Chapter 6

The Challenge of Iron Stress in Cyanobacteria

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Abstract

Iron is an essential nutrient for most living organisms. Due to the low solubility of ferric iron at physiological pH, the transition from an anaerobic atmosphere to the actual oxidant environment caused a dramatical decrease of iron bioavailability. Therefore, most organisms had to adapt their lifestyle to survive under an iron-depleted environment. In cyanobacteria, the electron transport chains involved in photosynthesis and respiration, as well as the enzymes involved in nitrogen metabolism have a high content of iron. Hence, cyanobacterial iron requirements are much higher than those of heterotrophic organisms. In this chapter, we revise different strategies developed by this important group of microorganisms to cope with iron deficiency, as well as the regulatory networks involved in the homeostasis of this indispensable element.

Keywords: cyanobacteria, iron stress, regulation, photosynthesis, nitrogen metabolism, cross-talk, cyanotoxin production

1. Introduction

The biological importance of iron almost entirely resides in its incorporation into proteins, either as a mono- or binuclear species, or as part of iron-sulfur clusters and heme groups. Through these forms, iron acts as a cofactor of a plethora of crucial enzymes and electron carriers involved in major biological processes including photosynthesis, respiration, tricarboxylic acid cycle, DNA biosynthesis and nitrogen fixation, among others [1]. Despite iron is the fourth most abundant element on earth crust, its bioavailability is extremely limited because of its poor solubility in the actual oxygenic atmosphere. Hence, whereas free Fe^{3+}

concentration ranges from 10^{-9} to 10^{-18} M, virtually all living microorganisms require a minimum effective concentration of 10^{-8} M to live and growth, and at least 10^{-7} to 10^{-5} M to achieve optimal growth [1].

Iron limitation is a challenge of particular importance in cyanobacteria, being one of the main limiting factors of ocean primary productivity [2]. Cyanobacteria have an absolute dependence of iron for growth and optimal development of their major physiological processes, particularly photosynthesis and nitrogen fixation. Iron serves as a cofactor for every membrane-bound protein complex and other mobile electron carriers within the photosynthetic apparatus [3], which determines an iron quota about 10 times higher than that exhibited by a similarly sized non-photosynthetic bacterium [4]. Additionally, diazotrophic cyanobacteria have significant further iron requirements compared with other phototrophs due to the abundance of iron-containing enzymes in the nitrogen-fixation machinery [5]. Although iron plays a key role in cyanobacterial physiology, an excess of free intracellular iron is extremely deleterious because it catalyzes the formation of reactive oxygen species (ROS) through Fenton reactions, leading to oxidative stress [6]. Likewise, iron starvation leads to significant increase in ROS and induces oxidative stress in cyanobacteria [7]. Hence, iron uptake and metabolism must be tightly regulated in order to ensure suitable supply maintaining the intracellular concentration within nontoxic levels [8, 9].

To cope with the usually frequent periods of iron starvation in nature, cyanobacteria have evolve efficient strategies which imply changes in the transcription of a plethora of genes, resulting among other changes in a deep rearrangement of the photosynthetic machinery [10] and the induction of the mechanisms involved in iron uptake. Thus, the transcription of genes coding for several TonB-dependent outer membrane transporters, periplasmic ferric-binding proteins, ATP-binding permeases as well as enzymes involved in siderophore biosynthesis will depend on iron availability [9, 11, 12].

Since an effective balance between iron acquisition and protection against oxidative stress is crucial for cell survival, as occurs in most Gram-negative and several Gram-positive bacteria, in cyanobacteria iron homeostasis is controlled by a global transcriptional regulator known as Fur, which stands for ferric uptake regulator [9, 13, 14]. Fur typically acts as a transcriptional repressor, which senses intracellular free iron and modulates transcription in response to iron availability [1]. Fur not only controls the expression of iron acquisition and storage systems, but also a wide set of genes and operons belonging to a broad range of functional categories, thereby contributing to couple iron availability to major physiological processes in cyanobacteria [14–17]. In this chapter, we revise the strategies of these photosynthetic bacteria to face the challenge of iron starvation. We put special emphasis in the transcriptional and physiological changes triggered by iron starvation in this group of microorganisms. Details on cyanobacterial iron metabolism and control of iron homeostasis as well as their connections with other cellular processes are discussed.

2. Classical strategies to overcome iron starvation situations

Cyanobacteria evolved very efficient mechanisms to cope with iron deficiency. Iron deprivation triggers a variety of responses that range from upregulation of the iron acquisition systems to

reduction or substitution of structures or molecules. At the physiological level, Strauss [18] categorized the responses as retrenchment (reduction of cell size, loss of phycobilisomes, ultrastructural changes and pigment changes), compensation (as the synthesis of flavodoxin, playing ferredoxin role, expression of *isiA* gene) and acquisition (induction of iron acquisition systems). Accommodation to iron deficiency requires changes in the expression of a large number of genes of many metabolic pathways, some of them not obviously related with iron metabolism, such as respiration, photosynthesis, nitrogen metabolism, glycolysis, tricarboxylic acids cycle, amino acid synthesis, synthesis of toxins and antioxidant defenses. Those changes highlight the responses associated to iron deficiency [9, 19]. It is important to consider that the responses are going to be different depending on the stress threshold: moderate, severe or extreme.

2.1. Rearrangement of photosynthetic electron transport chain under iron starvation conditions

Many photosynthetic components are iron-containing proteins, and also iron is involved in chlorophyll synthesis. Chlorophyll level is affected by iron availability, so the photosynthetic machinery may be diminished or even dismantled if the deficiency occurs suddenly, as in laboratory experiments. In general, populations living in limiting environments adapt its chlorophyll synthesis to the bioavailability, and the chlorophyll per cell is lower. Iron deficiency adaptation implies a reduction of the linear photosynthetic electron transport and enhances respiratory electron transport [20, 21] as well as a concomitant increase of the cyclic photophosphorylation [22]. Moreover, under iron deficiency, several responses to oxidative stress have been described, evidencing the link between iron starvation and oxidative stress, with photosystems specially affected [7, 23]. Consistently, several photosynthetic and oxidative defense genes have been identified as regulated by iron availability [9, 14, 24]. Among the iron-induced genes, *isiAB* [13] and *idiAB* products are playing key roles in the adaptability of the photosynthetic machinery to optimize its function at low iron availability.

2.1.1. IsiA and IsiB proteins

In *Synechococcus* sp., the *isiAB* operon is transcriptionally regulated to be expressed under iron deficiency, and the monocistronic transcript of *isiA* is more abundant than the dicistronic one [25]. IsiA gene product was found to confer fitness of photosynthetic machinery under iron-limited environments. The product of *isiA* was described in iron-starved *Anacystis nidulans* as an induced chlorophyll-binding protein [26]. This protein was initially named CP43'due to its similarity to CP43, located at the photosystem (PS)II [25]. Initially, IsiA was proposed to play a role as an additional light-harvesting complex [27], and over the years, several functions have been suggested, summarized by Sun and Golbeck [28]: (i) IsiA is a chlorophyll storage protein for the rapid recovery of the cyanobacteria after stress [29]; (ii) it acts as an excitation energy dissipator, protecting PSs from photoinhibition [30]; (iii) it serves as a light-harvesting complex potentially for both PSs [27, 31] and (iv) IsiA replaces CP43 in PSII and permits a cyclic electron transfer pathway involving PSII and the cytochrome b_6f complex [32, 33].

It is interesting to note that *isiA* is not present in all cyanobacteria, and no homologs of *isiA* have been found in plants. In fact, the presence of *isiA* in cyanobacteria found in the iron-limited,

high-nutrient low-chlorophyll regions of the equatorial Pacific lead to the suggestion that the presence of this gene can be a natural biomarker for iron limitation in oceanic environments [34].

In most unicellular cyanobacteria downstream, *isiA* lies the *isiB* gene that encodes a small FMN-flavoprotein called flavodoxin. It is noticeable that, usually, in filamentous cyanobacteria, the flavodoxin gene is transcribed independently of *isiA* and lies in a different locus. Flavodoxin allows that the distribution of light energy as reducing power remains unaltered in iron deficient environments. When iron is not available, the synthesis of the iron-sulfur protein ferredoxin is repressed while flavodoxin is induced. Flavodoxin replaces ferredoxin as an electronic transporter in many of the reactions in which ferredoxin participates [35–39]; surprisingly, flavodoxin is not able to functionally replace heterocyst ferredoxin, even though electron transfer chain to nitrogenase is also an iron-dependent process [35]. Flavodoxin is not exclusive of cyanobacteria, and it may also be present in heterotrophic bacteria as well as in a few cases of algae [40]. Cyanobacteria which lack flavodoxin synthesis capability are particularly affected when iron is scarce, and ferredoxin downregulation under adverse conditions severely compromises survival [41]. Ferredoxin and flavodoxin are isofunctional proteins, but they do not share any significant similarity in primary, secondary or tertiary structures. These proteins can interact productively with the same redox partners [37, 38] and exhibit kinetics constants in the same range even though flavodoxin is slightly less efficient [37].

Flavodoxin expression is induced not only under iron deficiency but also under a wide range of several environmental stresses that result in ferredoxin downregulation [38, 42, 43], especially oxidative stress. Concerning the photosynthesis, flavodoxin behaved as an alternative intermediate for the photosynthetic electron transfer chain *in vivo*, acting, as ferredoxin does, as the main distributor of the reducing power [38, 44]. Under iron limitation, reduced flavodoxin also signals for the whole cell the presence of an active photosynthetic electron transfer chain through the thioredoxin electron transfer pathway. Reduced thioredoxins via thioredoxin reductase, regenerates, through reduction of their cysteine residues, the active forms of many target enzymes as peroxiredoxins, Calvin cycle enzymes and $NADP^+$-malate dehydrogenase, among others. Flavodoxin allows that this key process is still working under iron deficient conditions.

Since flavodoxin synthesis is one of the first responses to iron deficiency [45], flavodoxin was first proposed as an iron-deficiency biomarker in the marine diatom *Thalassiosira weissflogii* [46]. Similarly, in the green algae *Scenedesmus vacuolatus*, the ferredoxin/flavodoxin ratio [47, 48] was used as iron-stress molecular marker.

2.1.2. IdiA, IdiB and IdiC proteins

In cyanobacteria under iron and manganese limitation, the *idiA* gene expresses the iron deficiency-induced protein, IdiA [49]; No counterpart seems to exist in green algae and higher plants [22]. The transcriptional regulator IdiB regulates the expression of *idiA*, in a response controlled by iron availability [50]. IdiA plays an important role in protecting the acceptor side of PSII against oxidative damage, especially under iron-limiting growth conditions [51].

IdiA shows considerable sequence similarity to a family of bacterial periplasmic ABC transporter complexes involved in iron import known as FutA, SfuA, FbpA or HitA (http://genome.

microbedb.jp/cyanobase/). Although some IdiA-similar proteins have been found in the periplasm [52], IdiA is predominantly found associated to thylakoids [53], suggesting different functions for the distinct IdiA-similar proteins [52]. IdiA undergoes prominent structural changes upon iron deficiency and forms a tight and specific complex with dimeric PSII by interaction with CP43 and D1 [54], suggesting that IdiA protects the acceptor side of PSII, which is more exposed under iron limitation due to ongoing phycobilisome degradation [54].

In the *idi* operon, IdiB positively regulates transcription of *idiA* under iron starvation. IdiB encoding a member of the Crp/Fnr transcriptional regulators family [55] is transcribed under iron limitation and oxidative stress and controlled itself by iron-responsive Fur family members [56]. A third iron-regulated gene is *idiC*, belonging to the thioredoxin-like (2Fe–2S) ferredoxin family. Even though IdiC synthesis is constitutive, iron limitation induces a strongly enhanced expression of *idiC*. IdiC is loosely attached to the thylakoid and to other membranes, and its expression is enhanced during conditions of iron starvation or during the late growth phase [57]. Even though its role is still unclear, based on the similarity of IdiC to NuoE of the respiratory *Escherichia coli* NDH-1 complex, it has been suggested that IdiC is a component of the NADH-1 complex in *Synechococcus elongatus* and, thus, has a function in the electron donation from NAD(P)H to plastoquinone. Under stress conditions, when PSII resulted damaged, IdiC would prevent or reduce the oxidative stress deviating electron transport via alternative dehydrogenases, increasing PSI cyclic flow interconnected with respiratory routes [57].

2.2. Siderophore synthesis and induction of high affinity transporters

Derepression or induction of high affinity transporters to enhance iron acquisition as well as siderophore synthesis and cell surface enzymes production is a generalized response to iron starvation [1]. In cyanobacteria, siderophore-mediated iron uptake is thought to be an evolutionary advance that contributes to dominate iron-limited environments. Siderophores are strong Fe^{3+} chelators, and some of them synthetized by nonribosomal peptide synthetase systems. Siderophore production and secretion occurs, especially under iron starvation, when the intracellular iron concentration drops below a certain threshold required for functionality [58]. Siderophore-iron complexes are bound by outer membrane receptor proteins, the TonB-dependent transporters (TBDTs). These outer membrane receptors are generally induced by iron starvation and usually are not present or poorly expressed under iron-sufficient conditions [1]. The iron uptake, transport and storage mechanisms in cyanobacteria are reviewed in detail in Section 3.

2.3. Retrenchment

Retrenchment or downregulation of physiological rates is a progressive and reversible response, resulting in a modulation of the overall growth rate and changes in biochemical parameters. This mechanism is widely used in the adaptation of many organisms to adverse conditions. The most frequent response implies remodeling of bioenergetic pathways in response to iron availability (see Sections 2.1 and 5). As mentioned previously, low iron concentrations trigger a reduction in the level of iron-rich photosynthetic proteins in cyanobacteria while iron-rich mitochondrial proteins are preserved [22].

Cell size reduction and/or morphological changes as response to iron starvation have also been described. For example, thylakoidal membranes and carboxysomes decrease as well as glycogen storage granules increase were observed in *A. nidulans* R2 by electron microscopy [26]. Iron limitation causes morphological changes in the thylakoid packing, promoting unpacking [59]. This phenomenon may be related with phosphorylation of light-harvesting chlorophyll-binding protein of PSII (LCHII) in barley induced by iron deficiency [60]. Iron deficiency causes in cyanobacteria a reduction of cell size [61, 26], sometimes related with growth rate [26, 62].

3. Iron uptake, transport and storage

Siderophores are low-molecular-weight (generally <1000 Da) extracellular iron chelators produced by many prokaryotes and some eukaryotes including fungi, yeasts and plants. These secreted molecules often have a peptidic backbone, with modified amino acid side chains creating three main types of iron-coordinating ligands, that is hydroxamates, catecholates and carboxylates, which commonly form hexadentate octahedral complexes with one ferric ion [63, 64].

Most of the cyanobacterial siderophores appear to contain hydroxamate groups [65, 66], including the dihydroxamate siderophores schizokinen [65, 67] and synechobactin [68], though some species produce catecholate-type chelators such as anachelins [69, 70]. Hydroxamate-based siderophores are strong organic chelators showing a 1:1 stability constant with ferric iron of ~10^{30}, something greater than that of the Fe^{3+}-EDTA complex (~10^{25}); however, ferric-catecholate siderophore complexes almost duplicate this affinity (~10^{49}) [71]. Siderophores may coordinate other metals such as Zn^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Mn^{3+}, Co^{3+}, Al^{3+}, and Cr^{3+}, playing significant roles in the biogeochemical cycling, biological uptake, and protection against deleterious exposure to high concentrations of these elements [72, 73]. In fact, the cyanobacterial siderophore schizokinen binds Cu^{2+} and contributes to alleviate copper toxicity under high environmental copper concentration. Secreted schizokinen sequesters extracellular Cu^{2+}, but cupric-schizokinen is not recognized and internalized by cyanobacterial outer membrane transporters, thereby lowering the amount of copper taken up by the cells [74]. A similar detoxifying effect of cyanobacterial dihydroxamate siderophores has been observed with cadmium [75].

Among freshwater cyanobacteria, the model filamentous nitrogen-fixing heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120 as well as the bloom-forming, toxin-producing *A. flos-aquae* synthesize schizokinen as their major siderophores [76]. Hydroxamate-based siderophore production has also been described in the paddy field cyanobacterium *A. oryzae* [75], and in nontoxic strains of the bloom-forming cyanobacterium *Microcystis aeruginosa* [77]. A novel group of cyanobacterial catecholate-type siderophores known as anachelins has been described in *A. cylindrica* [69]. In marine environments, only the coastal cyanobacterium *Synechococcus* sp. has been reported to produce siderophores. Notably, a distinct suite of dihydroxamate siderophores termed synechobactins is produced by *Synechococcus* sp. PCC 7002 [68]. In addition, xenosiderophore uptake (i.e., aerobactin and desferrioxamine B) has been documented in cyanobacteria [65], though the uptake of self-secreted siderophores is more efficient [78].

The routes of siderophore biosynthesis have not been extensively studied in cyanobacteria. Siderophore biosynthesis occurs in heterotrophic bacteria by two main pathways: one is directed by a large family of modular multienzymes called non-ribosomal peptide synthetases (NRPSs) and polyketide synthetases (PKS), while the other is known as the NRPS-independent siderophore (NIS) pathway [79]. Biosynthesis of hydroxamate-based siderophores with similar structures to schizokinen and synechobactins (e.g., aerobactin) takes place by the second route, involving four enzymes encoded by the gene cluster *iucABCD*, usually organized as an operon [80]. In *Anabaena* sp. PCC 7120, the outer membrane transports for ferric-schizokinen SchT (Alr0397) has been characterized [11], which showed a high amino acid sequence similarity with the ferric-aerobactin IutA transporter from *E. coli*. Near to the gene *alr0397*, a cluster of four open reading frames (*all0394*, *all0393*, *all0392*, *all0390*) show similarity with *iuc* genes, suggesting a role in the biosynthesis of schizokinen [11]. Since the defining characteristic of the NIS biosynthetic pathways is the presence of one or more nucleotide triphosphate-dependent synthetases responsible for condensation reactions during siderophore biosynthesis, this route has also been proposed for hydroxamate-based siderophore biosynthesis in *A. variabilis* and *Synechococcus* sp. PCC 7002 [81, 82].

Another putative route of siderophore biosynthesis in *Anabaena* sp. PCC 7120 occurs presumably via a template-directed, nucleic acid-independent non-ribosomal mechanism which is mediated by the gene products of a cluster of nine open reading frames, from *all2641* to *all2649*, encoding seven NRPSs and two PKSs [83]. The expression of this NRPS-PKS gene cluster is transcriptionally repressed by the master regulator of iron homeostasis FurA [9], being induced under iron limitation or oxidative stress condition [83]. Since iron starvation induces oxidative stress in *Anabaena* sp. [7], maybe by dysfunction of the photosynthetic electron transport and some iron-containing antioxidant enzymes (e.g., SodB and DpsA), it has been postulated that release of siderophore biosynthesis to increase iron uptake during oxidative stress could restore both photosynthesis and ROS scavenging [83]. The protective effect of siderophores against oxidative stress has also been documented in heterotrophic bacteria [73].

De novo synthetized and re-used siderophores are secreted to the outside environment of bacterial cells by export systems which are not very well known in cyanobacteria. In *E. coli*, the export of enterobacterin siderophore involved different mechanisms comprising at least two components, the outer membrane channel tunnel protein TolC [84] which transports the siderophore from the periplasm to the outside, and several inner membrane transporters including the major facilitator superfamily (MFS) protein EntS [85] and the resistance nodulation cell division (RND) transport proteins AcrB, AcrD, AcrEF, MdtABC, and MdtEF [86]. In *Anabaena* sp. PCC 7120, the deletion mutant of the MFS-type inner membrane protein SchE (All4025) failed to secrete schizokinen siderophore to the external milieu [59]. Similar results were observed upon deletion of gene *hgdD* (*alr2887*) [59], encoding the only TolC-like protein in *Anabaena* sp. PCC 7120, termed HgdD, which is also required for protein and glycolipid secretion during heterocyst development [87] and secondary metabolite/antibiotic export [88]. Hence, hydroxamate siderophores appear to be exported in this model cyanobacterium through the mechanism SchE-HgdD.

Once bound to iron, ferric-siderophore complexes are efficiently taken up in Gram-negative bacteria through transport machinery which involves different outer and inner membrane-associated proteins as well as soluble periplasmic binding proteins [1, 12]. First, iron-loaded siderophores are recognized and translocated into the bacterial periplasm by TonB-dependent transporters (TBDTs) located in the outer membrane, in a process that is driven by the cytosolic membrane potential and mediated by the energy-transducing TonB-ExbB-ExbD system. Next, periplasmic binding proteins shuttle ferric-siderophores from the outer membrane transporter to ATP-binding cassette (ABC) permeases associated to the cytoplasmic membrane which delivers the iron-loaded siderophores to the citosol [1].

TBDTs are composed of a transmembrane β-barrel domain that encloses a globular plug domain, and a periplasmic exposed TonB box [89]. Bacteria often possess multiple TBDT receptors, each providing the bacterium with specificity for different siderophores [90], but also allowing uptake of other nutrients [89, 91, 92]. TBDTs involved in iron uptake are generally induced by iron starvation and usually are not present or poorly expressed under iron-sufficient conditions [1]. Twenty-two TBDTs have been identified in the genome sequence of *Anabaena* sp. PCC 7120, most of them integrated into gene clusters or even putative operons containing genes coding for proteins involved in iron transport [93]. A TBDT receptor involved in schizokinen uptake, SchT (Alr0397), has been described in *Anabaena* sp. PCC 7120 [11]. The expression of this outer membrane ferric-siderophore transporter is induced under iron-limitation [11], and it is transcriptionally regulated by FurA [94]. SchT appeared not essential for cyanobacterial growth under iron-limited conditions, suggesting the occurrence of other iron transporters in *Anabaena* sp. [11]. A second TBDT termed IacT (All4026), involved in iron and copper uptake, has been characterized in *Anabaena* sp. PCC 7120. IacT is not a schizokinen transporter; it appears to function under conditions in which the copper concentration exceeds the concentration of iron and seems to transport iron as ferric-citrate [59]. Finally, a third TBDT also involved in ferric-schizokinen uptake, IutA2 (Alr2581), has been recently described [78]. The *iutA2* mutant showed significant growth impairment under iron deprivation as well as alterations in ferric-schizokinen uptake.

Beyond the TBDTs SchT and IutA2, the iron-loaded schizokinen uptake machinery in *Anabaena* sp. PCC 7120 appears to comprise, at least, the gene products of *tonB3* (*all5036*), *exbB3*/*exbD3* (*all5047*, *all5046*), and *fhuCDB* (*all0389-all0387*). Whereas several *tonB*-like genes, *exb* clusters, and permease systems (i.e., *fhu*, *fut*, *fec*) have been annotated in the *Anabaena* genome, only the expression of the abovementioned ORFs were induced under iron-limiting conditions and reduced at high iron concentrations [12]. Additionally, mutants of the periplasmic ferric-siderophore binding protein FecB1 (All2583), but not of its homolog FutA, showed a slightly reduced uptake rate of ferric-schizokinen [78]. The *Anabaena* sp. PCC 7120 siderophore uptake system SchT/FhuBCD appears to be also involved in ferric-aerobactin uptake; however, the uptake of this hydroxamate siderophore produced by *E. coli* was ~10 fold slower than the uptake of ferric-schizokinen in the filamentous cyanobacterium [78].

Whereas some cyanobacterial species produce siderophores to scavenge iron under iron-limiting conditions, many cyanobacteria do not possess this ability, including some environmentally relevant lineages such as the planktonic freshwater cyanobacterium *Synechocystis* sp. [95], the

dominant picocyanobacterium *Prochlorococcus marinus* [82], and the open-ocean nitrogen fixers *Trichodesmium erythraeum* and *Crocosphaera watsonii* [82, 96]. However, some non-siderophore-producing cyanobacteria express all the components of iron-siderophore uptake machinery, being capable of incorporate xenosiderophores [97]. Reductive iron uptake appears extended in many non-siderophore-producing cyanobacteria. In this strategy, reduction of free or complexed ferric iron (e.g., ferric-citrate) into its ferrous form takes place prior to the transport across the plasma membrane either by iron-reducing superoxide radicals secreted to the extracellular milieu as has been described in *Trichodesmium* and *Lyngbya* [96, 98], or through the action of plasma membrane-associated respiratory terminal oxidases as occurs in *Synechocystis* sp. PCC 6803 [95]. Given their small sizes, hydrophilic ferrous and unchelated ferric ions may passively diffuse to the periplasmic space through nonspecific outer membrane porins [95]. However, due to its frequent environmental low concentration, ferric iron uptake usually requires TonB-ExbB-ExbD-dependent active transport systems [99]. Once into the periplasm, high affinity ferric-binding soluble proteins bind ferric ions such as FutA1 and FutA2 and shuttle them to inner membrane ferric permeases such as FutB and FutC [100, 101]. Alternatively, ferric ions are reduced by any of the abovementioned mechanisms and cross the inner membrane through ferrous iron transporters like FeoB [95].

Once inside the cell, ferric iron is reduced to ferrous iron, which has a much lower affinity for the siderophore and spontaneously dissociates [1]. Due to poor bioavailability or iron and its frequent intermittent supply in nature, bacteria have evolved efficient iron storage mechanisms involved ubiquitously multi-subunit proteins termed ferritins and bacterioferritins [102]. These proteins can accommodate up to 4500 iron atoms into a central cavity in a form that is unlike to participate in ROS generation reactions [102, 103]. In *Synechocystis* sp. PCC 6803, bacterioferritins BfrA and BfrB are responsible for the storage up to 50% of intracellular iron content [104], while the DPS family ferritin MrgA plays a pivotal role in both the mobilization of the stored iron within the cell [105], and the coordination between iron homeostasis and oxidative stress response [4]. By contrast, little is known about the mechanisms of iron storage in *Anabaena* species. Only four nonheme-binding ferritin family genes have been identified in *Anabaena* sp. PCC 7120 [104], including *alr3808* [106] and *all1173* [107], encoding two DNA-binding protein homologs to DpsA from *Synechococcus* sp. PCC 7942 [108]. DpsA from *Synechococcus* displays a weak catalase activity *in vitro* and is presumably involved in peroxide-consuming mechanism located on the chromosomal DNA, conferring resistance to peroxide damage during oxidative stress conditions or long-term nutrient limitation [108]. According to the CyanoBase [109], the genomes of other environmentally relevant cyanobacteria such as *P. marinus*, *C. watsonii*, *T. erythraeum*, and *M. aeruginosa* encode members of the ferritin/bacterioferritin superfamily.

4. Regulation of iron homeostasis

Regulators of the Fur (Ferric uptake regulator) family constitute the primary mechanism in the maintenance of iron homeostasis in cyanobacteria. The first evidence of the existence of a Fur protein in cyanobacteria was the isolation of a *fur* gene in *Synechococcus* PCC 7942 through an *E. coli*-based *in vivo* repression assay [13]. Apart from *Synechococcus*, Fur homologs

have been mainly identified and studied in *Synechocystis*, *Anabaena* and *Microcystis* [17, 110–112]. Cyanobacterial Fur proteins contain histidine rich motifs (HHXHXXCXXC) as potential metal binding sites, which share properties with Fur from other prokaryotes [113, 114]. In the classic model of operation for this transcriptional regulator, Fur functions as a repressor, using ferrous iron as a co-repressor. Under sufficient iron availability, a dimer of active Fe^{2+}-Fur complex binds to *cis* regulatory elements in the promoter of target genes and thereby prevents transcription [115]. However, other regulatory mechanisms have been described indicating that Fur can also bind to specific promoters in its apo form repressing transcription. Even apo- and holo-Fur activations have been reported [113, 116]. In the cyanobacterial genomes, it is common to find diverse ORFs that encode different Fur homologs which perform several functions. In this sense, in *Synechococcus* 7002 or *Anabaena* sp. PCC 7120, three *fur*-type genes exist, but only one of them, denoted as *furA*, appears directly involved in upregulation of iron uptake genes under iron limitation [9, 117, 118]. Recent studies confirmed that FurA is an essential, well-conserved protein among cyanobacteria. A significant depletion of *furA* expression levels impaired the photoautotrophic growth of *Anabaena* sp. under standard culture conditions in both, solid and liquid media [14]. FurA is the master regulator of iron homeostasis in *Anabaena* sp. PCC 7120 [9] and presumably in many other cyanobacterial species [14]. FurA modulates not only the expression of the iron metabolism machinery, but also regulates directly or indirectly the transcription of a plethora of genes and operons involved in a variety of physiological processes including photosynthesis, respiration, response to oxidative stress, nitrogen fixation, heterocyst differentiation, cellular morphology, tetrapyrrole biosynthesis pathway, phycobilisome degradation, chlorophyll catabolism, programmed cell death, light sensing and response, signal transduction systems, exopolysaccharide biosynthesis, and cyanotoxin production, among others [15, 16, 94, 119].

Cyanobacterial Fur regulators can function both as activator and repressor as observed in the transcriptional regulation by FurA of genes involved in the tetrapyrrole biosynthesis pathway in *Anabaena* sp. PCC 7120 [9]. In all these cases, regulation by Fur adapts the answer to provide iron in case of deficiency of this metal or to allow its storage or the use of proteins that depend on iron when this metal is sufficient [1]. Fur recognizes AT rich regions called Fur boxes located in the promoter region of iron responsive genes [120]. Although it is assumed that this regulator binds as a dimer to the promoter, a computational study of Fur proteins from *Synechocystis* sp. PCC 6803 proposed the binding of multimers of the Fur-like regulator onto its target DNA, which possesses internal repeats [121]. Lately, atomic force microscopy revealed the sequential binding of FurA to its own promoter boosted by DNA bending in *Anabaena* sp. PCC 7120 [122]. Cyanobacterial Fur-DNA recognition depends not only on metal levels. Apart from iron, a reduced form of FurA from *Anabaena* sp. PCC 7120 is required for *in vitro* optimal DNA-binding [112, 123]. Also, reduction of Fur from *M. aeruginosa* PCC 7806 increases the binding affinity to its target genes [124]. Cyanobacterial Fur homologs contain a variable number of cysteine residues in their primary sequence and the need for reducing power for this regulator to develop its function is based on the importance of the redox state of these residues. A cysteine mutational study of the five cysteines present in *Anabaena* sp. PCC 7120 Fur sequence revealed that C^{101}, a residue conserved in most bacterial

Fur homologs, is part of a thiol/disulfide redox switch that determines FurA ability to bind the metal co-repressor [125]. Moreover, this residue belongs to a CXXC motif responsible of the disulfide reductase activity exhibited by *Anabaena* FurA, suggesting that Fur is involved in the cyanobacterial redox-signaling pathway. Apparently, Fur connects the response to changes in the intracellular redox state and iron management in cyanobacteria [126].

The amount of Fur is controlled in cyanobacteria by mechanisms present in the three levels of the flow of genetic information [123]. At the transcriptional level, the TetR family transcriptional regulator PfsR regulates *fur* transcription in *Synechocystis* PCC 6803. A *pfsR* deletion mutant displayed stronger tolerance to iron-limiting conditions as compared with the wild type. Moreover, the transcripts of *pfsR* were enhanced by iron limitation and inactivation of the gene affected pronouncedly expression of *furA* gene and genes involved in iron transport and storage among others [127].

At the post-transcriptional level, *cis*-encoded antisense RNAs regulate Fur expression in cyanobacteria [128]. In *Anabaena* sp. PCC 7120, a large dicistronic transcript encoding the putative membrane protein Alr1690 and a *α-furA* RNA transcript complementary to *furA* is involved in the control of the cellular levels of the protein [129]. Also, *cis α-furA* RNAs are present in *M. aeruginosa* PCC 7806 and *Synechocystis* sp. PCC 6803 [130].

Regulation of the Fur level and its activity also take place post-translationally by different mechanisms in cyanobacteria. It has been reported that the membrane cytoplasmic FtsH1/FtsH3 protease heterocomplex, involved in the acclimation of cells to iron deficiency, controls the availability of *Synechocystis* sp. PCC 6803 Fur by degradation of apo-Fur in order to regulate transcription of iron responsive genes [131]. Moreover, cyanobacterial Fur can form a complex with heme that alters its ability to join to DNA. In particular, *Anabaena* sp. PCC 7120 FurA interacts strongly with heme in the micromolar range of concentration and inhibits the *in vitro* ability of this protein to bind to DNA [117]. The axial ligand of heme in the FurA-heme complex is a cysteine residue that belongs to a Cys-Pro motif (Heme regulatory motif) present in its primary sequence and the sequences of all cyanobacterial homologs but absent in most non cyanobacterial ones. The regulator undergoes a redox-dependent ligand switch so that heme could be involved in sensing redox variations within the cyanobacterial filament and alter the regulatory function of FurA [132].

A novel layer of complexity of iron homeostasis regulation in cyanobacteria involves RNA molecules as IsaR1. When iron is scarce, IsaR1 affects the photosynthetic apparatus in three different ways: (1) directly, inhibiting the expression of proteins important in photosynthesis; (2) indirectly, by suppression of pigment production; (3) preventing the expression of proteins that contain iron-sulfur clusters. Homologs of IsaR1 are conserved throughout the cyanobacterial phylum [133]. Also, the SufA and IscA proteins, proposed to function as scaffolds in the assembly of Fe/S clusters in bacteria, seem to play regulatory roles in iron homeostasis in cyanobacteria, according to experiments performed on single and double null-mutant strains of *Synechococcus* sp. [134]. Even the three PchR regulators (PchR1, PchR2, PchR3) present in *Synechocystis* PCC 6803 seem to play a prominent role in the protection against iron stress, among other stresses, in this cyanobacterium [135].

5. The regulation of iron homeostasis is tightly connected to central metabolic pathways

As mentioned previously, iron deficiency is one of the major causes of stress in cyanobacterial communities. Due to the occurrence of iron in most electron transport proteins conforming photosynthetic, respiratory and nitrogenase pathways, the adaptive strategies developed by the cyanobacteria are tightly related to the rearrangement and modulation of these processes. Furthermore, many of the different responses triggered by iron deprivation are aimed to prevent and alleviate oxidative stress and to the modulation of central metabolism.

5.1. Iron availability and the oxidative stress response

Oxidative stress is one of the many consequences of iron imbalance in cyanobacteria. Thus, the control of iron homeostasis is intimately linked to the regulation of many genes involved in the response to oxidative stress [4, 14, 24, 94]. Moreover, the master regulators involved in such processes in cyanobacteria, namely FurA and PerR/FurC, display a set of common targets [14, 136]. Furthermore, PerR/FurC is able to modulate *in vitro* FurA-DNA binding activity [117]. Transcriptomic analyses and differential proteomics focused on the definition of the FurA regulon in *Anabaena* PCC 7120 unveiled that around 13% of FurA targets with a known function were involved in detoxification of ROS [14]. Those FurA-regulated genes belong to different subcategories, such as electron transport proteins dedicated to restore oxidized thiols (*trxA*, *trxB*, the glutaredoxin-related protein *alr0799* and the glutathione S-transferases *alr3195* and *alr7354*, among others); detoxification of hydrogen peroxide (the Mn-catalase *katB* and the peroxiredoxins *all1541* and *alr4641*) or the protection of DNA (*dpsA*) [14, 106, 119, 137]. FurA also controls the expression of flavodoxin that is strongly induced under iron deficiency [13, 138]. Initially described as a substitute for ferredoxin I (Fd) in the photosynthetic electron transport to $NADP^+$ [45, 138] (reviewed in Sections 2.1.1 and 5.2), flavodoxin is also a powerful scavenger of ROS. Interestingly, the expression of flavodoxin in chloroplasts of tobacco unveiled that this flavoprotein is able to effectively interact with several Fd-dependent oxidoreductive pathways, including thioredoxin reduction [139]. The expression of flavodoxin in plastids protected target enzymes of central metabolic pathways from oxidative inactivation, such as the Calvin cycle components fructose-1,6-bisphosphatase (FBPase) and phosphoribulokinase (PRK). Therefore, the expression of flavodoxin triggered by iron deficiency relieves the oxidative stress in the cyanobacteria and contributes to the reconstitution of the electron transport chains rich in iron-containing proteins whose iron-sulfur clusters are immediate targets of free radicals, minimizing the effect of the oxidative damage on the photosynthetic rates and the nitrogen metabolism, among other metabolic pathways [139].

5.2. Influence of iron availability in the control of photosynthetic genes

As it has been shown previously, iron limitation has important consequences in the composition and performance of cyanobacterial photosystems. Several photosynthetic cyanobacterial specific genes induced under iron deficiency contribute to modify their photosynthetic machineries such as *isiA*, *isiB* (flavodoxin), *idiA*, *idiB* and *idiC* proteins (reviewed in Section 2.1.1). Fur controls the expression of *isiA* and isiB [13], whose transcription is induced by multiple stresses such as treatment with hydrogen peroxide or high salt [56, 136, 140].

Further transcriptomic studies evaluating the cyanobacterial response to iron deficiency unveiled that as a general trend, photosynthesis genes were repressed under low-iron conditions and induced upon the re-addition of iron. Many of those genes belonged to the *psa* and *psb* families, components of the phycobilisomes and genes involved in the synthesis of chlorophyll are also direct targets of FurA [14, 24, 141]. Furthermore, Fur is involved in the control of genes involved in carboxysome formation and Calvin cycle. Notably, a close relationship between light availability and iron requirements can be inferred from different studies, such as the differentially expressed genes in [142], the regulation of *furA* and the *alpha-furA* antisense RNA by light [143], or the need of an active photosynthetic electron transport chain for the expression of the *mcy* operon in *M. aeruginosa*, that in turn is controlled by FurA [124, 143, 144]. As *furA* from *M. aeruginosa*, the expression of the *Anabaena* sp. PCC7120 ortholog is controlled by an antisense RNA whose inactivation produces iron-deficient cells and severe structural disorders in the photosynthetic apparatus of *Anabaena*. Furthermore, disruption of the dicistronic message encoding the *alr1690-alpha-furA* tandem leads to lower photosynthetic performance indexes, unveiling that its expression is required for maintenance of a proper thylakoid arrangement, efficient regulation of iron uptake and optimal yield of the photosynthetic machinery [123, 145]. In addition, FurA modulates the transcription of the LexA regulator in *Anabaena* PCC7120. This regulator is critical to the survival of cyanobacterial cells facing inorganic carbon starvation, since most of the LexA-responsive genes were known to be involved in carbon assimilation or controlled by carbon availability [146].

5.3. Iron-responsive genes involved in cyanobacterial respiratory pathways

In addition to the photosynthetic electron transport chains, cyanobacterial thylakoids contain multiple respiratory electron transport complexes [147]. Thus, photosynthesis and respiration are tightly related in cyanobacteria since both pathways share several components, such as a quinone/quinol pool [148], plastoquinone, cytochrome b6f and plastocyanin/cytochrome [148, 149]. Furthermore, the cyanobacteria contain a second complete respiratory chain present in the cell membrane that also uses the same mobile quinone pool mediating electrons in the photosynthetic and thylakoidal respiratory processes. Several studies evidence the relationship between the iron pool and the respiratory activity. The major oxidase in cyanobacteria, COX, is encoded by the *cox* operon (*coxBAC*) and FurA regulated though the modulation of *cox*B [15]. Similarly, the transcription of *alr0869* (*ndhF*) and the subunit 5 of NADH dehydrogenase encoded by *all1127* are regulated by FurA as response to iron availability [15]. Furthermore, iron starvation in *S. elongatus* causes upregulation of several cytochrome oxidases and the increase of respiratory electron transport [22, 150], while an *Anabaena* mutant lacking of the *alr1690-alpha-furA* message that exhibits a reduced iron pool with respect to the wild-type strain has affected its respiratory activity [145].

5.4. Cross-talk between iron and nitrogen metabolism

The electron carriers involved in nitrogen metabolism are also rich in iron, especially the proteins involved in nitrogen fixation. Nitrogenase and nitrogenase reductase complex harbor around 40 atoms of Fe^{2+} distributed between the iron-molybdenum cofactor (FeMo-co) and the [8Fe-7S] P-cluster present in NifDK nitrogenase, and the [4Fe-4S] cubane in the NifH dinitrogenase reductase. In addition, most of the proteins involved in the assembly

of the metalloclusters embedded within the NifDK protein also contain diverse [Fe-S] centers [151, 152]. Thus, growing under nitrogen fixation conditions adds an additional iron stress to the cell. Therefore, optimal cyanobacterial performance requires a tight and coordinated regulation of iron and nitrogen metabolisms [137]. Nitrogen metabolism in cyanobacteria is controlled by the master regulator NtcA [153] that usually senses the C/N balance through the intracellular 2-oxoglutarate levels [154]. NtcA controls a wide regulon of genes involved in different functional categories [155, 156]. Among them, NtcA controls most steps required for nitrogen fixation in cyanobacteria, starting from heterocyst differentiation and development until *nif* genes expression. NtcA also controls key genes in nitrogen assimilation pathways in cyanobacteria [157]. Different studies evidence a tight relationship between iron and nitrogen metabolism. Interestingly, transcription of the *nifHDK* operon and excision of the 11 kb DNA fragment required for heterocyst differentiation was observed in iron-starved *Anabaena*, even though cells grew in the presence of combined nitrogen [138]. Further studies showed that the expression of FurA is highly induced in the heterocyst [137]. FurA participates in the regulation of *nif* genes, and the levels of this regulator are critical for the modulation of heterocyst differentiation by controlling the expression of NtcA and vice versa [14, 16]. Thus, several iron-responsive genes in cyanobacteria, such as *nblA*, *petH*, *pkn41*, *pkn42*, among others, are also modulated by NtcA [137, 158–161]. Conversely, in *Synechocystis sp.* PCC 6803, the NtcA-regulated genes *bgtB*, *glnA* and *urtB* are highly upregulated under iron limitation [162]. Different studies focused on the identification of the FurA and NtcA regulons in different cyanobacterial strains support that FurA and NtcA are interactive regulators and corroborate that both transcription factors share an important number of targets mainly related to photosynthesis and respiration, iron uptake and incorporation, oxidative stress response and nitrogen metabolism [137]. However, given that both FurA and NtcA are global regulators, it is not surprising that the nitrogen starvation response involves a large number of genes not only related to iron metabolism but also to heavy metal and oxidative stress adaptation, reinforcing the interrelationship of those processes [162].

6. Iron involvement in cyanotoxin production

Metabolic plasticity of cyanobacteria includes the synthesis of a broad variety of secondary metabolites, some of them potentially toxic for eukaryotic organisms, the so-called cyanotoxins [163]. When toxins are synthetized, the cyanobacteria divert large amounts of carbon and nitrogen to this process so that it might be obvious to think that cyanotoxin synthesis gives them some adaptive advantage. Cyanotoxin production is not universal or constant even among those species and strains holding the necessary genes. The conditions that induce cyanotoxin production in capable species have not been elucidated. Under certain environmental conditions, cyanobacteria can proliferate to form blooms consisting of significant biomass and covering large areas in fresh or marine water. It is necessary to separate the phenomenon of blooms occurrence from the fact of toxicity, although obviously the problem is detected when the population of toxic cyanobacteria synthetizing toxins is high.

6.1. Iron and blooms occurrence

Iron availability and biolimitation by iron of the phytoplankton are important subjects discussed for many years. After IronExII [2], it was definitively established that iron availability limits rates of cell division, as well as abundance and production of phytoplankton of the equatorial Pacific and likely in other "high nutrient, low chlorophyll regions" [55]. There is broad agreement that nutrient over-enrichment of freshwater and marine ecosystems promote cyanobacterial blooms. Phosphorus and nitrogen have traditionally been considered the key nutrients limiting primary productivity and algal biomass. But based on such accessibility (and light and temperature suitable for cyanobacterial growth), iron availability could be suggested to be the switch that triggers a bloom. Cyanobacteria compete very efficiently with other phytoplankton species for iron resources and often end up dominating the population. In addition to all, the adaptive strategies previously mentioned, in some cases, their competitive advantage is based on its ability to vertical migration [164].

6.2. Iron and cyanotoxin production

Cyanotoxins are a heterogenous group of molecules that include hepatotoxins, neurotoxins, dermatotoxins and cytotoxins, with diverse chemical nature such as cyclic peptides: cyclic peptides, alkaloids, non-proteic amino acids. The synthesis of most toxins is inducible, and the genes involved in its biosynthesis have been identified during these last years [165]. The genes conforming biosynthetic pathways, its regulation and the molecular mechanisms involved in toxicity are in each case different. However, NRPS are present in all the described toxic operons, involved in cyanotoxin synthesis. Many NRPS present in many bacteria are iron regulated [166, 167]. A substantial variety of siderophore structures, toxins and antimicrobial molecules with toxic effects are produced from similar NRPS assembly lines [167], and a large number of secondary metabolites are also synthesized as response to iron starvation.

Among cyanotoxins, microcystins are the most ubiquitous toxins causing several environmental and health problems. They are a family of cyclic heptapeptides, synthesized by a mixed PKS-NRPS system called microcystin synthetase encoded in *mcy* operon [168]. The role of microcystins in cyanobacteria is still unclear, but there are evidences that could confer to the toxic strains advantages for survival in iron-limited conditions. The microcystin synthesis has been linked to iron metabolism for many years. Lyck and colleagues [169] showed that during iron depletion, toxic strains of *Microcystis* maintained cell vitality much longer than the nontoxic strains. Moreover, Utkilen and Gjolme [170] found that toxic strains exhibited higher rates of iron uptake than nontoxic strains. They proposed that microcystin could be an intracellular chelator of Fe^{+2}, as well as predicted that the synthesis of the toxin would be controlled by the amount of free iron present in the cells. Structural similarities between microcystin and bacterial siderophores [167] led also to propose a putative role as an extracellular iron-scavenging molecule. Recently, it was shown that while the microcystin producing strain *M. aeruginosa* PCC 7806 and its close strain, the non-producing *M. aeruginosa* PCC 7005 grew similarly in BG11 in the presence of 17 μM iron, under severe iron deficient conditions (0.05 μM), the toxigenic strain grew slightly less than in iron-replete conditions, while the non-producing microcystin strain was not able to grow [171]. Taking together all these data suggest that microcystin production

could be another mechanism evolved by cyanobacteria related to iron homeostasis, on track to survive in iron-limited conditions. In agreement with this statement, it was shown that in *M. aeruginosa* PCC 7806, the *mcy* operon was regulated by Fur [124], and that the *mcy* operon transcription as well as microcystin content were enhanced under iron-limited conditions [172].

Recently, microcystin ability to bind iron and other metals has been demonstrated using various experimental approaches [171], corroborating a possible role of this molecule in iron metabolism. A putative role of microcystin acting as iron chelator involved in iron acquisition has been recurrently suggested. The main problem associated to this theory is the fact that microcystin seems to be an endotoxin although the results showed in bibliography are contradictory. When radioactive inorganic carbon is supplied to *M. aeruginosa* and the fate of intracellular microcystin pool is followed, no export of microcystin was observed [173]. However, the *mcyH* gene included in the *mcy* operon encoded an ABC transporter reported to be essential for microcystin synthesis, suggesting a possible export of microcystin outside of the cell [174]. On the other hand, electron microscopy of immuno-gold labeled microcystin showed that the vast majority of intracellular microcystin is located around the thylakoids [175–177]; hence, a possible role in protecting the photosynthetic machinery to photo-oxidation has been proposed. Recently, it has been described that microcystin can perform metal-driven oligomerization. Some environmental stresses such as low iron or high light conditions cause oxidative stress in the cell which triggers photo-oxidation phenomena. In this scenario, the PSs can be disassembly and then, microcystin could perform oligomerization and capture of iron avoiding metal-dependent Fenton reactions [171]. Another proposed role is related with colony formation performed by *Microcystis* cells. Solid evidences linking microcystin presence and enhanced colony formation and size have been reported [178].

7. Conclusion

Iron is at the core of cyanobacterial metabolic and regulatory networks, playing a central role in the control of electron delivery and distribution in the photosynthetic and respiratory electron transport chains, the reduction of nitrogenase and central metabolic pathways. The adaptive responses of cyanobacteria to iron limitation affect all those processes, though the iron demand of the cell is subject to a hierarchy in favor of photosynthesis. The high quota of iron in cyanobacteria, its ability to promote oxidative stress and its ubiquity in electron transport pathways require a tight control of iron homeostasis mainly performed by FurA. In order to optimize iron resources, the regulation of FurA activity and expression, as well as the genes composing the FurA regulon are strongly interconnected with other master regulators such as PerR and NtcA.

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Conflict of interest

The authors declare no conflicts of interest.

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References

[1] Andrews SC, Robinson AK, Rodriguez-Quinones F. Bacterial iron homeostasis. FEMS Microbiology Reviews. 2003;**27**(2-3):215-237

[2] Coale KH, Johnson KS, Fitzwater SE, Gordon RM, Tanner S, Chavez FP, Ferioli L, Sakamoto C, Rogers P, Millero F, et al. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature. 1996;**383**(6600):495-501

[3] Ferreira F, Straus NA. Iron deprivation in cyanobacteria. Journal of Applied Phycology. 1994;**6**(2):199-210

[4] Shcolnick S, Summerfield TC, Reytman L, Sherman LA, Keren N. The mechanism of iron homeostasis in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 and its relationship to oxidative stress. Plant Physiology. 2009;**150**(4):2045-2056

[5] Richier S, Macey AI, Pratt NJ, Honey DJ, Moore CM, Bibby TS. Abundances of iron-binding photosynthetic and nitrogen-fixing proteins of *Trichodesmium* both in culture and in situ from the North Atlantic. PLoS One. 2012;**7**(5):e35571

[6] Latifi A, Ruiz M, Zhang CC. Oxidative stress in cyanobacteria. FEMS Microbiology Reviews. 2009;**33**(2):258-278

[7] Latifi A, Jeanjean R, Lemeille S, Havaux M, Zhang CC. Iron starvation leads to oxidative stress in *Anabaena* sp. strain PCC 7120. Journal of Bacteriology. 2005;**187**(18):6596-6598

[8] Shcolnick S, Keren N. Metal homeostasis in cyanobacteria and chloroplasts. Balancing benefits and risks to the photosynthetic apparatus. Plant Physiology. 2006;**141**(3):805-810

[9] Gonzalez A, Bes MT, Valladares A, Peleato ML, Fillat MF. FurA is the master regulator of iron homeostasis and modulates the expression of tetrapyrrole biosynthesis genes in *Anabaena* sp. PCC 7120. Environmental Microbiology. 2012;**14**(12):3175-3187

[10] Morrissey J, Bowler C. Iron utilization in marine cyanobacteria and eukaryotic algae. Frontiers in Microbiology. 2012;**3**:43

[11] Nicolaisen K, Moslavac S, Samborski A, Valdebenito M, Hantke K, Maldener I, Muro-Pastor AM, Flores E, Schleiff E. Alr0397 is an outer membrane transporter for the siderophore schizokinen in *Anabaena* sp. strain PCC 7120. Journal of Bacteriology. 2008; **190**(22):7500-7507

[12] Stevanovic M, Hahn A, Nicolaisen K, Mirus O, Schleiff E. The components of the putative iron transport system in the cyanobacterium *Anabaena* sp. PCC 7120. Environmental Microbiology. 2012;**14**(7):1655-1670

[13] Ghassemian M, Straus NA. Fur regulates the expression of iron-stress genes in the cyanobacterium *Synechococcus* sp. strain PCC 7942. Microbiology. 1996;**142**(Pt 6):1469-1476

[14] Gonzalez A, Bes MT, Peleato ML, Fillat MF. Expanding the role of FurA as essential global regulator in cyanobacteria. PLoS One. 2016;**11**(3):e0151384

[15] Gonzalez A, Angarica VE, Sancho J, Fillat MF. The FurA regulon in *Anabaena* sp. PCC 7120: In silico prediction and experimental validation of novel target genes. Nucleic Acids Research. 2014;**42**(8):4833-4846

[16] Gonzalez A, Valladares A, Peleato ML, Fillat MF. FurA influences heterocyst differentiation in *Anabaena* sp. PCC 7120. FEBS Letters. 2013;**587**(16):2682-2690

[17] Martin-Luna B, Hernandez JA, Bes MT, Fillat MF, Peleato ML. Identification of a Ferric uptake regulator from *Microcystis aeruginosa* PCC7806. FEMS Microbiology Letters. 2006;**254**(1):63-70

[18] Straus NA. Iron deprivation: Physiology and gene regulation. In: Bryant DA, editor. The Molecular Biology of Cyanobacteria. Advances in Photosynthesis, Dordrecht: Springer; 1994;**1**

[19] Peleato ML, Bes MT, Fillat MF. Iron homeostasis and environmental responses in cyanobacteria: Regulatory networks involving Fur. In: de Brujin FJ, editor. Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria. 2016;**19**(1):1065-1078

[20] Michel KP, Berry S, Hifney A, Kruip J, Pistorius EK. Adaptation to iron deficiency: A comparison between the cyanobacterium *Synechococcus elongatus* PCC 7942 wild-type and a DpsA-free mutant. Photosynthesis Research. 2003;**75**(1):71-84

[21] Pietsch D, Staiger D, Pistorius EK, Michel KP. Characterization of the putative iron sulfur protein IdiC (ORF5) in *Synechococcus elongatus* PCC 7942. Photosynthesis Research. 2007;**94**(1):91-108

[22] Michel KP, Pistorius EK. Adaptation of the photosynthetic electron transport chain in cyanobacteria to iron deficiency: The function of IdiA and IsiA. Physiologia Plantarum. 2004;**120**(1):36-50

[23] Yingping F, Lemeille S, Talla E, Janicki A, Denis Y, Zhang CC, Latifi A. Unravelling the cross-talk between iron starvation and oxidative stress responses highlights the key

role of PerR (*alr0957*) in peroxide signalling in the cyanobacterium *Nostoc* PCC 7120. Environmental Microbiology Reports. 2014;**6**(5):468-475

[24] Singh AK, McIntyre LM, Sherman LA. Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiology. 2003;**132**(4):1825-1839

[25] Leonhardt K, Straus NA. An iron stress operon involved in photosynthetic electron transport in the marine cyanobacterium *Synechococcus* sp. PCC 7002. Journal of General Microbiology. 1992;**138**(Pt 8):1613-1621

[26] Sherman DM, Sherman LA. Effect of iron deficiency and iron restoration on ultrastructure of Anacystis nidulans. Journal of Bacteriology. 1983;**156**(1):393-401

[27] Riethman HC, Sherman LA. Purification and characterization of an iron stress-induced chlorophyll-protein from the cyanobacterium *Anacystis nidulans* R2. Biochimica et Biophysica Acta. 1988;**935**(2):141-151

[28] Sun J, Golbeck JH. The presence of the IsiA-PSI supercomplex leads to enhanced photosystem I electron throughput in iron-starved cells of *Synechococcus* sp. PCC 7002. The Journal of Physical Chemistry B. 2015;**119**(43):13549-13559

[29] Tetenkin VL, Golitsin VM, Gulyaev BA. Stress protein of cyanobacteria CP36: Interaction with photoactive complexes and formation of supramolecular structures. Biochemistry (Mosc). 1998;**63**(5):584-591

[30] Park YI, Sandstrom S, Gustafsson P, Oquist G. Expression of the *isiA* gene is essential for the survival of the cyanobacterium *Synechococcus* sp. PCC 7942 by protecting photosystem II from excess light under iron limitation. Molecular Microbiology. 1999;**32**(1):123-129

[31] Pakrasi HB, Goldenberg A, Sherman LA. Membrane development in the Cyanobacterium, *Anacystis nidulans*, during recovery from Iron starvation. Plant Physiology. 1985;**79**(1): 290-295

[32] De Las Rivas J, Barber J. Analysis of the structure of the PsbO protein and its implications. Photosynthesis Research. 2004;**81**(3):329-343

[33] Nogi T, Miki K. Structural basis of bacterial photosynthetic reaction centers. Journal of Biochemistry. 2001;**130**(3):319-329

[34] Bibby TS, Zhang YA, Chen M. Biogeography of photosynthetic light-harvesting genes in marine phytoplankton. PLoS One. 2009;**4**(2):e4601

[35] Razquin P, Peleato ML, Fillat MF, Gomez-Moreno C, Bohme H. Differential activities of heterocyst ferredoxin, vegetative cell ferredoxin, and flavodoxin as electron carriers in nitrogen fixation and photosynthesis in *Anabaena* sp. Photosynthesis Research. 1995; **43**:35-40

[36] Fillat MF, Edmondson DE, Gomez-Moreno C. Structural and chemical properties of a flavodoxin from Anabaena PCC 7119. Biochimica et Biophysica Acta. 1990;**1040**(2):301-307

[37] Vigara AJ, Inda LA, Vega JM, Gomez-Moreno C, Peleato ML. Flavodoxin as an electronic donor in photosynthetic inorganic nitrogen assimilation by iron-deficient *Chlorella fusca* cells. Photochemistry and Photobiology. 1998;**67**(4):446-449

[38] Lodeyro AF, Ceccoli RD, Pierella Karlusich JJ, Carrillo N. The importance of flavodoxin for environmental stress tolerance in photosynthetic microorganisms and transgenic plants. Mechanism, evolution and biotechnological potential. FEBS Letters. 2012; **586**(18):2917-2924

[39] Pierella Karlusich JJ, Ceccoli RD, Grana M, Romero H, Carrillo N. Environmental selection pressures related to iron utilization are involved in the loss of the flavodoxin gene from the plant genome. Genome Biology and Evolution. 2015;**7**(3):750-767

[40] Peleato ML, Ayora S, Inda LA, Gomez-Moreno C. Isolation and characterization of two different flavodoxins from the eukaryote *Chlorella fusca*. The Biochemical Journal. 1994;**302**(Pt 3):807-811

[41] Karlusich JJP, Ceccoli RD, Grana M, Romero H, Carrillo N. Environmental selection pressures related to Iron utilization are involved in the loss of the Flavodoxin gene from the plant genome. Genome Biology and Evolution. 2015;**7**(3):750-767

[42] Laudenbach DE, Straus NA. Characterization of a cyanobacterial iron stress-induced gene similar to *psbC*. Journal of Bacteriology. 1988;**170**(11):5018-5026

[43] Fulda S, Hagemann M. Salt treatment induces accumulation of Flavodoxin in the Cyanobacterium *Synechocystis* Sp Pcc-6803. Journal of Plant Physiology. 1995;**146**(4): 520-526

[44] Tognetti VB, Zurbriggen MD, Morandi EN, Fillat MF, Valle EM, Hajirezaei MR, Carrillo N. Enhanced plant tolerance to iron starvation by functional substitution of chloroplast ferredoxin with a bacterial flavodoxin. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(27):11495-11500

[45] Sandmann G, Peleato ML, Fillat MF, Lazaro MC, Gomez-Moreno C. Consequences of the iron-dependent formation of ferredoxin and flavodoxin on photosynthesis and nitrogen fixation on *Anabaena* strains. Photosynthesis Research. 1990;**26**(2):119-125

[46] Doucette GJ, Erdner DL, Peleato ML, Hartman JJ, Anderson DM. Quantitative analysis of iron-stress related proteins in *Thalassiosira weissflogii*: Measurement of flavodoxin and ferredoxin using HPLC. Marine Ecology Progress Series. 1996;**130**(1-3):269-276

[47] Inda LA, Peleato ML. Immunoquantification of flavodoxin and ferredoxin from *Scenedesmus vacuolatus* (Chlorophyta) as iron-stress molecular markers. European Journal of Phycology. 2002;**37**(4):579-586

[48] Inda LA, Peleato ML. Development of an ELISA approach for the determination of flavodoxin and ferredoxin as markers of iron deficiency in phytoplankton. Phytochemistry. 2003;**63**(3):303-308

[49] Michel KP, Thole HH, Pistorius EK. IdiA, a 34 kDa protein in the cyanobacteria *Synechococcus* sp. strains PCC 6301 and PCC 7942, is required for growth under iron and manganese limitations. Microbiology. 1996;**142**(Pt 9):2635-2645

[50] Michel KP, Kruger F, Puhler A, Pistorius EK. Molecular characterization of *idiA* and adjacent genes in the cyanobacteria *Synechococcus* sp. strains PCC 6301 and PCC 7942. Microbiology. 1999;**145**(Pt 6):1473-1484

[51] Exss-Sonne P, Tolle J, Bader KP, Pistorius EK, Michel KP. The IdiA protein of *Synechococcus* sp PCC 7942 functions in protecting the acceptor side of photosystem II under oxidative stress. Photosynthesis Research. 2000;**63**(2):145-157

[52] Tolle J, Michel KP, Kruip J, Kahmann U, Preisfeld A, Pistorius EK. Localization and function of the IdiA homologue Slr1295 in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Microbiology. 2002;**148**(Pt 10):3293-3305

[53] Michel KP, Exss-Sonne P, Scholten-Beck G, Kahmann U, Ruppel HG, Pistorius EK. Immunocytochemical localization of IdiA, a protein expressed under iron or manganese limitation in the mesophilic cyanobacterium *Synechococcus* PCC 6301 and the thermophilic cyanobacterium *Synechococcus elongatus*. Planta. 1998;**205**(1):73-81

[54] Lax JE, Arteni AA, Boekema EJ, Pistorius EK, Michel KP, Rogner M. Structural response of photosystem 2 to iron deficiency: Characterization of a new photosystem 2-IdiA complex from the cyanobacterium *Thermosynechococcus elongatus* BP-1. Biochimica et Biophysica Acta. 2007;**1767**(6):528-534

[55] Boyd PW, Law CS, Wong CS, Nojiri Y, Tsuda A, Levasseur M, Takeda S, Rivkin R, Harrison PJ, Strzepek R, et al. The decline and fate of an iron-induced subarctic phytoplankton bloom. Nature. 2004;**428**(6982):549-553

[56] Yousef N, Pistorius EK, Michel KP. Comparative analysis of *idiA* and *isiA* transcription under iron starvation and oxidative stress in *Synechococcus elongatus* PCC 7942 wild-type and selected mutants. Archives of Microbiology. 2003;**180**(6):471-483

[57] Pietsch D, Bernat G, Kahmann U, Staiger D, Pistorius EK, Michel KP. New insights into the function of the iron deficiency-induced protein C from *Synechococcus elongatus* PCC 7942. Photosynthesis Research. 2011;**108**(2-3):121-132

[58] Kranzler C, Rudolf M, Keren N, Schleiff E. Iron in cyanobacteria. In: Franck Chauvat CC-CE, editor. Genomics of Cyanobacteria. Vol. 65. Elsevier; 2013. pp. 57-105

[59] Nicolaisen K, Hahn A, Valdebenito M, Moslavac S, Samborski A, Maldener I, Wilken C, Valladares A, Flores E, Hantke K, et al. The interplay between siderophore secretion and coupled iron and copper transport in the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120. Biochimica et Biophysica Acta. 2010;**1798**(11):2131-2140

[60] Saito A, Shimizu M, Nakamura H, Maeno S, Katase R, Miwa E, Higuchi K, Sonoike K. Fe deficiency induces phosphorylation and translocation of Lhcb1 in barley thylakoid membranes. FEBS Letters. 2014;**588**(12):2042-2048

[61] Mann EL, Chisholm SW. Iron limits the cell division rate of *Prochlorococcus* in the eastern equatorial Pacific. Limnology and Oceanography. 2000;**45**(5):1067-1076

[62] Walworth NG, Fu FX, Webb EA, Saito MA, Moran D, McLlvin MR, Lee MD, Hutchins DA. Mechanisms of increased *Trichodesmium* fitness under iron and phosphorus co-limitation in the present and future ocean. Nature Communications. 2016;**7**:12081

[63] Winkelmann GN, Carrano CJ. Transition Metals in Microbial Metabolism. Amsterdam: Harwood Academic Publishers; 1997

[64] Chu BC, Garcia-Herrero A, Johanson TH, Krewulak KD, Lau CK, Peacock RS, Slavinskaya Z, Vogel HJ. Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. Biometals. 2010;**23**(4):601-611

[65] Goldman SJ, Lammers PJ, Berman MS, Sanders-Loehr J. Siderophore-mediated iron uptake in different strains of *Anabaena* sp. Journal of Bacteriology. 1983;**156**(3):1144-1150

[66] Singh A, Mishra AK. Influence of various levels of iron and other abiotic factors on siderophorogenesis in paddy field cyanobacterium *Anabaena oryzae*. Applied Biochemistry and Biotechnology. 2015;**176**(2):372-386

[67] Mullis KB, Pollack JR, Neilands JB. Structure of schizokinen, an iron-transport compound from *Bacillus megaterium*. Biochemistry. 1971;**10**(26):4894-4898

[68] Boiteau RM, Repeta DJ. An extended siderophore suite from *Synechococcus* sp. PCC 7002 revealed by LC-ICPMS-ESIMS. Metallomics. 2015;**7**(5):877-884

[69] Beiderbeck H, Taraz K, Budzikiewicz H, Walsby AE. Anachelin, the siderophore of the cyanobacterium *Anabaena cylindrica* CCAP 1403/2A. Zeitschrift für Naturforschung. Section C. 2000;**55**(9-10):681-687

[70] Wilhelm SW, Trick CG. Iron-limited growth of cyanobacteria: Multiple siderophore production is a common response. Limnology and Oceanography. 1994;**39**(8):1979-1984

[71] Butler A, Theisen RM. Iron(III)-siderophore coordination chemistry: Reactivity of marine siderophores. Coordination Chemistry Reviews. 2010;**254**(3-4):288-296

[72] Ahmed E, Holmstrom SJ. Siderophores in environmental research: Roles and applications. Microbial Biotechnology. 2014;**7**(3):196-208

[73] Johnstone TC, Nolan EM. Beyond iron: Non-classical biological functions of bacterial siderophores. Dalton Transactions. 2015;**44**(14):6320-6339

[74] Clarke SE, Stuart J, Sanders-Loehr J. Induction of siderophore activity in *Anabaena* spp. and its moderation of copper toxicity. Applied and Environmental Microbiology. 1987;**53**(5):917-922

[75] Singh A, Kaushik MS, Srivastana M, Tiwari DN, Mishra AK. Siderophore mediated attenuation of cadmium toxicity by paddy field cyanobacterium *Anabaena oryzae*. Algal Research. 2016;**16**:63-68

[76] Sonier MB, CD A, Treble RG, Weger HG. Two distinct pathways for iron acquisition by ironlimited cyanobacterial cells: Evidence from experiments using siderophores and synthetic chelators. Botany. 2012;**90**(3):181-190

[77] Nagai T, Imai A, Matsushige K, Fukushima T. Growth characteristics and growth modeling of *Microcystis aeruginosa* and *Planktothrix agardhii* under iron limitation. Limnology. 2007;**8**(3):261-270

[78] Rudolf M, Stevanovic M, Kranzler C, Pernil R, Keren N, Schleiff E. Multiplicity and specificity of siderophore uptake in the cyanobacterium *Anabaena* sp. PCC 7120. Plant Molecular Biology. 2016;**92**(1-2):57-69

[79] Barry SM, Challis GL. Recent advances in siderophore biosynthesis. Current Opinion in Chemical Biology. 2009;**13**(2):205-215

[80] De Lorenzo V, Bindereif A, Paw BH, Neilands JB. Aerobactin biosynthesis and transport genes of plasmid ColV-K30 in *Escherichia coli* K-12. Journal of Bacteriology. 1986; **165**(2):570-578

[81] Burrell M, Hanfrey CC, Kinch LN, Elliott KA, Michael AJ. Evolution of a novel lysine decarboxylase in siderophore biosynthesis. Molecular Microbiology. 2012;**86**(2):485-499

[82] Hopkinson BM, Morel FM. The role of siderophores in iron acquisition by photosynthetic marine microorganisms. Biometals. 2009;**22**(4):659-669

[83] Jeanjean R, Talla E, Latifi A, Havaux M, Janicki A, Zhang CC. A large gene cluster encoding peptide synthetases and polyketide synthases is involved in production of siderophores and oxidative stress response in the cyanobacterium *Anabaena* sp. strain PCC 7120. Environmental Microbiology. 2008;**10**(10):2574-2585

[84] Bleuel C, Grosse C, Taudte N, Scherer J, Wesenberg D, Krauss GJ, Nies DH, Grass G. TolC is involved in enterobactin efflux across the outer membrane of *Escherichia coli*. Journal of Bacteriology. 2005;**187**(19):6701-6707

[85] Furrer JL, Sanders DN, Hook-Barnard IG, McIntosh MA. Export of the siderophore enterobactin in *Escherichia coli*: Involvement of a 43 kDa membrane exporter. Molecular Microbiology. 2002;**44**(5):1225-1234

[86] Horiyama T, Nishino K. AcrB, AcrD, and MdtABC multidrug efflux systems are involved in enterobactin export in *Escherichia coli*. PLoS One. 2014;**9**(9):e108642

[87] Moslavac S, Nicolaisen K, Mirus O, Al Dehni F, Pernil R, Flores E, Maldener I, Schleiff E. A TolC-like protein is required for heterocyst development in *Anabaena* sp. strain PCC 7120. Journal of Bacteriology. 2007;**189**(21):7887-7895

[88] Hahn A, Stevanovic M, Mirus O, Schleiff E. The TolC-like protein HgdD of the cyanobacterium *Anabaena* sp. PCC 7120 is involved in secondary metabolite export and antibiotic resistance. The Journal of Biological Chemistry. 2012;**287**(49):41126-41138

[89] Noinaj N, Guillier M, Barnard TJ, Buchanan SK. TonB-dependent transporters: Regulation, structure, and function. Annual Review of Microbiology. 2010;**64**:43-60

[90] Schalk IJ, Mislin GL, Brillet K. Structure, function and binding selectivity and stereoselectivity of siderophore-iron outer membrane transporters. Current Topics in Membranes. 2012;**69**:37-66

[91] Perez AA, Rodionov DA, Bryant DA. Identification and regulation of genes for cobalamin transport in the cyanobacterium *Synechococcus* sp. strain PCC 7002. Journal of Bacteriology. 2016;**198**(19):2753-2761

[92] Napolitano M, Rubio MA, Santamaria-Gomez J, Olmedo-Verd E, Robinson NJ, Luque I. Characterization of the response to zinc deficiency in the cyanobacterium *Anabaena* sp. strain PCC 7120. Journal of Bacteriology. 2012;**194**(10):2426-2436

[93] Mirus O, Strauss S, Nicolaisen K, von Haeseler A, Schleiff E. TonB-dependent transporters and their occurrence in cyanobacteria. BMC Biology. 2009;**7**:68

[94] González A, Bes MT, Barja F, Peleato ML, Fillat MF. Overexpression of FurA in *Anabaena* sp. PCC 7120 reveals new targets for this regulator involved in photosynthesis, iron uptake and cellular morphology. Plant & Cell Physiology. 2010;**51**(11):1900-1914

[95] Kranzler C, Lis H, Finkel OM, Schmetterer G, Shaked Y, Keren N. Coordinated transporter activity shapes high-affinity iron acquisition in cyanobacteria. The ISME Journal. 2014;**8**(2):409-417

[96] Roe KL, Barbeau KA. Uptake mechanisms for inorganic iron and ferric citrate in *Trichodesmium erythraeum* IMS101. Metallomics. 2014;**6**(11):2042-2051

[97] Babykin MM, Obando TSA, Zinchenko VV. TonB-dependent utilization of dihydroxamate xenosiderophores in *Synechocystis* sp. PCC 6803. Current Microbiology. 2018;**75**(2):117-123

[98] Rose AL, Salmon TP, Lukondeh T, Neilan BA, Waite TD. Use of superoxide as an electron shuttle for iron acquisition by the marine cyanobacterium *Lyngbya majuscula*. Environmental Science & Technology. 2005;**39**(10):3708-3715

[99] Jiang HB, Lou WJ, Ke WT, Song WY, Price NM, Qiu BS. New insights into iron acquisition by cyanobacteria: An essential role for ExbB-ExbD complex in inorganic iron uptake. The ISME Journal. 2015;**9**(2):297-309

[100] Katoh H, Hagino N, Grossman AR, Ogawa T. Genes essential to iron transport in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Journal of Bacteriology. 2001;**183**(9): 2779-2784

[101] Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, et al. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature. 2003;**424**(6952):1042-1047

[102] Andrews SC. Iron storage in bacteria. Advances in Microbial Physiology. 1998;**40**:281-351

[103] Castruita M, Saito M, Schottel PC, Elmegreen LA, Myneni S, Stiefel EI, Morel FM. Overexpressin and characterization of an iron storage and DNA-binding Dps protein from *Trichodesmium erythraeum*. Applied and Environmental Microbiology. 2006; **72**(4):2918-2924

[104] Keren N, Aurora R, Pakrasi HB. Critical roles of bacterioferritins in iron storage and proliferation of cyanobacteria. Plant Physiology. 2004;**135**(3):1666-1673

[105] Shcolnick S, Shaked Y, Keren N. A role for *mrgA*, a DPS family protein, in the internal transport of Fe in the cyanobacterium *Synechocystis* sp. PCC6803. Biochimica et Biophysica Acta. 2007;**1767**(6):814-819

[106] Hernández JA, Pellicer S, Huang L, Peleato ML, Fillat MF. FurA modulates gene expression of *alr3808*, a DpsA homologue in *Nostoc* (*Anabaena*) sp. PCC 7120. FEBS Letters. 2007;**581**(7):1351-1356

[107] Wei X, Mingjia H, Xiufeng L, Yang G, Qingyu W. Identification and biochemical properties of Dps (starvation-induced DNA binding protein) from cyanobacterium *Anabaena* sp. PCC 7120. IUBMB Life. 2007;**59**(10):675-681

[108] Pena MM, Bullerjahn GS. The DpsA protein of *Synechococcus* sp. strain PCC7942 is a DNA-binding hemoprotein. Linkage of the Dps and bacterioferritin protein families. The Journal of Biological Chemistry. 1995;**270**(38):22478-22482

[109] Fujisawa T, Narikawa R, Maeda SI, Watanabe S, Kanesaki Y, Kobayashi K, Nomata J, Hanaoka M, Watanabe M, Ehira S, et al. CyanoBase: A large-scale update on its 20th anniversary. Nucleic Acids Research. 2017;**45**(D1):D551-D554

[110] Bes MT, Hernandez JA, Peleato ML, Fillat MF. Cloning, overexpression and interaction of recombinant Fur from the cyanobacterium *Anabaena* PCC 7119 with *isiB* and its own promoter. FEMS Microbiology Letters. 2001;**194**(2):187-192

[111] Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, et al. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Research. 1996;**3**(3):109-136

[112] Hernández JA, Artieda M, Peleato ML, Fillat MF, Bes MT. Iron stress and genetic response in cyanobacteria: *fur* Genes from *Synechococcus* PCC 7942 and *Anabaena* PCC 7120. Annales de Limnologie. 2002;**38**(1):3-11

[113] Troxell B, Hassan HM. Transcriptional regulation by ferric uptake regulator (Fur) in pathogenic bacteria. Frontiers in Cellular and Infection Microbiology. 2013;**3**:59

[114] Fillat MF. The FUR (ferric uptake regulator) superfamily: Diversity and versatility of key transcriptional regulators. Archives of Biochemistry and Biophysics. 2014;**546**:41-52

[115] Lee JW, Helmann JD. Functional specialization within the Fur family of metalloregulators. Biometals. 2007;**20**(3-4):485-499

[116] Deng Z, Wang Q, Liu Z, Zhang M, Machado AC, Chiu TP, Feng C, Zhang Q, Yu L, Qi L, et al. Mechanistic insights into metal ion activation and operator recognition by the ferric uptake regulator. Nature Communications. 2015;**6**:7642

[117] Hernandez JA, Lopez-Gomollon S, Bes MT, Fillat MF, Peleato ML. Three fur homologues from *Anabaena* sp. PCC7120: Exploring reciprocal protein-promoter recognition. FEMS Microbiology Letters. 2004;**236**(2):275-282

[118] Ludwig M, Chua TT, Chew CY, Bryant DA. Fur-type transcriptional repressors and metal homeostasis in the cyanobacterium *Synechococcus* sp. PCC 7002. Frontiers in Microbiology. 2015;**6**:1217

[119] Gonzalez A, Bes MT, Peleato ML, Fillat MF. Unravelling the regulatory function of FurA in *Anabaena* sp. PCC 7120 through 2-D DIGE proteomic analysis. Journal of Proteomics. 2011;**74**(5):660-671

[120] Lavrrar JL, McIntosh MA. Architecture of a fur binding site: A comparative analysis. Journal of Bacteriology. 2003;**185**(7):2194-2202

[121] Garcin P, Delalande O, Zhang JY, Cassier-Chauvat C, Chauvat F, Boulard Y. A transcriptional-switch model for Slr1738-controlled gene expression in the cyanobacterium *Synechocystis*. BMC Structural Biology. 2012;**12**:1

[122] Pallares MC, Marcuello C, Botello-Morte L, Gonzalez A, Fillat MF, Lostao A. Sequential binding of FurA from *Anabaena* sp. PCC 7120 to iron boxes: Exploring regulation at the nanoscale. Biochimica et Biophysica Acta. 2014;**1844**(3):623-631

[123] Hernández JA, López-Gomollón S, Muro-Pastor A, Valladares A, Bes MT, Peleato ML, Fillat MF. Interaction of FurA from *Anabaena* sp. PCC 7120 with DNA: A reducing environment and the presence of Mn(2+) are positive effectors in the binding to *isiB* and *furA* promoters. Biometals. 2006;**19**(3):259-268

[124] Martin-Luna B, Sevilla E, Hernandez JA, Bes MT, Fillat MF, Peleato ML. Fur from *Microcystis aeruginosa* binds *in vitro* promoter regions of the microcystin biosynthesis gene cluster. Phytochemistry. 2006;**67**(9):876-881

[125] Botello-Morte L, Pellicer S, Sein-Echaluce VC, Contreras LM, Neira JL, Abian O, Velazquez-Campoy A, Peleato ML, Fillat MF, Bes MT. Cysteine mutational studies provide insight into a thiol-based redox switch mechanism of metal and DNA binding in FurA from *Anabaena* sp. PCC 7120. Antioxidants & Redox Signaling. 2016;**24**(4):173-185

[126] Botello-Morte L, Bes MT, Heras B, Fernandez-Otal A, Peleato ML, Fillat MF. Unraveling the redox properties of the global regulator FurA from *Anabaena* sp. PCC 7120: Disulfide reductase activity based on its CXXC motifs. Antioxidants & Redox Signaling. 2014;**20**(9):1396-1406

[127] Cheng D, He Q. PfsR is a key regulator of iron homeostasis in Synechocystis PCC 6803. PLoS One. 2014;**9**(7):e101743

[128] Georg J, Voss B, Scholz I, Mitschke J, Wilde A, Hess WR. Evidence for a major role of antisense RNAs in cyanobacterial gene regulation. Molecular Systems Biology. 2009;**5**:305

[129] Hernandez JA, Muro-Pastor AM, Flores E, Bes MT, Peleato ML, Fillat MF. Identification of a *furA* cis antisense RNA in the cyanobacterium *Anabaena* sp. PCC 7120. Journal of Molecular Biology. 2006;**355**(3):325-334

[130] Sevilla E, Martin-Luna B, Gonzalez A, Gonzalo-Asensio JA, Peleato ML, Fillat MF. Identification of three novel antisense RNAs in the fur locus from unicellular cyanobacteria. Microbiology. 2011;**157**(Pt 12):3398-3404

[131] Krynicka V, Tichy M, Krafl J, Yu J, Kana R, Boehm M, Nixon PJ, Komenda J. Two essential FtsH proteases control the level of the Fur repressor during iron deficiency in the cyanobacterium *Synechocystis* sp. PCC 6803. Molecular Microbiology. 2014;**94**(3):609-624

[132] Pellicer S, González A, Peleato ML, Martinez JI, Fillat MF, Bes MT. Site-directed mutagenesis and spectral studies suggest a putative role of FurA from *Anabaena* sp. PCC 7120 as a heme sensor protein. The FEBS Journal. 2012;**279**(12):2231-2246

[133] Georg J, Kostova G, Vuorijoki L, Schon V, Kadowaki T, Huokko T, Baumgartner D, Muller M, Klahn S, Allahverdiyeva Y, et al. Acclimation of oxygenic photosynthesis to Iron starvation is controlled by the sRNA IsaR1. Current Biology. 2017;**27**(10):1425-1436 e1427

[134] Balasubramanian R, Shen G, Bryant DA, Golbeck JH. Regulatory roles for IscA and SufA in iron homeostasis and redox stress responses in the cyanobacterium *Synechococcus* sp. strain PCC 7002. Journal of Bacteriology. 2006;**188**(9):3182-3191

[135] Soni B, Houot L, Cassier-Chauvat C, Chauvat F. Prominent role of the three *Synechocystis* PchR-like regulators in the defense against metal and oxidative stresses. Open Biochemistry Journal. 2012;1-1

[136] Singh AK, Li H, Sherman LA. Microarray analysis and redox control of gene expression in the cyanobacterium *Synechocystis* sp. PCC 6803. Physiologia Plantarum. 2004;**120**(1):27-35

[137] Lopez-Gomollon S, Hernandez JA, Pellicer S, Angarica VE, Peleato ML, Fillat MF. Cross-talk between iron and nitrogen regulatory networks in *Anabaena* (*Nostoc*) sp. PCC 7120: Identification of overlapping genes in FurA and NtcA regulons. Journal of Molecular Biology. 2007;**374**(1):267-281

[138] Razquin P, Schmitz S, Fillat MF, Peleato ML, Bohme H. Transcriptional and translational analysis of ferredoxin and flavodoxin under iron and nitrogen stress in Anabaena sp. strain PCC 7120. Journal of Bacteriology. 1994;**176**(23):7409-7411

[139] Tognetti VB, Palatnik JF, Fillat MF, Melzer M, Hajirezaei MR, Valle EM, Carrillo N. Functional replacement of ferredoxin by a cyanobacterial flavodoxin in tobacco confers broad-range stress tolerance. Plant Cell. 2006;**18**(8):2035-2050

[140] Vinnemeier J, Kunert A, Hagemann M. Transcriptional analysis of the *isiAB* operon in salt-stressed cells of the cyanobacterium *Synechocystis* sp. PCC 6803. FEMS Microbiology Letters. 1998;**169**(2):323-330

[141] Hernandez-Prieto MA, Schon V, Georg J, Barreira L, Varela J, Hess WR, Futschik ME. Iron deprivation in *Synechocystis*: Inference of pathways, non-coding RNAs, and regulatory elements from comprehensive expression profiling. G3: Genes, Genomes, Genetics. 2012;**2**(12):1475-1495

[142] Thompson AW, Huang K, Saito MA, Chisholm SW. Transcriptome response of high- and low-light-adapted *Prochlorococcus* strains to changing iron availability. The ISME Journal. 2011;**5**(10):1580-1594

[143] Martin-Luna B, Sevilla E, Gonzalez A, Bes MT, Fillat MF, Peleato ML. Expression of fur and its antisense alpha-fur from *Microcystis aeruginosa* PCC7806 as response to light and oxidative stress. Journal of Plant Physiology. 2011;**168**(18):2244-2250

[144] Sevilla E, Martin-Luna B, Bes MT, Fillat MF, Peleato ML. An active photosynthetic electron transfer chain required for *mcyD* transcription and microcystin synthesis in *Microcystis aeruginosa* PCC7806. Ecotoxicology. 2012;**21**(3):811-819

[145] Hernandez JA, Alonso I, Pellicer S, Luisa Peleato M, Cases R, Strasser RJ, Barja F, Fillat MF. Mutants of *Anabaena* sp. PCC 7120 lacking *alr1690* and *alpha-furA* antisense RNA show a pleiotropic phenotype and altered photosynthetic machinery. Journal of Plant Physiology. 2010;**167**(6):430-437

[146] Domain F, Houot L, Chauvat F, Cassier-Chauvat C. Function and regulation of the cyanobacterial genes *lexA*, *recA* and *ruvB*: LexA is critical to the survival of cells facing inorganic carbon starvation. Molecular Microbiology. 2004;**53**(1):65-80

[147] Mullineaux CW. Co-existence of photosynthetic and respiratory activities in cyanobacterial thylakoid membranes. Biochimica et Biophysica Acta. 2014;**1837**(4):503-511

[148] Schmetterer G. Cyanobacterial respiration. In: Bryant DA, editor. The Molecular Biology of Cyanobacteria. Dordrecht: Kluwer Academic; 1994. pp. 409-435

[149] Peschek GA, Obinger C, Paumann M. The respiratory chain of blue-green algae (cyanobacteria). Physiologia Plantarum. 2004;**120**(3):358-369

[150] Nodop A, Pietsch D, Hocker R, Becker A, Pistorius EK, Forchhammer K, Michel KP. Transcript profiling reveals new insights into the acclimation of the mesophilic freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 to iron starvation. Plant Physiology. 2008;**147**(2):747-763

[151] Hernandez JA, Curatti L, Aznar CP, Perova Z, Britt RD, Rubio LM. Metal trafficking for nitrogen fixation: NifQ donates molybdenum to NifEN/NifH for the biosynthesis of the nitrogenase FeMo-cofactor. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(33):11679-11684

[152] Rubio LM, Ludden PW. Biosynthesis of the iron-molybdenum cofactor of nitrogenase. Annual Review of Microbiology. 2008;**62**:93-111

[153] Herrero A, Muro-Pastor AM, Flores E. Nitrogen control in cyanobacteria. Journal of Bacteriology. 2001;**183**(2):411-425

[154] Muro-Pastor MI, Reyes JC, Florencio FJ. Cyanobacteria perceive nitrogen status by sensing intracellular 2-oxoglutarate levels. The Journal of Biological Chemistry. 2001;**276**(41): 38320-38328

[155] Picossi S, Flores E, Herrero A. ChIP analysis unravels an exceptionally wide distribution of DNA binding sites for the NtcA transcription factor in a heterocyst-forming cyanobacterium. BMC Genomics. 2014;**15**:22

[156] Su Z, Olman V, Mao F, Xu Y. Comparative genomics analysis of NtcA regulons in cyanobacteria: Regulation of nitrogen assimilation and its coupling to photosynthesis. Nucleic Acids Research. 2005;**33**(16):5156-5171

[157] Flores E, Herrero A. Nitrogen assimilation and nitrogen control in cyanobacteria. Biochemical Society Transactions. 2005;**33**(Pt 1):164-167

[158] Cheng Y, Li JH, Shi L, Wang L, Latifi A, Zhang CC. A pair of iron-responsive genes encoding protein kinases with a Ser/Thr kinase domain and a his kinase domain are regulated by NtcA in the Cyanobacterium *Anabaena* sp. strain PCC 7120. Journal of Bacteriology. 2006;**188**(13):4822-4829

[159] Luque I, Zabulon G, Contreras A, Houmard J. Convergence of two global transcriptional regulators on nitrogen induction of the stress-acclimation gene *nblA* in the cyanobacterium *Synechococcus* sp. PCC 7942. Molecular Microbiology. 2001;**41**(4):937-947

[160] Valladares A, Muro-Pastor AM, Fillat MF, Herrero A, Flores E. Constitutive and nitrogen-regulated promoters of the *petH* gene encoding ferredoxin:NADP+ reductase in the heterocyst-forming cyanobacterium *Anabaena* sp. FEBS Letters. 1999;**449**(2-3):159-164

[161] Yingping F, Lemeille S, Gonzalez A, Risoul V, Denis Y, Richaud P, Lamrabet O, Fillat MF, Zhang CC, Latifi A. The Pkn22 Ser/Thr kinase in *Nostoc* PCC 7120: Role of FurA and NtcA regulators and transcript profiling under nitrogen starvation and oxidative stress. BMC Genomics. 2015;**16**:557

[162] Giner-Lamia J, Robles-Rengel R, Hernandez-Prieto MA, Muro-Pastor MI, Florencio FJ, Futschik ME. Identification of the direct regulon of NtcA during early acclimation to nitrogen starvation in the cyanobacterium *Synechocystis* sp. PCC 6803. Nucleic Acids Research. 2017;**45**(20):11800-11820

[163] Carmichael WW, Azevedo SM, An JS, Molica RJ, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. Environmental Health Perspectives. 2001;**109**(7):663-668

[164] Molot LA, Watson SB, Creed IF, Trick CG, McCabe SK, Verschoor MJ, Sorichetti RJ, Powe C, Venkiteswaran JJ, Schiff SL. A novel model for cyanobacteria bloom formation: The critical role of anoxia and ferrous iron. Freshwater Biology. 2014;**59**(6):1323-1340

[165] Dittmann E, Fewer DP, Neilan BA. Cyanobacterial toxins: Biosynthetic routes and evolutionary roots. FEMS Microbiology Reviews. 2013;**37**(1):23-43

[166] Crosa JH. Signal transduction and transcriptional and posttranscriptional control of iron-regulated genes in bacteria. Microbiology and Molecular Biology Reviews. 1997; **61**(3):319-336

[167] Crosa JH, Walsh CT. Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiology and Molecular Biology Reviews. 2002;**66**(2):223-249

[168] Tillett D, Dittmann E, Erhard M, von Dohren H, Borner T, Neilan BA. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: An integrated peptide-polyketide synthetase system. Chemistry & Biology. 2000;**7**(10):753-764

[169] Lyck S, Gjolme N, Utkilen H. Iron starvation increases toxicity of *Microcystis aeruginosa* CYA 228/1 (Chroococcales, Cyanophyceae). Phycologia. 1996;**35**:120-124

[170] Utkilen H, Gjolme N. Iron-stimulated toxin production in *Microcystis aeruginosa*. Applied and Environmental Microbiology. 1995;**61**(2):797-800

[171] Ceballos-Laita L, Marcuello C, Lostao A, Calvo-Begueria L, Velazquez-Campoy A, Bes MT, Fillat MF, Peleato ML. Microcystin-LR binds Iron, and Iron promotes self-assembly. Environmental Science & Technology. 2017;**51**(9):4841-4850

[172] Pernil R, Picossi S, Mariscal V, Herrero A, Flores E. ABC-type amino acid uptake transporters Bgt and N-II of *Anabaena* sp. strain PCC 7120 share an ATPase subunit and are expressed in vegetative cells and heterocysts. Molecular Microbiology. 2008;**67**(5): 1067-1080

[173] Rohrlack T, Hyenstrand P. Fate of intracellular microcystins in the cyanobacterium *Microcystis aeruginosa* (Chroococcales, Cyanophyceae). Phycologia. 2007;**46**(3):277-283

[174] Pearson LA, Hisbergues M, Borner T, Dittmann E, Neilan BA. Inactivation of an ABC transporter gene, *mcyH*, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. Applied and Environmental Microbiology. 2004; **70**(11):6370-6378

[175] Shi L, Carmichael WW, Miller I. Immonugold localization of hepatotoxins in cyanobacterial cells. Archives of Microbiology. 1995;**163**(1):7-15

[176] Young FM, Thomson C, Metcalf JS, Lucocq JM, Codd GA. Immunogold localisation of microcystins in cryosectioned cells of *Microcystis*. Journal of Structural Biology. 2005;**151**(2):208-214

[177] Gerbersdorf SU. An advanced technique for immuno-labelling of microcystins in cryosectioned cells of *Microcystis aeruginosa* PCC 7806 (cyanobacteria): Implementations of an experiment with varying light scenarios and culture densities. Toxicon. 2006;**47**(2): 218-228

[178] Sedmak B, Elersek T. Microcystins induce morphological and physiological changes in selected representative phytoplanktons. Microbial Ecology. 2005;**50**(2):298-305