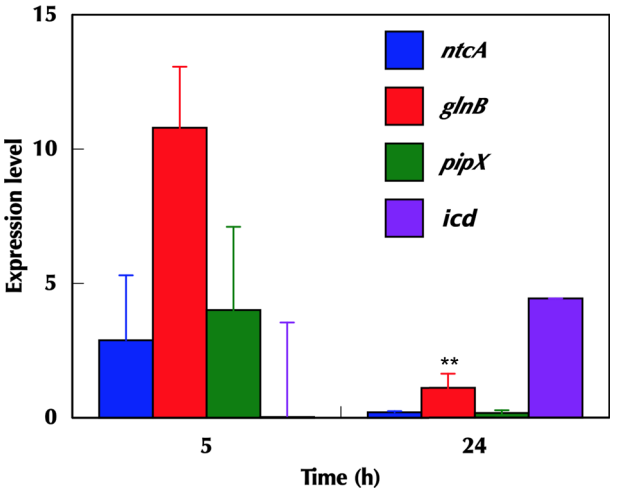

Figure 4. Effect of azaserine addition on gene expression in *Prochlorococcus* sp. strain MIT9313. Azaserine (100 μM) was added to cultures at 0 time, keeping an aliquot growing under standard conditions without addition. Samples were obtained at the indicated times to determine gene expression by qRT-PCR. The expression levels represent the response of azaserine-treated cells with respect to the control, for each sample. The expression levels correspond to *ntcA* (blue), *glnB* (red), *pipX* (green) and *icd* (purple). Error bars represent the standard deviation.

In summary, MSX addition provoked striking differences between MIT9313 and SS120, but the effect of azaserine was alike in both strains.

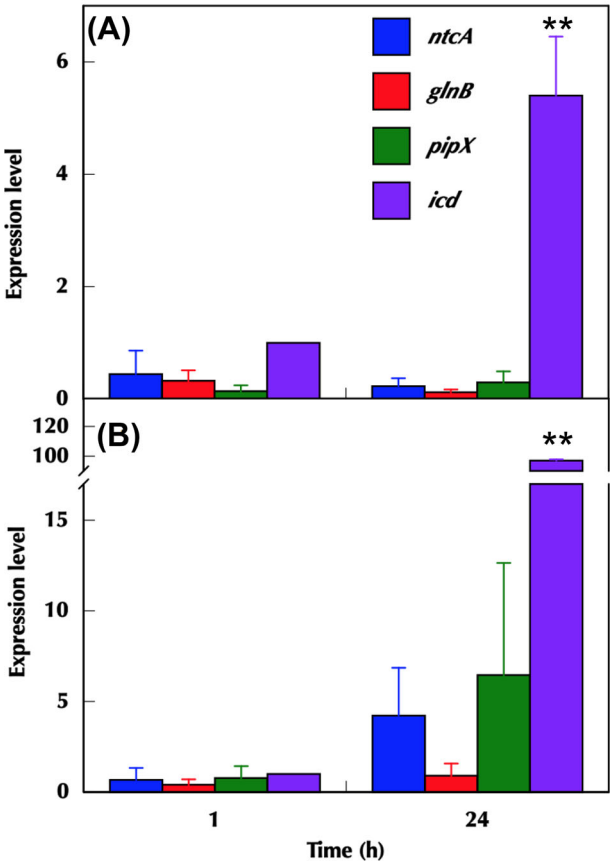

Figure 5. Effect of DCMU and DBMIB addition on gene expression in *Prochlorococcus* sp. strain MIT9313. (**A**) DCMU (0.3 μM) and (**B**) DBMIB (0.06 μM) were added to cultures at 0 time, keeping an aliquot growing under standard conditions without addition. Samples were obtained at the indicated times to determine gene expression by qRT-PCR. The expression levels represent the response of inhibitor-treated cells with respect to the control, for each sample. The expression levels correspond to *ntcA* (blue), *glnB* (red), *pipX* (green) and *icd* (purple). Error bars represent the standard deviation.

Effects of inhibitors of the photosynthetic electron chain

The addition of photosynthetic inhibitors (DCMU and DBMIB, blocking the photosynthetic electron flow before and after the plastoquinone pool, respectively) was studied in order to check whether the electron transport was involved in the control of the C/N balance in *Prochlorococcus* sp. MIT9313, and more specifically, whether the redox state of plastoquinone could be directly responsible for this control. Both inhibitors were added separately at the indicated concentrations, and samples from *Prochlorococcus* sp. MIT9313 cultures were taken after 1 and 24 h of the inhibitors' addition. A control culture with no addition was used in all experiments.

DCMU had little effect on the expression of the studied genes (Fig. 5A), with minor up- or downregulation depending on the gene, with the exception of *icd*, which was significantly upregulated after 24 h ($P = 0.0019$). However, addition of DBMIB induced an increase in all cases, going from 2-fold increase in the case of *glnB* to a remarkable ca. 100-fold increase in the case of *icd* ($P = 0.0001$). This suggests that electron flow is indeed involved in the control of the C/N balance; taking into account the striking effect of DBMIB, and the lack of effect of DCMU, it seems that reduction of the plastoquinone pool induces gene expression.

Interestingly, we observed similar effects for both inhibitors on *ntcA* and *glnB* expression in *Prochlorococcus* sp. SS120 (López-Lozano *et al.* 2009). However, DBMIB effect on *icd* expression clearly differs between both strains: *icd* levels strongly decreased in the SS120 strain, while they showed a huge increase in the MIT9313 strain (Fig. 5B). This points out to fundamental differences in the control of *icd* expression in *Prochlorococcus* depending on the studied strain, since isocitrate dehydrogenase is the enzyme linking the C and N metabolisms in cyanobacteria (Domínguez-Martín *et al.* 2014). Remarkable differences in the integration of the C/N metabolisms were also observed in previous transcriptomic studies (Tolonen *et al.* 2006). Our work corroborates these results and provides additional evidence to support a modulation of the C/N balance control systems in *Prochlorococcus*; early branching low-light strains as MIT9313 show a *icd* responsiveness to the redox state of the plastoquinone pool that seems to have been lost in more late-branching ecotypes as SS120. This idea fits nicely with the hypothesis of general streamlining in the C/N control systems in *Prochlorococcus*, previously proposed by our team (García-Fernández, Tandeau de Marsac and Diez 2004), as an adaptive mechanism to very oligotrophic oceans. Related processes have also been described by our team in the regulation of glutamine synthetase in the marine strain *Synechococcus* sp. WH7803 strain (Domínguez-Martín, Díez and García -Fernández 2016).

Concluding remarks

The results described in this work evidence clear differences in the expression of key genes in the regulation of C/N in *Prochlorococcus* sp. strain MIT9313, which are especially remarkable when compared to similar experiments in other low-light adapted strain as SS120: the expression of *glnB* under nitrogen starvation (Fig. 1B); the expression of *ntcA* after MSX addition (Fig. 3); and the expression of *icd* after DBMIB addition (Fig. 5B). All these responses are almost opposite to those found in *Prochlorococcus* sp. SS120, where *glnB*, *ntcA* and *icd* transcript levels decrease or remain unchanged when cells are subjected to the same conditions (López-Lozano *et al.* 2009). This might be another example of a progressive process of simplification in the regulatory systems of *Prochlorococcus*, where regulatory proteins become less responsive to changes in the environment: i.e. the lack of phosphorylation of the P_{II} protein (Palinska *et al.* 2002) or the decreased responsiveness of NtcA to 2-oxoglutarate (Domínguez-Martín *et al.* 2017). In this view, late-branching strains of the low-light *Prochlorococcus* ecotypes (as SS120) show regulatory patterns that are streamlined with respect to early-branching, low-light adapted strains as *Prochlorococcus* sp. strain MIT9313.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

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