The Maintenance of Iron Homeostasis Among Prokaryotic Phototrophs

Sébastien Zappa and Carl E. Bauer

Abstract Like all prokaryotes, photosynthetic bacteria have had to solve the difficulty of acquiring poorly available iron in oxic environments and, at the same time, have had to manage the potential deleterious Fenton chemistry effects caused by this metal. In addition, photosynthesis requires a lot of iron for both the synthesis of the photosystem and the photosynthetic process itself. As a result of this iron need, phototrophs are good model organisms to study bacterial iron homeostasis. This review focuses on transcriptomic changes induced by iron limitation centering on major functional features of iron homeostasis such as the acquisition, storage, and regulation. We review evidence that iron limitation induces significant stress that triggers global transcriptional changes resulting in upregulation of iron import and storage while decreasing photosynthesis. Studies on transcription factors that regulate genes involved in iron homeostasis will also be covered, with the Ferric Uptake Regulator being the most understood. Finally, we will discuss the interference between iron and copper homeostasis, especially since iron transport systems make up a primary defense against copper poisoning.

Keywords Iron • Iron homeostasis • Iron transport • Iron regulation • Iron stress response • Fur • Irr • Feo • TonB • Siderophore • EfeUOB • FutABC • PfsR • Copper homeostasis

Introduction

Studies as early as 1956 showed that iron affects the synthesis of the purple bacterial photosystem. Specifically, Lascelles reported that iron supplementation stimulates production of bacteriochlorophyll in the purple bacterium *Rhodobacter sphaeroides* (Lascelles 1956). Conversely it has also been shown that iron limitation leads to an absence of pigmentation and impaired growth under photosynthetic conditions (Peuser et al. 2011). Surprisingly, there are few other reports on the effect of iron availability on synthesis of the purple bacterial photosystem.

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In contrast to limited mechanistic studies with purple bacteria, the effect of iron on synthesis of the cyanobacterial photosystem has been an active area of research. Numerous studies have shown that iron deprivation leads to a general decreases in chlorophyll and phycocyanin synthesis in cyanobacteria (Öquist 1974; Guikema and Sherman 1983; Sandmann 1985; Sandström et al. 2002). Adaptation of the photosynthetic apparatus to iron deficiency also affects the composition and ratio of individual photosystem components. Briefly, the Fe-S-containing ferredoxin is replaced by the flavodoxin IsiB, and the photosystem I (PSI) is surrounded by IsiA proteins. Formation of the IsiA-PSI supercomplex compensates the decrease of PSI in terms of light-harvesting capacity. More details and references can be found in the following articles (Chauhan et al. 2011; Ryan Keogh et al. 2012; Fraser et al. 2013; Cheng and He 2014; Wahadoszamen et al. 2015). IsiA and IsiB are encoded by the *isiAB* operon and make up a central part of the response to iron limitation, often considered as a hallmark of the cyanobacterial iron response, and will be discussed in this review.

The effect of iron limitation on pigment synthesis in photosynthetic species is understandable given that many enzymes involved in the bacteriochlorophyll, chlorophyll, and phycocyanin synthesis require iron as a cofactor for catalysis (Beale and Cornejo 1983; Frankenberg and Lagarias 2003; Sirijovski et al. 2007; Sarma et al. 2008). In addition, iron is an important component of photosystem I (PSI) and photosystem II (PSII) as well as an essential component of the electron transport chain, many components of which use heme as an electron carrier (Ferreira and Straus 1994; Keren et al. 2004). Given the poor solubility of iron in oxic environments and its toxicity through Fenton chemistry, bacterial fitness is tied to the necessity of efficient import and storage systems that also prevent the buildup of an intracellular free iron pool, in other words an efficient iron homeostasis (Touati 2000; Andrews et al. 2003; Chiancone et al. 2004). The literature on the effect of iron on synthesis and function of photosystems is expansive and thus beyond the scope of a single review. Consequently, this review focuses on mechanisms that photosynthetic prokaryotes use to acquire, sequester, and regulate appropriate amounts of intracellular iron.

The Cellular Response to Iron Limitation

There are currently only few reports of global studies of the effect of iron limitation on gene expression in anoxygenic photosynthetic prokaryotes. A study on *Rhodobacter* (*R.*) *sphaeroides* showed that acclimation to iron-free medium induces a lack of pigmentation, as a result of the downregulation of the photosynthetic genes *pucAB* and *puc2AB*, encoding light-harvesting complex II. In addition, iron limitation increased the cellular amount of reactive oxygen species (ROS) (Peuser et al. 2011). Overall, 384 transcriptomic changes were observed: 33 downregulations concerning photosynthesis, flagellum biosynthesis, and chemotaxis genes and 351 upregulated genes dealing with iron uptake and storage but also Fe-S cluster

assembly and repair (Peuser et al. 2012). Unexpectedly, no significant changes were observed regarding genes involved in the oxidative stress response, despite the increase of cellular ROS. Finally, it is remarkable that the ferrous iron uptake system *feoAB* did not respond to iron deficiency while it did to H_2O_2 (Peuser et al. 2012). Another study on the effect of iron limitation on iron transport and heme gene expression deals with *R. capsulatus* (Zappa and Bauer 2013a). That study demonstrated that iron limitation led to an overall increase in ferrous and ferric transport gene expression and an increase in siderophore and heme uptake receptor expression. There was also a decrease in expression in a putative ferrous iron efflux pump indicating that *R. capsulatus* cells that are starved for iron adjust their membrane transport components in a way that maximizes iron acquisition and retention. There is also a general increase in heme gene expression which presumably reflects stress on heme synthesis caused by inactivation of enzymes in the heme pathway that use iron as a cofactor as well as limitation of iron availability for insertion into protoporphyrin IX (Zappa and Bauer 2013a).

In contrast to limited understanding of global effects of iron limitation in anoxygenic photosynthetic prokaryotes, there have been global studies on the effects of iron limitation with cyanobacteria. Major findings are summarized in Fig. 1. A study with *Synechocystis* sp. PCC 6803 demonstrated that an iron limitation kinetic shift resulted in 1076 transcription units that were differentially expressed during at least one time point. These include 644 mRNAs and 434 noncoding RNAs (307 asRNAs, 125 sRNAs) (Hernández-Prieto et al. 2012). A highly dynamic pattern was observed

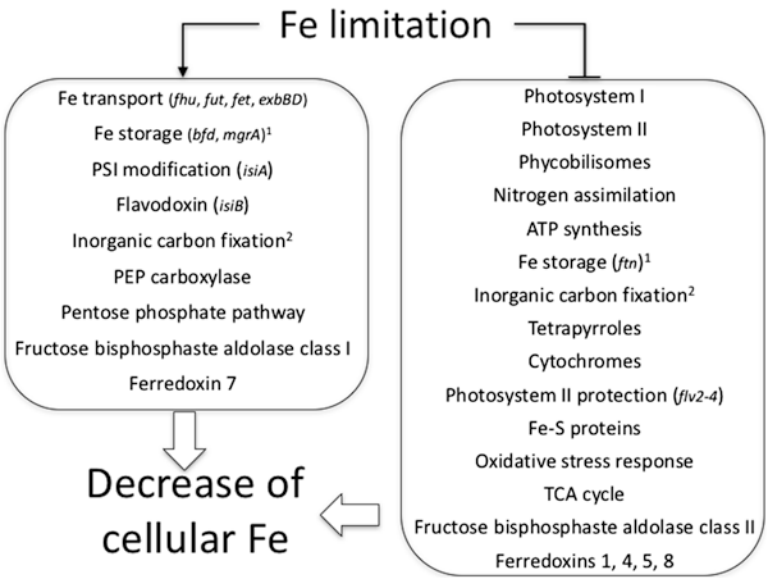

Fig. 1 Iron limitation induces global transcriptomic and metabolic changes. [1]Iron storage response varies depending on species studied (e.g., *Synechocystis* vs. *Trichodesmium*). [2]Inorganic carbon change in *Synechocystis* varies depending on studies

with a transient initial upregulation followed by downregulation. The affected genes included those involved in photosynthesis (PSI, PSII, phycobilisomes, ATP synthase complex, carbon fixation, porphyrin and chlorophyll synthesis) but also involved in oxidative phosphorylation, which in cyanobacteria borrows several components to photosynthesis (plastoquinone pool, soluble electron carriers, cytochrome b_6f, ATP synthase). Downregulation of cytochrome b_6f under iron limitation has been consistently observed (Sandström et al. 2002; Shi et al. 2007; Thompson et al. 2011; Fraser et al. 2013). Decrease in PSI gene expression is stronger than that of PSII genes, which is consistent with the PSI/PSII ratio as is classically observed (Hernández-Prieto et al. 2012). Other functional group had less dynamic, more sustained expression patterns. Typically, iron transport (*fhuA*, *futA*, *fetA*, *exbD*)- and storage (*bfd*)-related genes along with the *isiAB* operon were upregulated. The latter operon encodes the IsiA protein, involved in PSI modification, and the flavodoxin IsiB, both involved in the iron stress/sparing response. On the opposite, the *flv4-2* operon, encoding the PSII protection flavodiiron protein complex Fl2-Fl4, was downregulated. Also with sustained pattern was a transcription increase of nitrogen assimilation genes (*nirA*, *glnN*, *glnA*, *ntcA*), while inorganic carbon fixation genes decrease (*cmpABCD*). As both processes necessitate iron-containing enzymes, the observed difference in transcription change may be a signature of cellular prioritization (Hernández-Prieto et al. 2012).

Antisense RNA appears to be involved in different ways: from promoting mRNA stability of nitrogen assimilation genes to fine-tuning either the basal mRNA level of iron import genes or the transient expression of *isiA* and *flv4* (Hernández-Prieto et al. 2012). In addition, among the 125 sRNA differentially expressed upon iron limitation, four showed potential regulatory functions in photosynthesis and respiration. In a few cases, different patterns of expressions were observed between a UTR and its associated gene indicating that the UTR may constitute riboswitches which adds another layer of regulation. Such cases include ferrous iron transport (*feoA*), bicarbonate transport (*cmpA*), Fe-S cluster assembly (*sufB*), and phycobilisome-dependent light energy dissipation (slr1964) (Hernández-Prieto et al. 2012).

By following a set of 106 proteins in *Synechocystis* sp. PCC 6803, a proteomic approach confirmed the bulk of the above transcriptomic data. Most photosynthesis components were downregulated (PSI, PSII, phycobilisomes, cytochrome b_6f, soluble electron carriers), as were Fe-S-containing proteins (Vuorijoki et al. 2016). Upregulated photosynthesis elements were the IsiA and IsiB iron stress proteins and the phycobilisome-interacting orange carotenoid protein, involved in photoprotection. On the other hand, carbon fixation components were upregulated which is not consistent with previously reported transcriptomic data (Hernández-Prieto et al. 2012; Vuorijoki et al. 2016). Other upregulated components included iron transport (FeoB, FhuA) and storage (MgrA) proteins, sigma factors SigB and SigC, Fe-S biosynthesis repressor SufR, and nitrogen assimilation factor NtcA. Downregulation was observed for FurA, hydrogenase HoxH, and sigma factor SigA. TCA cycle was overall downregulated as a consequence of multiple Fe-S-containing enzymes, while phosphoenolpyruvate carboxylase and pentose phosphate pathway enzymes were upregulated (Vuorijoki et al. 2016).

An additional proteomic characterization of iron stress was reported for the marine non-heterocystous filamentous diazotroph *Trichodesmium erythraeum* IMS101. The global iron-sparing response in this species results in a reduction of 55–60% for the iron requirement of the cell, with the largest part of this saving coming from the reduction in nitrogenase and PSI contents (Snow et al. 2015). Statistical analysis of the iron-stressed proteome showed that with higher the iron content, individual proteins were more affected by iron limitation. Otherwise, ferrous iron transporter FeoA-FeoB could not be detected unlike the ferric iron transporter FutA/IdiA (Snow et al. 2015). Numerous enzymes involved in oxidative stress response (Ni-SOD, TrxA, TrxB, peroxiredoxin) were upregulated under iron deprivation. So was a PilA homologue, this major pilin protein was described to take part in growth on iron oxides for *Synechocystis* sp. PCC 6803 (Lamb et al. 2014). The nitrogen fixation and photosynthesis pathways were repressed, while IsiA, the flavodoxin IsiB, and the plastocyanin were overexpressed. Typical PSI/PSII decrease and IsiA/PSI increase under iron deficiency were confirmed in this study (Snow et al. 2015). Severe decrease of the iron storage ferritin under iron limitation indicates that the cellular iron pool becomes fully associated with functional iron proteins in these conditions. On the opposite, iron-replete cells are estimated to have 84% of the cellular iron in the ferritins (Snow et al. 2015). Interestingly, the class II fructose bisphosphate aldolase enzymes, which are often Fe^{2+} dependent, were substituted by class I enzymes. RuBisCo, ATP synthase, and cytochrome b_6f/PSII were unchanged or only mildly changed (Snow et al. 2015). Heme synthesis was also found affected by iron depletion although with various patterns. Indeed, *hemA* is downregulated or unchanged in *Prochlorococcus* sp. MED4 and MIT9313 strains, respectively (Thompson et al. 2011). This is contrasted by upregulation of several heme synthesis genes in *Anabaena* sp. PCC 7120 (González et al. 2012; Snow et al. 2015). In the same species, two heme oxygenases are activated by iron depletion, while in *Trichodesmium erythraeum* IMS101, heme oxygenase is repressed (Snow et al. 2015). This highlights that there are different strategies of iron recycling in different cyanobacterial species.

Iron availability also impacts the expression of several cyanobacterial ferredoxins. *Synechocystis* sp. PCC 6803 exhibits nine ferredoxins, Fed1–9. Iron starvation downregulates Fed1, Fed4, Fed5, and Fed8. On the other hand, Fed7 is upregulated by iron-deplete and downregulated under iron-replete conditions. Fed9 was not described as transcriptionally affected by iron, but it does interact with the flavodiiron protein Flv3. Both Fed7 and Fed9 are important in response to oxidative stress and metal availability, particularly to iron (Cassier-Chauvat and Chauvat 2014).

Finally, one note of caution regarding global changes needs to be made. A comparison of four iron limitation transcription studies with *Synechocystis* sp. PCC 6803 indicates that only 28 genes display significant differential changes in all four studies: 21 are upregulated, 3 downregulated, and 4 vary form one study to the other (Hernández-Prieto et al. 2012). This underlines the high sensitivity of the cellular response to variability in experimental conditions and potential genetic drift of laboratory strains.

Iron Transport

The import of iron can occur with several different classes of transporters (Andrews et al. 2003; Cartron et al. 2006; Cornelis and Andrews 2012; Zappa and Bauer 2013b). Briefly, poorly soluble Fe^{3+} iron can be imported as Fe^{3+} bound to a siderophore by a TonB-dependent siderophore uptake system or as free Fe^{3+} by a metal ABC transporter. Uptake of the more soluble Fe^{2+} can occur via the Feo system and the EfeUOB system, although other studies also show that the latter system can also import soluble Fe^{3+} as well as extract Fe^{2+} from heme (Létoffé et al. 2009; Miethke et al. 2013). By far, siderophore uptake has received most of the attention over past decades but more and more is known about these alternative pathways of iron import. None of these iron import systems are unique to photosynthetic bacteria as they are widely dispersed among the bacterial domains. Iron import systems identified in phototrophic bacteria are depicted in Fig. 2.

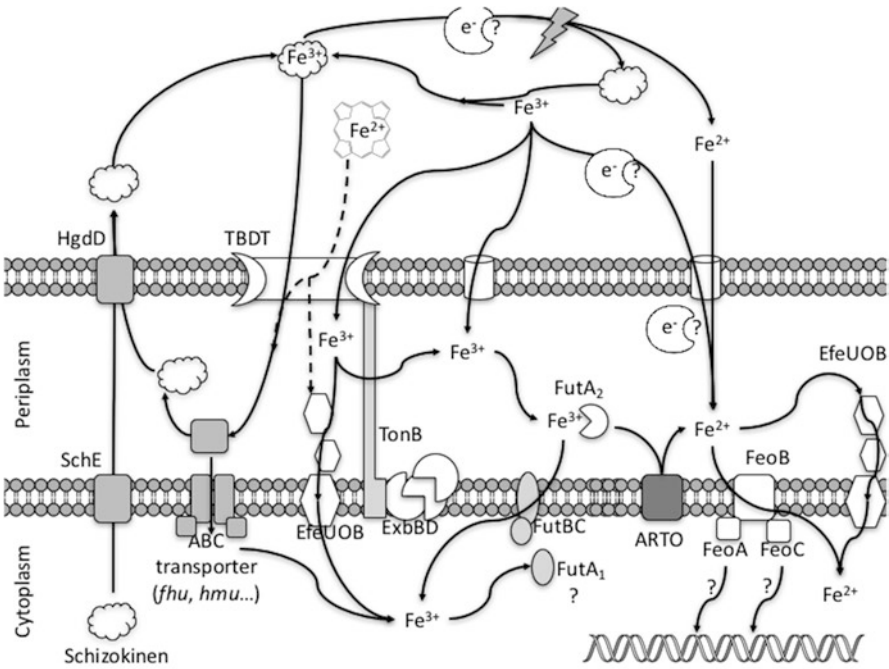

Fig. 2 Overview of iron transport systems identified in phototrophic prokaryotes. Represented are the export and import of siderophores, with the example of *Anabaena* schizokinen. TBDT-TonB-ExbBD systems can be involved in the uptake of ferrisiderophores, heme, and ferric iron. EfeUOB system can be involved in the import of iron as ferric, ferrous iron as well as extracting iron from heme. Ferric iron import can be achieved by the FutABC system while Feo system uptakes ferrous iron. Reductive pathway, involving a putative extracellular or periplasmic reductase, is also pictured as well as the abiotic photoreduction

Siderophore Synthesis and Uptake Systems

Siderophore synthesis and uptake have been frequently reviewed, so readers interested in this topic are directed to the following references (Köster 2001; Krewulak and Vogel 2008, 2011; Hopkinson and Morel 2009; Sandy and Butler 2009; Hider and Kong 2010; Chu et al. 2010; Morrissey and Bowler 2012). Siderophores are small ligands of 500–1500 Da that exhibit a high affinity for Fe^{3+} ($Kf > 10^{30}$ M). Siderophores are produced by a wide range of organisms (bacteria, fungi, diatoms, graminaceous plants) in all sorts of environments (terrestrial, soil, freshwater, open ocean, coastal ocean water) with more than 500 siderophores identified. Most siderophores are synthesized by nonribosomal peptide synthetases (NRPSs) or polyketide synthases (PKSs) with chemical structures classified as catechol, hydroxamate, or alpha-hydroxycarboxylate. Siderophores chelate the relatively insoluble Fe^{3+}, which enables organisms with adequate siderophore uptake systems to import this oxidized form of iron. Organisms can produce one or several siderophores, along with the corresponding uptake systems, but organisms can also scavenge siderophores that are produced by other species (in this case siderophores that are scavenged from another species are called xenosiderophores). In Gram-negative bacteria, the typical siderophore uptake system consists of an outer membrane ferrisiderophore receptor that interacts with a siderophore-specific TonB-ExbB-ExbD complex to provide the energy for translocation of the siderophore into the periplasm. Once in the periplasm, an ABC transporter cassette, comprised of a periplasmic siderophore-binding protein, permease, and ATPase, then imports the ferrisiderophore into the cytoplasm.

Siderophore-based iron import has been described for photosynthetic organisms. In the purple bacterium *R. sphaeroides*, no synthesis of hydroxamate- or catecholate-type siderophore could be detected, but this bacterium was shown to uptake ferric citrate and ferric parabactin supplied to the growth medium (Moody and Dailey 1984, 1985). Genome sequence analysis of model purple bacteria also shows the presence of numerous siderophore uptake systems. For example, there are 4 in *R. sphaeroides*, 7 in *R. capsulatus*, 24 in *Rhodopseudomonas (Rh.) palustris* but none in *R. ferrooxidans* (Larimer et al. 2004; Zappa and Bauer 2013b). Unlike *R. sphaeroides* and *R. capsulatus*, *Rh. palustris* is able to synthesize a siderophore, namely, rhizobactin. Based on the ratio of siderophore synthesis versus siderophore uptake systems in their respective genomes, it is reasonable to speculate that these three organisms rely more or less heavily on xenosiderophores. In *R. sphaeroides* and *R. capsulatus*, siderophore uptake systems were also shown to be transcriptionally upregulated under iron-limiting conditions (Peuser et al. 2012; Zappa and Bauer 2013a).

In cyanobacteria, the production of siderophores and/or use of xenosiderophores under iron scarcity have been known for a long time (Trick and Kerry 1992; Ferreira and Straus 1994; Michel and Pistorius 2004). Siderophore production has been reported for numerous species such as *Anabaena* sp., *Anabaena cylindrical*, *Anabaena oryzae*, *Microcystis aeruginosa*, *Microcystis wesenbergii,*

Synechococcus sp., but overall cyanobacteria have been described as poor siderophore producers (Simpson and Neilands 1976; Lammers and Sanders-Loehr 1982; Ghassemian and Straus 1996; Xing et al. 2007; Nicolaisen et al. 2010; Alexova et al. 2011; Wang et al. 2014; Singh and Mishra 2015). Characterized siderophores of cyanobacterial origin include the suite of synechobactins produced by the marine species *Synechococcus* sp. PCC 7002, schizokinen synthesized by the freshwater species *Anabaena* sp. PCC 7120, and the anachelins isolated from the other freshwater species *Anabaena cylindrica*. While synechobactins and schizokinen are similar citrate-based siderophores, anachelins are peptide siderophores. Synechobactins differ schizokinen by the presence of fatty acid tails (Hopkinson and Morel 2009).

Siderophore used in cyanobacteria has not been extensively characterized, but genome analysis suggests that, for marine species, the process might be more implemented in coastal rather than open-ocean species (Hopkinson and Morel 2009). From an ecological standpoint, cyanobacterial production of siderophores avoids being overgrown by eukaryotic algae (Murphy et al. 1976). Currently, the best molecular model of cyanobacterial siderophore usage was assembled in *Anabaena* sp. PCC 7120. This strain was shown to produce at least two siderophores, including schizokinen. The schizokinen exporter SchE, a protein of the major facilitator superfamily, and HgdD, a TolC-like protein, are responsible for the export of schizokinen (Nicolaisen et al. 2010). Two TonB-dependent outer membrane transporters (TBDT) were identified: the schizokinen transporter SchT and the Fe^{3+} and Cu^{2+} transporter IatC (Nicolaisen et al. 2008, 2010). Several TonB-exbBD systems were tested, and $TonB_3$-$ExbB_3D_3$ was shown to import schizokinen from the outer membrane receptor SchT to the periplasm. Finally, a FhuBCD cassette was identified as the final step of schizokinen uptake, with FhuD being the periplasmic schizokinen-binding protein, FhuB the permease, and FhuC the ATP-binding protein. The *tonB*$_3$ and *fhu* genes were found to be essential for the cells to grow under normal iron levels (Stevanovic et al. 2012). Systematic study of TBDT and TonB-ExbBD systems revealed a complex network where some are responsive to high iron levels at low cell density, while others are upregulated under iron-limiting condition at high cell density. The latter category makes up the core of the iron deficiency response (Stevanovic et al. 2013). Upregulation of *fhuC* was observed under iron limitation, although to a much lesser extent than *schE* and *schT* (Rudolf et al. 2015). Kinetics showed that endogenously produced schizokinen is imported faster than xenosiderophores by a 100-fold factor in *Anabaena* sp. PCC 7120 and *Anabaena flos-aqua* (Sonier et al. 2012; Rudolf et al. 2015). Different transporters are dedicated to import schizokinen and xenosiderophores in the periplasm, but final internalization through the plasma membrane involves the same transporter in *Anabaena* sp. PCC 7120 (Rudolf et al. 2015).

In *Anabaena* sp. PCC 7120, two gene clusters potentially involved in siderophore synthesis were found: one involved in hydroxamate-type siderophore synthesis, in the vicinity of the SchT-encoding gene, and a large gene cluster of 76 kb, with NRPS/PKS signature genes (Jeanjean et al. 2008; Nicolaisen et al.

2008). Transcription of the genes of the 76 kb cluster was found upregulated in iron-limiting conditions or in the presence of oxidative stress (Jeanjean et al. 2008). Likewise, genes involved in siderophore synthesis and uptake are upregulated in *Nostoc* when iron was scarce (Yingping et al. 2014). In addition, screening for TonB-dependent outer membrane receptors in 32 cyanobacterial genomes revealed the presence of 22 TonB-dependent receptors in *Anabaena* sp. PCC 7120, including 14 FhuA type, 3 IutA type, and 1 ViuA type, which are related to hydroxamate, citrate-hydroxamate, and catecholate siderophore import, respectively. Variability of the transcriptomic response to iron levels highlights that these TBDT are specialized in either quick response to high iron at low cell density, genuine response to iron limitation, or copper detoxification (Stevanovic et al. 2013). Nevertheless, the number of TBDT varies a lot between cyanobacterial genomes: 33 in *Gloeobacter violaceus*, 22 in *Anabaena* sp. PCC 7120, 10 in *Anabaena variabilis*, 6 in *Synechococcus* sp. PCC 7002, 4 in *Synechocystis* sp. PCC 6803, and none in *Prochlorococcus*. Some were also found in the "*Candidatus Synechococcus* spongiarum" group, cyanobacterial symbionts of sponges, and seem to be remnants of ancestral features of nonsymbiotic ancestors (Burgsdorf et al. 2015). Interestingly, no FecA-type transporter was identified, although this ferric citrate transporter is widely distributed in other bacterial classes (Mirus et al. 2009). So far, only three cyanobacterial TonB-dependent receptors were experimentally studied: the two aforementioned SchT and IacT in *Anabaena* sp. and FdTonB in *Fremyella diplosiphon*. As the latter was found unresponsive to iron levels, it brings to two the number of cyanobacterial TonB-dependent transporters involved in iron homeostasis with experimental confirmation, both in the same species. Finally, in *Synechocystis* sp., a putative FhuA-type siderophore transporter that was identified in silico (slr1406) was experimentally shown to be upregulated in iron-limiting condition (Mirus et al. 2009; Shcolnick et al. 2009). Still, this highlights how siderophore uptake in cyanobacteria is far from being fully characterized.

Besides classic activation under iron scarcity, it is interesting to note that light quality can affect iron uptake. Indeed, cyanobacteria can adapt to the color of light via a process known as complimentary chromatic adaptation. DNA microarray showed that genes responding to chromatic adaptation in *Fremyella diplosiphon* have homologues in other cyanobacteria that are responsive to iron scarcity, such as *atpB* that putatively encodes a siderophore uptake protein (Stowe-Evans et al. 2004). Finally, in marine environments, import of Fe^{3+} from siderophore could involve mechanisms other than the TonB-dependent uptake. Extracellular reduction of the ferrisiderophore complex could be a primary process in these conditions: instead of actually internalizing the complex, it is dissociated by reduction by an extracytoplasmic reductase or by photoreduction. Once reduced, free Fe^{2+} is set free to diffuse passively through an outer membrane porin before reaching Fe^{2+} transporter FutABC or FeoB (Hopkinson and Morel 2009; Sandy and Butler 2009). Extracellular reduction of ferric iron from siderophore prior to iron uptake was also shown in *Anabaena* sp. PCC 7120 (Kranzler et al. 2011).

Ferric Iron ABC Transporters

As an alternative to siderophore-based Fe^{3+} uptake systems, some species can also contain a ferric iron ABC transporter. In such a situation, ferric iron has to first reach the periplasm, for example, by passive diffusion through the outer membrane via a porin. Then, the Fe^{3+} ABC transporter operates using a Fe^{3+}-binding periplasmic protein, an inner membrane permease and an ATPase. Well-studied examples of the Fe^{3+} ABC transporter are the FbpABC (AfuABC), SfuABC, and HitABC systems (Andrews et al. 2003). Unlike TonB-dependent uptake systems, these transporters are less specific and were shown to be able to import Fe^{2+} and/or Mn^{2+} in addition to or instead of Fe^{3+} (see Zappa and Bauer (2013b) for references). In purple bacteria, screening of *Rhodobacter* genomes revealed the presence of an FbpABC homologue in *R. capsulatus*, *R. sphaeroides*, and *R. ferrooxidans*, where the permease-encoding gene is duplicated (Zappa and Bauer 2013b). Thus, this *fbpAB*$_1$*B*$_2$*C* operon might be inherited from a common ancestor to *Rhodobacter* species. Among this genus, another Fe^{3+} ABC transporter was found, but only in *R. sphaeroides*. It is homologous to the SitABCD transporter originally described in *Salmonella enterica* serovar Typhimurium (Kehres et al. 2002). And as in the latter organism, some elements suggest that it might be involved in Mn^{2+} rather than Fe^{3+} transport. Firstly, *R. sphaeroides* does not harbor the Mn^{2+}-specific transporter MntH, while the other *Rhodobacter* representatives do (Zappa and Bauer 2013b). Second, the specificity of this transporter to Fe^{3+} is not stable across the organisms harboring it. Third, its transcription is not upregulated under iron scarcity but instead is upregulated by manganese scarcity (Peuser et al. 2012).

More is known about Fe^{3+} ABC transporters in cyanobacteria than those of anoxygenic bacteria. The FutA/IdiA Fe^{3+} ABC transporter was identified in the genomes of 28 unicellular cyanobacteria of the *Prochlorococcus*, *Synechococcus*, and *Synechocystis* genera (Morrissey and Bowler 2012). Biochemical characterization has been primarily done on the *Synechocystis* sp. PCC 6803 homologue. The $FutA_1A_2BC$ consists of two Fe^{3+}-binding proteins $FutA_1$ and $FutA_2$, a permease FutB, and an ATPase FutC with *fut* gene expression upregulated under iron-limiting conditions. Deletion of *futA*$_1$ or *futA*$_2$ also reduces cellular iron content, with the *futA*$_1$ deletion being the most severe. Interestingly, while $FutA_2$ is a highly abundant periplasmic protein and is accepted as the Fe^{3+} periplasmic receptor of the ABC cassette, the role of $FutA_1$ remains unclear. Unlike $FutA_2$, $FutA_1$ is not located in the periplasm but is instead predominantly located in the cytoplasm. However, deletion of *futA*$_1$ does severely reduce the concentration of Fe^{3+}-$FutA_2$ in the periplasm, so it does exert an indirect control on iron import by the $FutA_2BC$ complex (Katoh et al. 2000, 2001a, b; Badarau et al. 2008; Shcolnick et al. 2009; Morrissey and Bowler 2012).

Originally identified as IdiA, $FutA_1$ has also been suggested to protect the acceptor side of PSII from H_2O_2 production under iron limitation by direct protein-protein interaction (Ting et al. 2002; Michel and Pistorius 2004). $FutA_2$ is also connected to oxidative stress as it is regulated by PerR, the Fur-like transcription factor involved in

oxidative stress response (Shcolnick et al. 2009). Other FutABC uptake systems were identified in other cyanobacteria, and most cyanobacterial FutA sequences make up a homologous clade (Tom-Yew et al. 2005). Regarding experimental clues, *Microcystis aeruginosa* has one FutA homologue that seems localized at the periphery of the cell and that was shown to be expressed under iron limitation (Alexova et al. 2011). A homologous Fe^{3+} transport system designated as IdiA/FutB/FutC was found to be conserved through several *Prochlorococcus* genomes, and IdiA, the Fe-binding periplasmic protein, was also shown to be transcriptionally upregulated under iron scarcity (Thompson et al. 2011). In *Anabaena* sp. PCC 7120, putative ferric iron transporters $iutA_1$, $iutA_2$, and $hutA_1$ were reported to only mildly respond to iron limitation (Rudolf et al. 2015). In the same species, various elements of ferric iron transporters were found to be upregulated in the presence of either high iron at low cell density ($fecD_2$, $fecD_3$) or low iron at mild cell density ($fecD_1$, $fecC_1$, *futB*). This reveals involvement of homologous systems in various cellular responses (Stevanovic et al. 2013).

Finally, ExbB-ExbD pathways are usually associated with TonB-dependent uptake, which was extensively characterized for siderophores. Nevertheless, three ExbB-ExbD systems were identified in the non-siderophore-producing *Synechocystis* sp. PCC 6803, all of which being upregulated under iron limitation and involved in inorganic Fe^{3+} uptake (Jiang et al. 2015). Redundancy and lethality induced by mutation of the three systems suggest that inorganic Fe^{3+} uptake may be the main iron source for this cyanobacterium and this class of phototrophs (Jiang et al. 2015). In *Anabaena* sp. PCC 7120, the various ExbBD systems respond to either high or low iron which indicate specialization (Stevanovic et al. 2013).

Ferrous Iron Uptake

While less is known about ferrous iron transport than its ferric counterpart, some pathways for Fe^{2+} transport have been identified, such as the Feo or EfeUOB systems. The former is widely distributed among bacteria and some archaea. It is encoded most of the time as an *feoAB* operon in which FeoB encodes an Fe^{2+} permease and FeoA, a cytoplasmic partner of FeoB with a function that remains to be elucidated. FeoA seems to be a multifunctional protein that is essential in some species for Fe^{2+} transport, while in others it is only required for full efficient Fe^{2+} transport. An additional FeoA partner can occur, as well as a third element FeoC that has also an elusive role. It is presumed to be a transcription factor that regulates *feoABC* operon expression but also to protect FeoB from proteolysis. FeoA, FeoB, and FeoC were shown to interact together in *Vibrio cholerae*. Multiple Feo systems in the same bacteria have been described, and this situation can lead to specialization of each system such as general cellular needs vs. magnetosome needs and iron import vs. manganese import (hence the appellation Feo vs. Meo) (Hantke 2003; Cartron et al. 2006; Perry et al. 2007; Lau et al. 2013, 2016; Stevenson et al. 2016).

The Efe system has also shown an involvement in ferrous iron transport. First identified in pathogenic bacteria, the Efe system is actually widespread. This system

is expressed as an operon, *efeUOB* or *efeUOBM*, and consists of a permease EfeU that is homologue to the yeast Ftr1p iron permease, a Dyp-type peroxydase EfeB that is presumed to be periplasmic and to bind heme, and a periplasmic cupredoxin-containing EfeO. A cupredoxin-less EfeO homologue can occur and is named EfeM. Mechanism of this pathway is not yet clear as it differs from one organism to another, sometimes enabling the uptake of Fe^{3+} or heme iron in addition to Fe^{2+} (see Zappa and Bauer (2013b) for references) (Turlin et al. 2013; Miethke et al. 2013). In the *Rhodobacter* genus, a $FeoA_1A_2BC$ cassette was identified in the three representatives *R. capsulatus*, *R. sphaeroides*, and *R. ferrooxidans*. A second and simpler FeoAB cassette was also found in the *R. capsulatus* genome. While the $FeoA_1A_2BC$ cassette is likely to come from a common *Rhodobacter* ancestor, the FeoAB one could have been acquired later by gene duplication or horizontal gene transfer. The other Fe^{2+} uptake pathway, EfeUOB, is present in the genome of *R. capsulatus* but absent in the two other *Rhodobacter* species (Zappa and Bauer 2013b). In this cassette, EfeU and EfeO appear encoded as a fusion EfeUO protein according to the genome sequence. But manual sequencing finally established that those two partners as distinct genes (Zappa and Bauer 2013a). The Feo system of *R. sphaeroides* was not transcriptionally induced under iron limitation, while both *R. capsulatus* Feo systems were upregulated in the absence of iron (Peuser et al. 2012; Zappa and Bauer 2013a). In addition, transcription of *R. capsulatus efeU* was shown to be induced under iron scarcity (Zappa and Bauer 2013a).

In marine cyanobacteria, the Feo system seems absent from the genomes of *Prochlorococcus* and open-ocean *Synechococcus* species while present in coastal *Synechococcus* species (Morrissey and Bowler 2012). In the freshwater cyanobacterium *Synechocystis* sp. PCC 6803, a *feoB* gene was reported, and its transcription was showed to be induced by either low iron concentrations or ROS in the growth medium (Katoh et al. 2001a; Latifi et al. 2005; Shcolnick et al. 2009). Likewise, a FeoB homologue in *Microcystis aeruginosa* was found to be upregulated under iron limitation (Alexova et al. 2011). In *Anabaena* sp. PCC 7120, *feoB* is only weakly induced by iron limitation, while all *feo* elements were activated under high iron (Stevanovic et al. 2013; Rudolf et al. 2015). The Feo system in this strain was proposed to be part of the immediate response to high iron at low cell density (Stevanovic et al. 2013). Interestingly, the Feo transporter is presumed to be associated downstream of a siderophore-independent Fe^{3+} reduction pathway (Kranzler et al. 2014).

Reductive Ferric Iron Uptake Pathway

Recent observations about the capability of cyanobacteria to directly reduce inorganic Fe^{3+} to Fe^{2+} have questioned the paradigm that siderophores are the main route of iron uptake in oxygenic phototrophs. Indeed, evidences of extracellular ferric iron reduction by cyanobacteria were reported using *Synechocystis* sp. PCC 6803 (Kranzler et al. 2011; Thorne et al. 2015). In *Anabaena* sp. PCC 7120 and (most likely) *Anabaena flos-aquae*, reductive Fe^{3+} uptake was also shown to be uncoupled

from the schizokinen uptake (Sonier et al. 2012; Rudolf et al. 2015). Heavy energetic costs of siderophore synthesis and import are also inversely correlated to the cell density. Rudolf and co-authors proposed that, for filamentous bacteria like *Anabaena* sp. PCC 7120, siderophore-based iron uptake is cost-effective only under low iron and high cell density. On the other hand, Fe^{3+} reduction to Fe^{2+} followed by Fe^{2+} transport is more adapted to a less dense population. While the expensive siderophore system is tightly regulated, the iron reduction pathway seems to be poorly regulated and may thus constitute the "default mode" of iron uptake (Rudolf et al. 2015). Such a reductive pathway was proven to be prevalent in eight cyanobacterial species, where its "default mode" character was confirmed. Whatever species studied, reductive Fe^{3+} uptake is consistently 10,000-fold more efficient than the uptake of the siderophore ferrioxamine B (Lis et al. 2015). In addition, *Anabaena* sp. PCC 7120 imports inorganic Fe^{3+} by reducing it 1000 times more efficiently than its endogenously produced siderophore schizokinen (Lis et al. 2015).

A mechanism of Fe^{3+} reduction has been suggested to involve the aforementioned $FutA_1A_2BC$ system, an alternate respiratory terminal oxidase (ARTO), and the Feo system (Kranzler et al. 2014). According to this model (Fig. 2), Fe^{3+} could enter the periplasm through a porin where it is chelated by $FutA_2$. This accumulation of Fe^{3+}-$FutA_2$ complex would enable a gradient pushing Fe^{3+} toward the periplasm where Fe^{3+} would be reduced by ARTO with the resulting Fe^{2+} imported into the cytoplasm via the Feo system (Kranzler et al. 2014). This model suggests regulatory activities for $FutA_1$ and FutC and matches the previously described property of $FutA_2$ as a cellular iron partitioning protein (Waldron et al. 2007; Kranzler et al. 2014).

Finally, a few studies suggest the involvement of pili in Fe^{3+} transport in *Synechocystis* sp. PCC 6803. Type IV pili were previously described as "bacterial nanowires" as they can conduct electricity (Lovley and Malvankar 2015). In this cyanobacterium, a $\Delta pilA_1$ mutant showed impaired growth on iron oxides and goethite. It also displays some iron-deficient signatures such as lower phycobilisome contents (Lamb et al. 2014). Moreover, deletion of the transcription factor LexA induces a transcription response that is very similar to the one triggered by iron limitation: upregulation of iron transporters and downregulation of photosynthesis-related genes except *isiAB* (Kizawa et al. 2016). PilA-encoding genes, including $pilA_1$, were strongly affected by *lexA* deletion with some being directly controlled as shown by LexA binding to the promoters of $pilA_7$ and $pilA_9$ (Kizawa et al. 2016). As Lamb and co-authors reported only about the $\Delta pilA_1$ phenotype, it would seem worth at this point to extend the study to other *pilA* genes.

Heme Uptake

Many cells can also scavenge iron from heme often acquired from the environment. Cellular use of heme iron can occur via two mechanisms: import and cytoplasmic degradation of heme and periplasmic deferrochelation of heme. The first pathway is enabled by TonB-dependent heme uptake systems, similar to the ones involved in

siderophore uptake. Once heme is internalized in the cytoplasm, a heme oxygenase is required in order to open the tetrapyrrole ring and extract Fe^{2+}. This uptake system has been well characterized in pathogenic bacteria, but is not restricted to pathogens as this salvage pathway also benefits symbiotic and free-living marine bacteria (Nienaber et al. 2001; Hopkinson et al. 2008; Runyen-Janecky et al. 2010; Anzaldi and Skaar 2010; Braun and Hantke 2011; Septer et al. 2011; Burgsdorf et al. 2015). In the second pathway, the iron atom is extracted from heme in the periplasm without modifying the tetrapyrrole structure, releasing protoporphyrin IX. It is performed by the aforementioned EfeUOB system (Létoffé et al. 2009; Turlin et al. 2013). Genome analysis of *Rhodobacter* model strains revealed the presence of a complete TonB-dependent heme uptake system in *R. capsulatus*, HmuRSTUV, but not in *R. sphaeroides* or *R. ferrooxidans* (Zappa and Bauer 2013b). The heme uptake receptor was shown to be induced under iron-limiting conditions (Zappa and Bauer 2013a). In *R. sphaeroides*, a protein annotated as being involved in heme uptake was observed to be induced under iron limitation (Peuser et al. 2012). This protein shows relatively poor sequence conservation with HmuP, a regulator of the Hmu system in *Bradyrhizobium japonicum,* also poorly conserved in *R. capsulatus* (Zappa and Bauer 2013b). *Rh. palustris* genome also revealed the presence of a heme uptake system (Larimer et al. 2004). Finally, the other pathway for heme iron usage, EfeUOB, could be identified in *R. capsulatus* where it is upregulated under low concentration of iron (Zappa and Bauer 2013b, a).

In cyanobacteria, studies of heme uptake are even more in their infancy. In an effort to characterize the occurrence and diversity of TonB-dependent transporters, putative heme uptake systems were identified, the cyanobacteria *Acaryochloris marina*, *Anabaena* sp. PCC 7120, and three strains of *Synechococcus* (Mirus et al. 2009). These are based on sequence analysis and will require experimental confirmation. Interestingly, a TonB-based heme uptake pathway that was identified to be relatively well distributed in marine bacteria was found totally absent from the marine cyanobacterial genomes (Hopkinson et al. 2008).

Elemental Iron Storage

Storage of iron can be achieved using three types of proteins: ferritins (Ftn), bacterioferritins (Bfr), and *D*NA-binding *p*roteins from *s*tarved cells (DPSs). These are homo-oligomers of 24- or 12-mers that form a protective ball of protein around amorphous iron and inorganic phosphate or ferrihydrite. Di-iron centers enable the oxidation of cytoplasmic Fe^{2+} into insoluble Fe^{3+} for storage when it is translocated inside the complex, while heme groups reduce the stored Fe^{3+} to mobilize it and export it as soluble Fe^{2+} to the cytoplasm (Andrews 2010a, b; Bou-Abdallah 2010).

As heme-containing proteins, bacterioferritins are considered a member of the cytochrome class of proteins. Before genome sequencing and sequence analysis could enable their quick identification, they were often isolated and named as cytochromes (cytochrome b_{558} in *R. sphaeroides*, cytochrome b_{557} in *R. capsulatus*). By keeping the iron away from cellular machinery, bacterioferritins add a detoxification property to their storage function. By accumulating and releasing iron upon cellular needs, Ftn,

Bfr, and DPS are considered as massive cytochrome complexes forming small organelles. While Ftn and Bfr seem to be involved in genuine iron storage, DPSs are usually associated with DNA providing protection from oxidative stress. The Ftn, Bfr, and DPS superfamily of proteins has been well documented (Andrews 2010a, b; Bou-Abdallah 2010).

A Bfr was first observed in 1962 in *R. sphaeroides* and isolated 23 years later as cytochrome b_{558}, a year after the isolation of a Bfr homologue in *Rhodospirillum rubrum* (Meyer and Cusanovich 1985). It contains one heme per two subunits. Genome analysis proved that *R. sphaeroides* has actually two Bfr-encoding genes in addition to a membrane-bound Ftn (Mbfa). *R. capsulatus* has only one Bfr and one Mbfa, while *R. ferrooxidans* has only one Mbfa. In addition, recent studies in *Bradyrhizobium japonicum* and *Agrobacterium tumefaciens* showed that Mbfa functions as an iron efflux pump (Bhubhanil et al. 2014; Sankari and O'Brian 2014). Pumping excess iron out of the cell is definitely a good way to regulate iron equilibrium. All three *Rhodobacter* representatives exhibit an Mbfa sequence, which can be a signature of an ancestral feature from iron-rich environments that was retained for toxicity issue. None of the three *Rhodobacter* species exhibit a DPS-encoding gene (Zappa and Bauer 2013b). As DPSs were characterized as oxidative stress defense instead of iron storage units, it might be interesting to see if the absence of DPS is a trait of anoxygenic photosynthetic bacteria that evolved in more reducing environments. One of the *R. capsulatus* Bfr has been crystallized and extensively studied (Cobessi et al. 2002). This protein consists of a cytoplasmic 24-subunit complex containing 900–1000 Fe atoms and 600 phosphate molecules per Bfr complex. The cellular content of *R. capsulatus* Bfr decreased in the absence of iron and increased when iron was added back to the growth medium (Ringeling et al. 1994). However, the transcription of the Bfr gene did not show much changes depending on iron availability, suggesting posttranscriptional regulation of Bfr in this organism (Zappa and Bauer 2013a). A Bfr homologue from *R. sphaeroides*, Bfr1 (orf1546), is associated with a ferredoxin and is transcriptionally upregulated when iron is scarce. Transcription of an additional Bfr2 (orf3342) gene also was insensitive to iron, while Mbfa appeared only weakly iron regulated (Rodionov et al. 2006; Peuser et al. 2011, 2012).

In oxygenic phototrophs, the Ftn family is well studied. For example, in *Synechocystis* sp. PCC 6803, two Bfr's, BfrA and BfrB, sequester 50% of the total cellular iron content. Both Bfr's are needed for optimal growth as single or double mutants have reduced iron content, impaired growth characteristics, increased amount of IsiA (a hallmark of cyanobacterial response to iron deficiency), and reduced PSI content. Interestingly, BfrA has a di-iron center, while BfrB shows the sequence signature of a heme-binding site. Thus, it is suggested that BfrA and BfrB form an active complex by interacting as a heteroligomer that can take advantage of both a di-iron center and heme (Keren et al. 2004). The transcription of the Bfr-encoding genes does not vary much upon either iron availability or oxidative stress changes (Shcolnick et al. 2009).

In addition to the BfrAB complex, *Synechocystis* sp. PCC 6803 has a DPS representative, MrgA, that appears to provide a link between iron homeostasis and oxidative stress. Unlike BfrAB, MrgA does not seem to exert major iron storage function

(Shcolnick et al. 2007). But it is to provide crucial resistance to H_2O_2, especially during the reallocation of iron between its storage in Bfr and its target use as a cofactor. Moreover, MgrA was shown to be regulated by the oxidative stress regulator PerR (Shcolnick et al. 2009). With the exception of *Prochlorococcus* species that harbor Ftn, Bfr, and DPS, most cyanobacteria contain one or two Bfr and multiple DPSs (Morrissey and Bowler 2012; Ekman et al. 2014) (Thompson et al. 2011). The multicellular heterocyst *Nostoc punctiforme* harbors four DPSs and one Bfr with one of the DPSs transcriptionally activated under oxidative stress and a mutant in the corresponding gene unable to grow on H_2O_2. This DPS is therefor thought to be involved in protection from oxidative stress. Still in *Nostoc punctiforme*, the Bfr representative is found primarily in heterocysts where it may help to provide the high levels of iron needed for N_2 fixation (Ekman et al. 2014). A DPS representative, DpsA, was also studied in *Synechococcus* sp. PCC 7942 that was shown to be a DNA-binding hemoprotein localized associated with the thylakoid membrane. The mRNA level of DpsA was relatively high under normal iron levels and increased in iron-limiting conditions, with various degrees depending on studies (Dwivedi et al. 1997; Durham and Bullerjahn 2002; Michel et al. 2003). Deletion of DpsA seemed to affect severely PSII but also increases cellular sensitivity to oxidative stress (high light, paraquat), while PSI remains at normal activity. Overall, DpsA appeared as a cellular protector of oxidative stress induced by oxygenic photosynthesis. Nevertheless, it was also identified as part of the iron homeostasis components as the *dpsA* mutant showed some of the typical marks of iron deficiency such as altered transcription of the *isiAB* operon and amount of PSI-IsiA super complex. Consequently, DpsA in this species is thought to have a dual role of iron storage and oxidative stress (Dwivedi et al. 1997; Durham and Bullerjahn 2002; Michel et al. 2003). A DpsA homologue was also identified in *Anabaena* sp. PCC 7120. However, one cannot conclude yet whether DpsA acts as an iron storage protein or an oxidative stress detoxifier or both in this species (Hernández et al. 2007). Two DPS homologues were also found in *Thermosynechococcus elongatus*, DpsA-Te and Dps-Te with DpsA-Te exhibiting unusual properties such as the presence of two Zn^{2+} at the ferroxidase center and the use of O_2 to oxidize Fe^{2+} with an efficiency that compares to the classically used H_2O_2 (Alaleona et al. 2010). Finally, in *Fremyella diplosiphon*, five out of six ferritins or ferritin-like proteins were observed to be transcriptionally downregulated under iron limitation (Pattanaik et al. 2014).

Transcription Regulation of Iron Homeostasis

The regulation of cellular iron homeostasis involves both the control of activity of iron export and import transporters and the expression of iron importers and exporters encoding genes. An active area of research has established that the regulation of genes involved in iron transport and sequestration is complex and involves network of regulatory proteins. Some of these regulatory proteins are involved in the regulation of a specific iron homeostasis gene cluster and are thus termed “local regulators.” Others control a wide array of iron homeostasis

genes and are thus "global regulators." Local regulators can be AraC type, two-component systems, extracytoplasmic function (ECF) sigma factors, LysR type, and small RNA (see Zappa and Bauer (2013b) for references). Unlike global iron regulators, local transcription regulator dealing with iron homeostasis has not been studied in phototrophs. Anecdotally, genome analysis of *R. capsulatus* showed four AraC-like transcription factors located next to siderophore uptake gene clusters (Rodionov et al. 2006). In *Rh. palustris,* seven genes encode ECF sigma factors which are located in the vicinity of siderophore uptake system, siderophore synthesis, or putative heme uptake gene clusters (Larimer et al. 2004). Below is a discussion about known iron regulatory factors.

The Ferric Uptake Regulator (FUR)

For several decades Fur has been considered the hallmark of bacterial iron homeostasis regulation with detailed reviews on its activity previously covered (Rudolph et al. 2006; Lee and Helmann 2007; Carpenter et al. 2009; Fillat 2014; Frawley and Fang 2014). Homologues of Fur have been identified in a wide range of bacteria where it acts as a repressor of iron acquisition gene expression under iron-replete conditions. Fur directly binds Fe^{2+} to form an Fe^{2+}-Fur holoprotein that interacts with target DNA promoters. This basic mechanism has been extended since the first Fur studies as it is now known that apo-Fur can also activate gene expression. Holo-Fur can also control the transcription of a small RNA that subsequently regulates expression of iron homeostasis genes which is an additional layer of indirect control.

Bacteria of the *Rhizobiales* and *Rhodobacterales* orders also use an alternative related global regulator of iron homeostasis called Irr that appears to be a functional shift of Fur. In these organisms Fur seems to act as a manganese uptake regulator and is thus renamed Mur (Rudolph et al. 2006; Rodionov et al. 2006; Johnston et al. 2007; O'Brian 2015). As a result, among photosynthetic bacteria, the Fur/Mur regulator is presumed to have different functions whether it is a purple bacteria representative or a cyanobacterial one. In addition, Fur is a member of a larger family of regulators that encompasses more than Fur sensu stricto, Mur, and Irr. Other regulators in this family included Zur, Nur, and PerR that sense zinc, nickel, and peroxide, respectively (Fillat 2014). Substrate selectivity of the active site can be rather subtle since a single Glu to Asp substitution was shown to induce a transition of the iron sensing of Fur to the peroxide activity of PerR (Parent et al. 2013).

Fur/Mur in Purple Bacteria

In the *Rhodobacter* genus, a *fur* gene was identified in *R. sphaeroides* and *R. ferrooxidans*, but not in *R. capsulatus* (Zappa and Bauer 2013b). In *R. sphaeroides*, a deletion of *fur* induces stronger growth impairment under manganese than iron

limitation. A putative Mn/Fe uptake system, SitABCD, was shown to be controlled by Fur, so Fur might be involved in manganese homeostasis rather than iron and as such should be renamed Mur (Peuser et al. 2011).

Fur in Cyanobacteria

Many cyanobacterial genomes such as *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7002, *Nostoc punctiforme*, *Anabaena* sp. PCC 7120, and *Microcystis aeruginosa* typically have three Fur-encoding genes, often named *furA*, *furB*, and *furC* or *fur*, *zur*, and *perR* (Hernández et al. 2004a; Shcolnick et al. 2009; Alexova et al. 2011; Ekman et al. 2014; Yingping et al. 2014). Nevertheless, only two Fur homologues can be found in *Prochlorococcus*, while *Acaryochloris marina* MBIC1107 has 13 (Thompson et al. 2011; Hernández-Prieto et al. 2012; Ludwig et al. 2015). Cyanobacterial *furA* seems to be an essential gene as all attempts to inactivate it have failed in all species studied (*Synechococcus elongatus* PCC 7942, *Synechococcus* sp. PCC 7002, *Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120) (Ghassemian and Straus 1996; Michel et al. 2001; Kunert et al. 2003; Hernández et al. 2006; López-Gomollón et al. 2006; Ludwig et al. 2015). A recent approach in *Anabaena* sp. PCC 7120, consisting in putting *furA* under the control of a Co^{2+}/Zn^{2+}-inducible promoter, confirmed that turning off *furA* expression shuts down bacterial growth (González et al. 2016). The FurA homologue of *Anabaena* sp. PCC 7120 is by far the most well-characterized cyanobacterial Fur protein. As such, most of the following information deals with this Fur representative, although elements from other Fur homologues will be discussed. A summary of current knowledge about *Anabaena* sp. PCC 7120 FurA is presented in Fig. 3.

Biochemistry of FurA

The *Anabaena* sp. PCC 7120 FurA was originally identified by the signature sequence HHXHXXCXXC (Bes et al. 2001). Biochemical characterization showed that Mn^{2+} and DTT, while not necessary, strongly enhance the DNA-binding activity of FurA, while H_2O_2 inhibits DNA binding. Inactivation by H_2O_2 can be reversed by the addition of DTT (Hernández et al. 2005). In addition, FurA binds to heme with an affinity in the μM range with the formation of this complex preventing DNA binding. Based on the presence of a Cys-Pro motif, where the Cys is an axial ligand of heme, these data suggest that FurA may be a heme sensor involved in the response to oxidative stress (Hernández et al. 2004b; Pellicer et al. 2012). Interestingly, this Cys-Pro motif is present in all cyanobacterial homologues but absent in non-cyanobacterial ones. Heme could thus be involved in sensing redox variations within a cyanobacterial filament, from the microaerophilic environment of heterocysts to the reduced vegetative cells performing photosynthesis. Unlike classic Fur proteins, no structural Zn^{2+} was found in FurA. Oligomerization was found to increase with the increase of FurA concentration or with ionic strength, but to

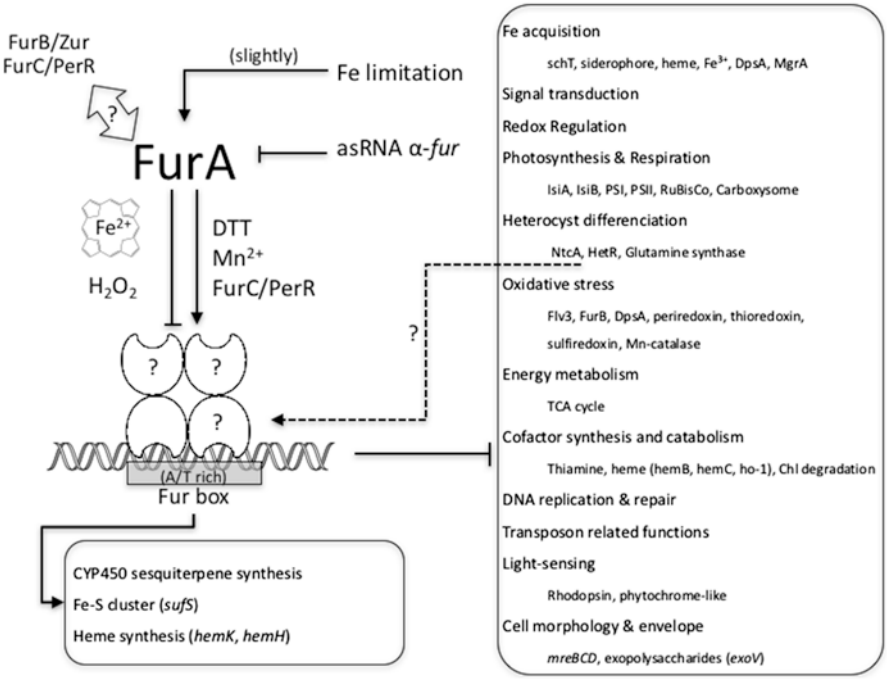

Fig. 3 The ferric uptake regulator FurA, in *Anabaena* sp. PCC 7120, is a global regulator. Represented are functions/genes where direct binding of FurA has been documented. FurA is primarily a repressor although few direct activation candidates were reported. Oligomerization differs between in vitro and in vivo studies

decrease in reducing conditions (Hernández et al. 2002). The FurA monomer has a diameter of 4 nm ± 1 with approximately 40% of α-helices and buried polar/charged residues. Dimerization seems to occur via hydrophobic interactions, which is consistent with the ionic strength effect, but disulfide bonds are observed in the trimer. Oxidizing conditions seem to disrupt the dimer form, which reassembles into trimers/tetramers. The odd number of cysteine residues suggests potential intermolecular bond formation and redox sensing property (Hernández et al. 2002, 2005; Lostao et al. 2010). Nevertheless, in vivo analysis shows that FurA is present mostly as a monomer with usually two but sometimes only one disulfide bond located at its two CXXC motifs and no intermolecular bond. Moreover, these two motifs were found to exert a disulfide reductase activity that has never been described in the Fur family and may extend its involvement in cellular processes such as redox signaling (Botello-Morte et al. 2014). Interaction with DNA occurs sequentially with first the binding by FurA monomer to the site 1 of the FurA-binding sequence, known as the "iron box." This introduces a 55 ± 14 bend in the DNA, which gives more exposure to the second site of the "iron box." From there, a second FurA monomer can bind to site 2 and form a dimer that releases the DNA bend. Or, a second FurA subunit can form a dimer with first FurA monomer without binding to site 2, which will

conserve the DNA bend. From either route, tri- or tetra-merization can occur thereafter (Pallarés et al. 2014). Finally, the "iron box" is not well defined, and, for now, it seems only safe to say that FurA binds to particular structures of A/T-rich DNA sequences of 19–23 bp (González et al. 2011, 2014).

FurA Regulation and the FurA Regulon

The *Anabaena* sp. *furA* gene was found to be expressed under iron-deplete or iron-replete conditions with only a slight increase in iron-limiting conditions (Hernández et al. 2002). A *furA cis* antisense RNA was also observed, consisting of the transcript of the nearby ORF alr1690 with the *α-furA* and the intergenic region. Deletion of this alr1690-*α-furA* region increased the level of FurA, highlighting a posttranscriptional regulation of FurA. Interestingly, the gene organization consisting of tail-to-tail *furA* and alr1690 is conserved in other cyanobacterial genomes such as *Nostoc punctiforme* and *Anabaena variabilis* (Hernández et al. 2006). In addition, regulation of *fur* by an antisense RNA has been observed also in *Microcystis aeruginosa* PCC 7806 and *Synechocystis* sp. PCC 6803, in a genomic context that is different from *Anabaena* sp. PCC 7120 (Sevilla et al. 2011). At the protein level, iron starvation was shown to downregulate FurA in *Synechocystis* sp. PCC 6803 (Vuorijoki et al. 2016).

An in silico analysis predicted that 215 proteins could be control by FurA in *Anabaena* sp. PCC 7120, which represent as much as 3.4% of the total ORF. These targets are scattered in different functional groups such as iron acquisition systems, signal transduction, redox regulation, photosynthesis and respiration, heterocyst differentiation, oxidative stress defenses, energy metabolism, fatty acid metabolism, synthesis of amino acids, cofactors and cell envelope, DNA replication, and repair- and transposon-related functions (González et al. 2014). Recent transcriptome analysis showed that 2089 genes display significantly different expressions when *furA* is turned off, consisting in 94 upregulations and 1595 downregulations which is inconsistent with the primary function of Fur as a repressor and reflects pleiotropic changes (González et al. 2016). These genes belong mostly to two functional groups—regulation and transport across membrane—and encompass processes such as iron homeostasis, photosynthesis, detoxification, light sensing, exopolysaccharide synthesis, chlorophyll catabolism, and transposons (González et al. 2016). In addition, a growing amount of target genes have been studied in vivo and/or in vitro. Moreover, as a *furA*-less strain of cannot be obtained, the regulon of FurA was mostly studied by overexpressing it. This was achieved by disrupting the aforementioned alr1690-*α-furA* RNA or by placing an extra-copy of *furA* under an inducible promoter. Finally, it is important to mention that, by disrupting alr1690-*α-furA*, one cannot discriminate the downstream effects of FurA overexpression from the alr1690 deletion. Another and more recent approach was to put *furA* under a Co^{2+}/Zn^{2+}-inducible promoter (González et al. 2016). But

whatever approach used, some trends could be revealed, sometimes highlighted by in vitro DNA-binding assay.

Increase of Fur through disruption of alr1690-*α-furA* revealed a wide array of effects such as a lower growth rate, lower chlorophyll and phycobiliprotein content, and lower cellular iron content but also modified ultrastructures (cell wall, thylakoid number and arrangement, shorter filaments, cell size, carboxysome number) (Hernández et al. 2010). All of this highlights signs of both iron-specific and generalized stress-generating pleiotropic responses when FurA is overexpressed. Such morphological alterations were also observed with the promoter-based FurA-overexpressing approach, as seen by shorter filament thylakoid distribution, although the pigment content did not decrease in this context (González et al. 2010). Among genes that could be involved in these morphological changes, the bacterial actins MreB and MreC were found upregulated in the FurA-overexpressing background. This was confirmed biochemically with FurA binding the upstream region of *mreBCD* in vitro (González et al. 2010). In addition, pleiotropic effects appeared to result from FurA binding to the promoters of genes involved in DNA replication, TCA cycle, signal transduction, and thiamine biosynthesis (González et al. 2011). In *Microcystis aeruginosa* PCC 7806, several genes involved in the synthesis of microcystins showed the presence of "iron boxes." The production of these toxins was found triggered by low iron level, and FurA was found to bind the promoter of these genes (Martin-Luna et al. 2006; Alexova et al. 2011).

FurA and Iron Homeostasis

While complete segregation of *furA*-deleted chromosome could not be achieved, heteroallelic mutants in *Synechococcus* sp. PCC 7942 were shown to exhibit iron deficiency signature symptoms such as the constitutive production of hydroxamate siderophores and flavodoxin (Ghassemian and Straus 1996). Similar approach in *Synechococcus* sp. PCC 7002 also established Fur as a repressor of iron uptake, involved in the iron-sparing response (Ludwig et al. 2015). This highlights the involvement of FurA in iron homeostasis. Moreover, in *Anabaena* sp. PCC 7120 overexpression of FurA triggered by alr1690-*α-furA* deletion was showed to lower the cellular iron content (Hernández et al. 2010). The schizokinen siderophore transporter SchT was found upregulated in the FurA-overexpressing background. Direct control is strongly suggested by FurA binding to the upstream region of *schT* (González et al. 2010). Putative transporters of siderophore, heme or Fe^{3+} ABC type, were observed to be under the direct regulation of FurA. The synthesis of siderophore is also part of the FurA regulon, as seen by a siderophore synthesis gene cluster that fails to be activated under iron limitation in the FurA overexpression strain. Moreover, four "iron boxes" were found in that cluster, and FurA binds to all of them in vitro with variable affinity (González et al. 2012). In addition, a gene coding for a ferritin family protein DpsA was identified in *Anabaena* sp. PCC 7120,

and its promoter displays an "iron box." FurA was shown to bind to that sequence, and the level of DpsA was reduced in the alr1690-*α-furA* mutant at both transcriptional and translational levels (Hernández et al. 2007). Still, regarding iron storage, another DPS, homologous to *Synechocystis* MrgA, was found under the direct control of FurA (González et al. 2014). Finally, FurA involvement in iron homeostasis can be further illustrated by the regulation of iron-containing enzyme and/or iron-containing cofactor synthesis (Fe-S, heme). For example, direct binding of FurA was established with the promoters of genes encoding NADH dehydrogenases (*all1127* and *alr0869-ndhF*), cytochrome *c* oxidase subunit II (*alr0950-coxB*), and flavodiiron protein Flv3 (*all3895-flv3*) (González et al. 2014). In addition, iron-containing enzymes can consist of Fe-S cluster-harboring enzymes. In *Microcystis aeruginosa* PCC 7806, an *α-fur* and a *fur-α-sufE* antisense RNA were identified. As SufE is involved in Fe-S cluster assembly, these data suggest a co-regulation of iron homeostasis and Fe-S cluster synthesis by antisense RNAs (Sevilla et al. 2011). Finally, a direct binding of FurA to the promoter of Alr2679 and Alr2680 was observed. These genes encode polyketide synthases potentially involved in siderophore or cyanotoxin synthesis (González et al. 2016).

FurA and Nitrogen Fixation

One fascinating property of FurA is the connection that it enables between iron homeostasis and nitrogen fixation. Nitrogen fixation is a very iron intensive process and is very sensitive to O_2. *Anabaena* sp. PCC 7120 copes with nitrogenase iron oxidation by spatial separation of oxygenic photosynthesis in vegetative cells from nitrogen fixation in heterocysts. Overexpression of FurA leads to partial arrested morphogenesis with cell differentiation blocked at the rather early stage of proheterocysts with more space between proheterocysts and phycobilisome degradation in proheterocysts. Heterocyst differentiation is also regulated by NtcA and HetR with FurA and NtcA found to regulate each other at the transcription level. These two regulators thus work in concert as a cellular development switch. Nitrogen was found to upregulate *furA* expression, while *furB* and *furC* were stable. In addition, NtcA was shown to activate FurA in proheterocysts and in heterocysts while repressing it in vegetative cells. Biochemically, NtcA was found to bind to the *furA* promoter and vice versa, and FurA showed DNA-binding activity with the *hetR* promoter. Moreover, integration of nitrogen into carbon metabolism is realized by the glutamine synthase gene *glnA* that is downregulated by decreasing either nitrogen or iron availability. NtcA and FurA can also both bind to the *glnA* promoter. Other actors of heterocyst differentiation proved to be controlled by FurA such as the DNA-binding protein Abp1, HetC, PatA, and Alr1728 with expression of Abp1 decreased in a FurA-overexpressing strain (López-Gomollón et al. 2007a, b; González et al. 2011, 2013, 2014). Recently, direct bindings of FurA were observed on the promoters of *ccbP* and *nblA*, encoding a Ca^{2+}-binding protein involved in heterocyst development and a phycobilisome degrading protein, respectively (González et al. 2016). The latter is known to be upregulated under nitrogen starvation.

FurA, Photosynthesis, and Respiration

FurA appeared in several studies as a regulator of both photosynthesis and respiration in cyanobacteria. In an alr1690-*α-furA* mutant, respiration and photosynthesis were both altered. On the one hand, respiration was found more efficient under lower than normal iron levels in the alr1690-*α-furA* strain, which is the opposite of WT cells. In that regard, the alr1690-*α-furA* strain copes better with low iron conditions than did WT cells. On the other hand, the alr1690-*α-furA* mutant also exhibited partial inhibition of the photosynthetic electron transport chain on the acceptor side of PSI and a decreased cyclic electron transport. It appeared that adaptation processes to perform photosynthesis under iron-limiting conditions are regulated more efficiently in WT cells than in the alr1690-*α-furA* mutant (Hernández et al. 2010). Influence of iron deficiency on photosynthetic electron chain and respiration was also observed in *Synechococcus* sp. PCC 7942 (Michel et al. 2003). In a FurA overexpression background, major changes in gene expression affected proteins of PSI (PsaA, PsaB) and PSII (PsbA, PsbB, PsbZ) reaction centers. Such changes are likely to impair the equilibrium between the photosystem components that is required for efficient photosynthesis. These consist in the increase of *psaA*, *psaB*, *psbA*, and *psbB* expression, while *psbZ* expression decreased. In addition, FurA was shown to bind the promoters of genes coding for NADH dehydrogenases, NAD(P) transhydrogenase, RuBisCo, IsiA, flavodoxin IsiB, the PSI PsaK subunit, the PSII reaction center protein D1 CP43 protein PsbC homologue, and the ß-carboxysome shell protein CccM (López-Gomollón et al. 2007a; González et al. 2010, 2011, 2014).

Interestingly, FurA and NtcA may connect "photosynthesis and respiration" to "iron homeostasis and nitrogen fixation." Indeed, multiple "iron boxes" were identified upstream NtcA-regulated genes and were confirmed to interact with FurA in vitro. Such genes include PSI subunit XI (*psaL*), PSII 11 kDa protein (*psbZ*), PSII chlorophyll-binding protein (*isiA*), cytochrome oxidase ($coxB_2$, $coxA_2$), and ferredoxin $NADP^+$ reductase (*petH*) (López-Gomollón et al. 2007a). In *Microcystis aeruginosa* PCC 7806, the expression of FurA appeared to require an intact photosynthetic electron chain (Martin-Luna et al. 2011). FurA was also identified as a regulator of tetrapyrrole synthesis and degradation. Overall, iron limitation altered the transcription of both heme synthesis and heme degradation genes. While FurA represses heme synthesis genes *hemB* and *hemC* and heme oxygenase gene *ho1*, it activates heme synthesis genes *hemK* and *hemH*. Direct DNA binding was observed with each of these promoters (González et al. 2012).

FurA, Oxidative Stress, and Redox Regulation

Cyanobacterial FurA was also showed to interact directly with the promoters of genes responding to redox stress, such as *dpsA* and *furB* (Hernández et al. 2007; López-Gomollón et al. 2009). Transcription of two peroxiredoxins and a thioredoxin reductase was also modified in FurA-overexpressing background with direct control by FurA interacting with the respective promoter regions (González et al.

2011). Moreover, FurA was showed to be a direct repressor of the flavodiiron Flv3 protein, the sulfiredoxin SrxA, and the Mn catalase, involved in oxidative stress defense (González et al. 2014, 2016). In addition, overexpression of FurA leads to a decrease in catalase and SOD activities, while cellular ROS levels were stable. At the transcription level, mRNA levels of two thiol peroxidases and the Fe-SOD were reduced, while transcripts of glutathione reductase and Mn-SOD increased. None of these genes showed direct control by FurA (González et al. 2010, 2014). In *Microcystis aeruginosa* PCC 7806, the expression of *fur* itself was found to be redox controlled. On the one hand, oxidative stress generated by blocking the photosynthetic electron chain at PSII (Q_B) decreased *fur* mRNA levels, while *α-fur* could not be detected. The same pattern was observed in darkness. On the other hand, photooxidative stress generated by excess of light (with intact electron flow) triggered an increase of *fur* mRNA. Exposure to H_2O_2 induced a decrease of *fur* mRNA along with an increase of *α-fur*. And the presence of superoxides using methyl viologen was found to increase *fur*, *α-fur*, and the Fur protein level (Martin-Luna et al. 2011).

What About FurB and FurC?

In *Anabaena* sp. PCC 7120, FurA, FurB, and FurC show overall poor sequence identity with each other besides exhibiting characteristic Fur signature sequences. Their respective antibodies also do not cross-react (Hernández et al. 2004a). FurB and FurC were subsequently identified as Zur and PerR homologues, respectively (Napolitano et al. 2012; Yingping et al. 2014). The former is zinc uptake regulator while the latter is an oxidative stress regulator. Interestingly, FurC seems to diverge from other phylogenetic Fur clusters in cyanobacteria (Ludwig et al. 2015). Detailed description of these regulators would be out of the scope of this review, but a few properties linking them to FurA activity are worth being mentioned. While FurA and FurB bind each of the three *fur* promoters, FurC does not bind to any. The DNA-binding activity of FurA is enhanced by Mn^{2+} and DTT and impaired by H_2O_2. On the other hand, FurB DNA-binding activity was improved in the absence of metal and the presence of DTT, while H_2O_2 had no influence. Finally, combinatory effects of the three Fur paralogues with each other were observed. For example, although not binding to any *fur* promoter, the presence of FurC seems to reduce DNA-binding activity of FurB while enhancing DNA-binding activity of FurA (Hernández et al. 2004a, 2005). Potential cross talk between Fur representatives within the same organism, by hetero-oligomerization, for example, has not been further studied. In *Anabaena* sp. PCC 7120, FurB and FurC were found downregulated in the FurA overexpression strain (González et al. 2010). In addition, *furA* is upregulated in the absence of FurB/Zur and consistently downregulated in a FurB/Zur-overexpressing environment (Sein-Echaluce et al. 2015). The reciprocal regulation of FurA and FurB/Zur and the occurrence of common target genes involved in the oxidative stress response raise the question of potentially compensatory roles (Sein-Echaluce et al. 2015).

Conclusions About FurA: Always More Complex

While Fur in *Synechococcus* sp. PCC 7002 seems to be confined to the iron homeostasis response, FurA in *Anabaena* sp. PCC 7120 is involved in a broad spectrum of cellular processes that go beyond typical iron homeostasis, encompassing photosynthesis, respiration, and nitrogen fixation (Ludwig et al. 2015; González et al. 2016). Following technological improvements, the characterization of FurA has been continuously expanding, and its regulon has been consequently growing. Lately, it has been showed to control transposon activity, Fe-S cluster (*sufS*), exopolysaccharide (*exoV*), and sesquiterpene (alr4686) biosynthesis (González et al. 2016). In addition, it is involved in light sensing process by regulating rhodopsin- and phytochrome-like proteins (Asr, AphC, Alr356) (González et al. 2014, 2016). Also, direct control on a lethal leaf spot-1 homologue questions the involvement of FurA in chlorophyll catabolism and programmed cell death (González et al. 2016). Besides an expanding regulon, the mechanism of action of FurA proved to be more complex. Indeed, recent studies showed that, while being primarily a repressor, it can also act as an activator. Two genes, a cysteine desulfurase-encoding *sufS* and a CYP450 sesquiterpene synthesis-encoding gene, were found to be upregulated in the presence of FurA. The dual role of direct repressor/activator has seldom been observed in Fur proteins (Fillat 2014; González et al. 2016). Indirect activation by Fur can occur through sRNA, while direct activation was shown only in a handful of organisms (Fillat 2014). Lastly, posttranslational regulation of Fur was demonstrated in *Synechocystis* sp. PCC 6803, where a heterocomplex of membrane proteases FtsH1/3 degrades apo-Fur in iron-deplete conditions. Doing so, it prevents apo-Fur to bind DNA with its residual DNA-binding efficiency. But apo-Fur degradation can also prevent it to scavenge traces of iron, reconstituting holo-Fur, and bind to DNA. Either way, it suppresses Fur repression under iron limitation and enables full expression of target genes (Krynická et al. 2014).

The Iron Response Regulator (IRR)

In *Rhizobiales* and *Rhodobacterales*, the function of Fur diverged from an iron to a manganese regulator. The master regulator of iron homeostasis in this group of organisms is iron response regulator (Irr), which senses the iron level as a function of intracellular heme status. Extensively studied in *Bradyrhizobium japonicum*, and to a lesser extent in other *Rhizobiales*, only a few data are available regarding photosynthetic bacteria (Rudolph et al. 2006; Small et al. 2009; Zappa and Bauer 2013b; O'Brian 2015).

The *Rhodobacter* genus shows a conserved Irr-encoding gene in the three model species *R. capsulatus*, *R. sphaeroides*, and *R. ferrooxidans* (Zappa and Bauer 2013b). In *R. sphaeroides*, deletion of *irr* induces a very moderate growth defect under iron-limiting conditions, although it appeared to control iron uptake, utiliza-

tion, and storage at the transcriptional level. In addition, Irr seems to increase the sensitivity to oxidative stress by repressing the catalase KatE. Irr was showed to be involved in the expression of the bacterioferritin Bfr1 (orf1546) and membrane-bound ferritin Mbfa. As mentioned earlier, the latter may actually be an iron efflux pump. Moreover, it was proven to be under the direct control of Irr, confirming in silico modeling. Also, a weak downregulation of a cytochrome *c* peroxidase was observed under iron limitation, and Irr does bind the upstream region of the corresponding gene *ccpA*. This direct regulation of a cytochrome is relevant given the heme-binding properties of Irr that were confirmed in this species (Rodionov et al. 2006; Peuser et al. 2012). In *R. capsulatus*, where in the absence of Fur/Mur, Irr could be expected to play a major role, deletion of *irr* did not change the phenotype with regard to iron availability, but no thorough characterization was undertaken (Zappa and Bauer 2013a).

PfsR: Enter a New Player

First discover in a high light-sensitive mutant of *Synechocystis* sp. PCC 6803, PfsR, is a transcription factor of the TetR family that is involved in the global response to both light and iron stresses, hence its name photosynthesis, Fe homeostasis, and stress response regulator (Jantaro et al. 2006; Cheng and He 2014). The Δ*pfsR* mutant is more tolerant to iron limitation than the wild-type strain where it accumulates more photosynthetic pigments (chlorophyll a, carotenoids, phycocyanins) and shows an attenuated decrease of the photosystems (Cheng and He 2014). While accumulating a mere 15% more iron than wild-type cells in iron-replete conditions, Δ*pfsR* retains 240% more iron when iron is depleted. Regarding protein content, Δ*pfsR* displays more PSI (PsaC, PsaD) and PSII (PsbA, PsbB) components but also more IsiA and cytochrome c_{550} than wild-type cells under iron-limiting conditions. Overall, this mutant has a higher photosynthetic rate and efficiency. Interestingly, under iron depletion, the transcription of *pfrR* increases but in a transient manner (Cheng and He 2014). Differential transcription patterns were observed between WT and Δ*pfsR*, especially concerning ferric (*futA*$_1$, *futB*, *futC*) and ferrous (*feoB*) iron transport, iron storage (*bfrA*, *bfrB*), heme oxygenase (*ho-1*, *ho-2*), iron regulation (*furA*), and iron stress response (*isiA*). Overall, PfsR acts as a repressor of these genes, but no direct binding on the promoters of these target genes was reported. However, PfsR does bind to its own promoter, so one might expect direct self-regulation and indirect regulation of the iron homeostasis genes (Cheng and He 2014). Interestingly, the high derepression of *isiA* (approximately 30-fold) in Δ*pfsR* is counterintuitive as high level of IsiA is a hallmark of iron starvation, but Δ*pfsR* seems to both ramp up *isiA* expression and thrive in iron-limiting conditions. In fact, the derepression of *isiA* may actually explain the better tolerance for iron scarcity (Cheng and He 2014). In summary, with PfsR controlling the expression of *furA*, the recent discovery of this new iron homeostasis regulator may put the FurA master regulation into new perspectives.

Copper and Iron Homeostasis

Excess copper can be toxic by displacing native metal ions from active sites, such as Fe-S clusters. Moreover, like iron, copper can generate dangerous reactive oxygen species in oxic conditions through Fenton chemistry (Osman and Cavet 2008; Macomber and Imlay 2009). Copper was also shown to inhibit heme synthesis at the Fe-S-containing coproporphyrin oxidase step resulting in decreased defense against oxidative stress (Djoko and McEwan 2013). In addition, copper is toxic to the photosynthetic machinery by inhibiting PSII, altering the thylakoid membrane, and substituting Mg in Chl (Bhargava et al. 2008). In this section, highlights will be given on how excess copper can interfere with key components of photosynthesis: iron homeostasis, tetrapyrrole, and cytochrome synthesis.

Copper and the Repression of Siderophore Uptake

Most of the experimental characterization of cross talk between iron and copper homeostasis has been studied in *Anabaena* sp. PCC 7120. The siderophore schizokinen is produced when iron is limiting but it was shown to also bind copper. While Fe^{3+}-schizokinen is imported to the cytoplasm, Cu^{2+}-schizokinen is maintained in the growth medium, thereby alleviating potential copper toxicity if imported in the cell (Clarke et al. 1987; Ferreira and Straus 1994). The production of siderophores usually massively exceeds the amount of bondable extracellular iron, which may reflect a distress strategy to maximize iron mobilization and avoid the deleterious effect of copper (Clarke et al. 1987). Indeed, it seems that copper homeostasis can be easily disturbed, as *Anabaena* sp. PCC 7120 cells grown under iron-deficient copper-sufficient conditions were showed to have reduced cellular iron content, while the copper content was almost fourfold higher (Nicolaisen et al. 2008). Also, hydroxamate siderophore synthesis was found triggered by either low iron or high copper levels. The transport of iron-schizokinen and the overall iron demand were found to be reduced in iron-deplete/copper-replete conditions compared to both metal deplete conditions (Nicolaisen et al. 2008, 2010). In addition, deletion of the schizokinen exporter SchE increases copper toxicity (Nicolaisen et al. 2010). While SchT was proven to be dedicated to schizokinen uptake, a second TonB-dependent transporter IacT appeared to be involved in citrate-based iron and copper transport with a less obvious mechanism. It is hypothesized that under iron-deplete/copper-deplete conditions, Fe^{3+}-schizokinen is imported by the SchT-Fhu system, while under iron-deplete/copper-replete conditions, then schizokinen binds to copper. This "neutralizes" copper from potential harmful effect, and, in order to fulfill the cellular requirements, the iron and copper transporter IacT takes over the SchT-Fhu system (Nicolaisen et al. 2010; Stevanovic et al. 2012). In addition, *Anabaena* sp. PCC 7120 has two copper transport systems of the CusBA type, one being induced at high copper levels and the other one repressed at high iron

levels. Interestingly, both are repressed when *iacT* is overexpressed (Nicolaisen et al. 2010). Overall, this model elegantly describes the dialogue between SchT and IatC, involved in Fe^{3+}-schizokinen and Fe^{3+}-citrate/Cu^{2+} import, respectively, as a function of extracellular Fe^{3+}/Cu^{2+} concentrations and equilibrium in *Anabaena* sp. PCC 7120. Although the switch is known to be iron/copper dependent, regulatory mechanisms have not been investigated.

But the iron/copper homeostasis interdependence is likely to involve more actors, even in the same organism. Indeed, other putative siderophore and heme uptake systems in the same organism were found expressed only under copper limitation, not iron limitation, in *Anabaena* sp. PCC 7120 (Mirus et al. 2009). Likewise, in the same organism a large gene cluster involved in siderophore synthesis was found to be responsive to copper (Jeanjean et al. 2008). Finally, in the process of identifying the Fe^{3+}-schizokinen import system (aforementioned SchT-Fhu-$TonB_3$-$ExbB_3D_3$), TonB-ExbBD-encoding genes were experimentally found to respond to copper levels (*exbB*$_1$*D*$_1$, *tonB*$_4$, *tonB*$_1$), as were ferric iron transporters (*futB*, *fecD*$_1$, *fecC*$_1$, *fecD*$_2$) (Stevanovic et al. 2012). A set of TBDT was found responsive to high iron/high copper levels with a high basal expression under iron limitation. These transporters may be essential for the copper detoxification response (*viuA*, *iutA*, *btuB*$_2$, *alr2185*, *all2148*, *schE*) (Stevanovic et al. 2013).

Copper Toxicity Issues

A recent example of copper toxicity was shown in anoxic conditions. While iron and copper are well known to be toxic in oxic conditions, where they can produce Fenton chemistry-induced reactive oxygen species, these two metals have been shown to exert synergic bacteriostatic effect in anaerobic condition, as shown on the purple bacteria *Rh. palustris* and *R. capsulatus*. While the mechanism is not clear, it seems that the presence of iron impairs some copper detoxification components (Bird et al. 2013). Moreover, copper has been shown to impair the synthesis of heme in *Neisseria gonorrhoeae* (Djoko and McEwan 2013). The same mechanism is likely to occur in phototrophic organisms in tetrapyrrole synthesis at steps performed by Fe-S-containing enzymes such as HemN and BchE (Bhargava et al. 2008; Hassani et al. 2010; Azzouzi et al. 2013). This highlights the importance of copper detoxification mechanisms to protect iron-based reactions.

Copper-Dependent Iron Transport

The aforementioned iron transporter EfeUOB system involves a cupredoxin-containing protein, EfeO. As such, with this transport system, fully functional import of iron actually relies on the presence of copper. Interestingly, EfeO shares similarities with the FET3/FTR1 transporter in yeast which is homologous to the

copper-dependent iron transporters in the eukaryotic phototroph *Chlamydomonas reinhardtii* (La Fontaine et al. 2002; Herbik et al. 2002; Terzulli and Kosman 2010). Being out of the scope of this review, no further details will be given, but it is worth mentioning that EfeUOB system was also identified in the purple non-sulfur bacterium *R. capsulatus* (Zappa and Bauer 2013b).

Copper and the Iron-Sparing Response

In cyanobacteria, two well-studied key copper-containing enzymes are plastocyanin and the caa_3-type cytochrome *c* oxidase. During photosynthesis, plastocyanin transfers electrons from the cytochrome b_6f complex to the PSI and copper limitation induces the use of cytochrome c_6 instead of plastocyanin. Iron limitation can lead to an unintuitive phenomenon from the release of siderophores that bind both iron and copper (Zhang et al. 1992; Ferreira and Straus 1994). Indeed, as copper-siderophores are not imported into the cell, copper availability is severely reduced. This generates copper limitation that represses plastocyanin in favor of cytochrome c_6. In summary, iron depletion induces the use of an iron-containing cytochrome instead of the iron-sparing plastocyanin. Despite looking inconsistent, this mechanism seems to perform well due to the fact that the cytochrome c_6 pool is restricted. Actually, some cyanobacterial species exhibit the cytochrome c_6 alone, having lost the plastocyanin gene. The cost of this imperfection does not outbalance the benefits of siderophores (Ferreira and Straus 1994). Moreover, deletion of cytochrome c_6 in *Synechocystis* sp. PCC 6803 does not result in much phenotypic change, even in copper-deficient conditions where cytochrome c_6 is supposedly required for effective photosynthesis. But interestingly, this mutant exhibited an elevated expression of *isiAB*, which is a hallmark of iron stress in cyanobacteria (Ardelean et al. 2002).

The cytochrome c_6-plastocyanin switch involves two copper transporters, a copper chaperone and also the periplasmic Fe^{3+}-binding protein $FutA_2$ (Tottey et al. 2001, 2002; Waldron et al. 2007). The $\Delta futA_2$ mutant is impaired in cytochrome c_6. As $FutA_2$ binds Fe^{3+} preferentially to Cu^{2+} and the $\Delta futA_2$ mutant shows abnormal distribution of metals in the cell, it was suggested that $FutA_2$ is involved in metal partitioning in the cell and failure to achieve the right cellular distribution of metals impairs biochemical switches (Waldron et al. 2007). Also illustrating this copper-iron dialogue is the sensitivity to iron starvation of the copper chaperone deleted strain (Tottey et al. 2002). In *Anabaena* sp. PCC 7120, *isiA* transcription was showed to be activated under copper limitation (Jeanjean et al. 2008).

Conclusion: Intertwined Metal Homeostasis

The occurrence of multiple iron transporters with overlapping functions exemplifies the competition for iron in the environment between bacterial populations. While siderophore-based iron uptake has been a long-lasting paradigm,

recent research established that the reduction of inorganic Fe^{3+} is likely to be the main route of iron intake in cyanobacteria. This feature is consistent with the occurrence of numerous cyanobacteria that have no identified siderophore synthesis systems, such as *Synechocystis*, *Prochlorococcus*, and *Synechococcus*, even though they require a lot of iron. Prevalence of this pathway tends to indicate a common origin, and its stable and high efficiency between species suggests that this pathway may have reached its maximum potential (Lis et al. 2015). Thus, competition between cell populations is dependent on other iron homeostasis features such as assuming the energetic costs of synthesizing and importing siderophores. Another strategy for dealing with iron competition is to reduce iron needs which has been achieved in eukaryotic phototrophs (Sunda and Huntsman 2015).

The mechanism of siderophore-based uptake is well detailed at least for schizokinen. A mechanism with regard to the reductive pathway has also emerged involving both $FutA_1A_2BC$ and Feo system, along with ARTO. Nevertheless, the ExbB-ExbD systems seem to be as important and spread across species as the reductive pathway. However, it is not known yet if these systems that are described as Fe^{3+} importers are actually importing Fe^{3+} or if the latter is reduced beforehand (Jiang et al. 2015).

The relatively restricted Fur regulon in *Synechococcus* sp. PCC 7002 as compared to the large and still expanding FurA regulon in *Anabaena* sp. PCC 7120 illustrates the diversity of cyanobacterial strategy to regulate fluctuation of environmental iron availability.

Iron is a source of oxidative stress, but its limitation also results in an unbalanced oxygenic photosystem that cannot deal with variation of light intensity. Moreover, due to heavy use of iron in photosystems, light-induced damages could trigger the release of iron in the cell, increasing oxidative stress. The recently characterized regulator PfsR seems to act at the crossroad of iron and light availability (Jantaro et al. 2006; Cheng and He 2014).

We also discussed the interconnection between the Fe and Cu networks in phototrophs. Strong evidences link Fe and Mn homeostasis in non-phototrophic bacteria (O'Brian 2015; Guan et al. 2015). A similar Fe/Mn dialogue seems to occur in photosynthetic bacteria, especially since Mn was reported to be involved in oxidative stress response under iron limitation (Kaushik et al. 2015). Both PSI and Mn_4CaO_5 cluster containing PSII are impaired under Mn limitation in *Synechocystis* sp. PCC 6803 (Salomon and Keren 2011). Importantly, *Synechocystis* sp. PCC 6803 cells acclimated to low Mn do not display the typical iron stress response (Salomon and Keren 2015). Since the used low Mn concentration is actually environmentally relevant, it put the several decade-old research on iron response into new perspectives. Likewise, the occurrence of three Fur representatives in cyanobacteria highlights the cross talk between iron and zinc homeostasis along with the oxidative stress response. The recent discovery of posttranslational regulation of Fur in *Synechocystis* sp. PCC 6803 by Zn^{2+}-containing protease (FtsH1/3) underlines the iron and zinc homeostasis interdependency (Krynická et al. 2014).

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