Stress signalling in Cyanobacteria: a mechanistic overview

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Abstract: Cyanobacteria are highly diverse, widely distributed photosynthetic bacteria inhabiting various environments ranging from deserts to the cryosphere. Throughout this range of niches, they have to cope with various stresses and kinds of deprivation which threaten their growth and viability. In order to adapt to these stresses and survive, they have developed several global adaptive responses which modulate the patterns of gene expression and the cellular functions at work. Sigma factors, two-component systems, transcriptional regulators and small regulatory RNAs acting either separately or collectively, for example, induce appropriate cyanobacterial stress responses. The aim of this review is to summarize our current knowledge about the diversity of the sensors and regulators involved in the perception and transduction of light, oxidative and thermal stresses and nutrient starvation responses. The studies discussed here point to the fact that various stresses affecting the photosynthetic capacity are transduced by common mechanisms.

Keywords: Cyanobacteria; Gene expression; Regulation; Signalling; Stress

1. Introduction

The domain of Bacteria includes an ancient, monophyletic phylum of organisms called Cyanobacteria which are able to undergo oxygenic photosynthesis. Their metabolism makes them leading players in the biosphere because of their impact on the global carbon and nitrogen cycles: they are thought to account for 30% of the global primary production, and in view of the N₂-fixing ability of some strains, they are held to be the main source of combined nitrogen in the marine environment. They therefore play an important role in the fields of agriculture, aquatic ecology and environmental protection. In addition, due to the great progress made in the field of genetic manipulations and the recent emergence of synthetic biology, Cyanobacteria are now being applied successfully in many biotechnological processes such as bioremediation, high-value secondary metabolite synthesis, and biofuel (including ethanol and hydrogen) production. From their early emergence up to the present day, cyanobacteria have succeeded in colonizing a wide range of aquatic to terrestrial ecological niches. This impressively wide pattern of distribution is due to their ability to cope with many kinds of starvation and stress, such as nutrient deprivation, light and temperature fluctuations, and oxidative, thermal and osmotic stresses. In response to environmental changes of various kinds, the ability to trigger and coordinate suitable adaptive mechanisms depends on the ability of these bacteria to rapidly sense the physical stimuli present and to appropriately transduce the signals perceived into gene expression modulation processes.

The cellular mechanisms developed by cyanobacteria for adapting to stress conditions have been studied in detail, in several strains. In addition to the publications included in this special issue, these aspects have been addressed in the following reviews [1-4]. The aim of the present review is to sum up the latest knowledge available on the perception and regulatory mechanisms involved in stress transduction in cyanobacteria. During the last decade, the development of DNA microarray technology and proteome analysis combined with systematic mutagenesis has made it possible to
identify several regulators involved in the stress response of the unicellular cyanobacterium model *Synechocystis* PCC 6803, as reviewed in [5]. Here we give an update of this knowledge and summarize recent progress in studies on stress regulation in other cyanobacterial strains. Among the mechanisms known to regulate gene expression, two-component systems, Sigma factors and regulatory RNAs have often been found to participate in stress transduction processes in Cyanobacteria. A general scheme of their function is presented in **Figure 1**.

**Figure 1:** Two-component systems, Sigma factors and small regulatory RNA.

**A: Two-component systems**

Two-component systems (TCSs), which occur widely in bacteria, play essential roles in signalling events. Most TCSs are composed of two proteins, a histidine kinase sensor and a response regulator. The sensor perceives specific signals via its sensor domain (pink), resulting in the activation of the kinase activity and the autophosphorylation of a conserved histidine residue in the transmitter domain (blue). In response, the phosphoryl group is transferred to a cognate response regulator. The phosphorylation occurs on a conserved aspartate residue present in the receiver domain (green). Most response regulators are transcription regulators that activate or repress gene transcription.
processes in response to the signals perceived. Upon being phosphorylated, their activity is either activated or inhibited.

B: Sigma factors

Sigma factors are key players in the initiation of transcription in prokaryotes. They associate with the catalytic core RNA polymerase, enabling it to ensure the essential steps in the process of initiation. In addition to the housekeeping sigma factor (Group 1), several alternative factors make it possible for the organisms to adapt to changes in the physiological and environmental conditions. In addition to the housekeeping sigma factor, Cyanobacteria contain various Group 2 sigma factors. They recognize the −35 (TTGACA) and -10 (TATAAT) sequences (indicated by the blue boxes) in a similar way to the Group 1 sigma factor without playing an essential role during the exponential growth phase [6].

C: Small regulatory RNAs

Small regulatory RNAs (rRNA) are 50-500 nucleotide non-coding, highly structured RNA molecules that affect gene regulation via several mechanisms. They are expressed either as an antisense to their target RNA (cis-expressed) or by a separate (trans expressed). Via their interactions with their target mRNAs, they exert either a positive or negative effect on the translation of the latter. They can also interact with proteins and regulate their activities (not shown in the figure).

2. Light stress:

Since Cyanobacteria use solar energy for their growth, the perception of light and the physiological changes that occur in response to light variations are the most important adaptative responses they have to orchestrate. They are equipped with light-absorbing antenna called phycobilisomes, which are part of the main systems of acclimation to light. In particular, several strains are able to vary the composition of their phycobilisomes to respond to changes in the quality of light, based on a mechanism known as complementary chromatic acclimation (CCA). In addition, motile strains use a process of phototaxis to move towards the optimal light conditions required for their photosynthesis. CCA and phototaxis are advantageous adaptative responses that do not induce any general stress responses. They will therefore not be discussed here (these topics have been reviewed in: [1,7-9]).

In addition to qualitative changes in light, Cyanobacteria can also be exposed in their environment to quantitative daily changes. Irradiances far above the light saturation level of the photosynthetic machinery are harmful as they induce photoinhibition and photo destruction of the photosystems. In addition, when challenged by high light (HL) stress, photosynthetic organisms generate reactive oxygen species which are deleterious to all the macromolecules in the organism (see below). Adaptive responses to HL and oxidative stresses are therefore tightly intricated, which makes it difficult to identify the signal transduction systems specific to each type of condition. Short term acclimation to strong light can be achieved by quenching excess light energy and redistributing between the photosystems the excitation energy required. By contrast, long term adaptation often requires a particular process of modulation of the patterns of gene expression. The transcription of a number of genes is induced in response to HL stress, and the promoters of several of these genes harbor a conserved tandem sequence known as the HL regulatory region (HLRR) [10-12]. The use of this sequence in DNA/protein interaction assays has been a decisive step towards identifying the regulatory mechanisms involved in the transmission of light stress. This is how the RpaB protein was found to be the master HL stress regulator in *Synechocystis* PCC 6803 (called *Synechocystis* hereafter) and *Synechococcus elongatus* PCC 7942 (called *Synechococcus* hereafter) [12-15]. The *rpaB* gene, which is largely conserved in cyanobacterial genomes, encodes a response regulator protein belonging to
the two-component systems. Genetic and biochemical experiments have shown that RpaB acts as a repressor of HL induced genes during growth under normal light [16-18]. In *Synechococcus* the histidine kinase NblS has been found to be the sensor partner of RpaB in the HL transduction signal [16]. NblS and its orthologue Hik33 (DpsA) in *Synechocystis* are the most highly conserved histidine kinases in the genomes of Cyanobacteria [19]. The role of NblS/Hik33 in the response to HL was actually established long before that of RpaB [20,21], but establishing the proof of its direct involvement in this response has been rather a tedious job due to the fact that this kinase is a pleiotropic regulator involved in the transduction of multiple stresses (see below). In *Synechococcus*, A second response regulator (SsrA) is phosphorylated by NblS. ssrA gene expression is induced by HL under the control of RpaB. Once it has been produced, SsrA might quench the phosphotransfer from NblS to RpaB [16,18]. In addition, the kinase activity of NblS and Hik33 have been found to be stimulated through their interactions with SipA (NblS-interacting protein A) [22]. As the sipA gene is also conserved in cyanobacterial genomes, SipA might contribute importantly to modulating the NblS control in vivo. Since the sipA gene is also conserved in cyanobacterial genomes, the data observed in *Synechocystis* and *Synechococcus* might well apply to other strains. The NblS pathway involved in HL control is therefore a hierarchical cascade where two response regulators cooperate, and in which the activity of the sensor is finely tuned to ensure optimal acclimation to HL in cyanobacteria.

Multiple rpoD genes encoding sigma factors have been identified in the genomes of cyanobacteria, and it has been suggested that many of them may act only under specific growth conditions [6]. In *Synechococcus*, the expression of the rpoD3 gene is induced in response to HL stress under the control of RpaB, and the rpoD3 deletion mutant is unable to survive this type of stress [15]. RpoD3 (as well as its orthologues in other Cyanobacteria) might therefore be the specific sigma factor enabling the polymerase to transcribe HL-induced genes.

In addition to the control process exerted at the initiation of transcription described above, the regulation of gene expression in response to HL also occurs at the translational level. In *Synechocystis*, a small regulatory RNA called PsrR1 (photosynthesis regulatory RNA1), which is conserved in the cyanobacterial genomes, has been found to be expressed in response to HL stress under the control of RpaB [23]. Based on computational and genetic findings, it has been established that PsrR1 regulates several photosynthetic genes negatively by interacting with their ribosome binding sites, and thus by inhibiting the translation of these genes [24]. The existence of this negative regulation process is consistent with the fact that phycobilisome and photosystem reduction is one of the main physiological processes of adaptation to HL [3]. A second non-coding RNA (RblR) accumulates under HL conditions in *Synechocystis*. RblR acts as an anti-sense RNA to the *rbcL* mRNA encoding the large subunit of the Rubisco. The idea that this RNA may enhance carbon assimilation has been suggested, based on the phenotypes of mutants strains over or down-expressing the *rblR* gene [25], but how exactly the regulation of the *rblR* transcription process induced in response to HL stress is achieved has not yet been established. In the marine cyanobacterium *Synechococcus* sp. WH7803, the expression of six non-coding RNA genes is regulated in response to HL, several of which possess photosynthesis genes as predictive targets [26]. All these data provide strong evidence that non-coding RNAs play an important role in regulation of genes involved in HL stress response. Elucidating the identity of all the non-coding RNA-target genes, the molecular mechanisms involved
and how they are integrated into the global process of acclimation to HL is certainly the most challenging perspective ahead of us in this field (Figure 2).

**Figure 2: Regulation of high light stress acclimation**

The response regulator RpaB represses gene transcription under normal light conditions. In response to high light (HL) stress, RpaB is phosphorylated, resulting in its inactivation and the subsequent induction of HL inducible genes. The Hik33/NblS (DspA) kinase is involved in the perception of HL stress. The putative phosphorylation of RpaB by Hik33 is thought to be inhibited by the SsrA protein. The *psrR1* gene belonging to the RpaB regulon expresses an rRNA that inhibits the translation of several genes under HL stress conditions.

3. **Oxidative stress:**

Reactive oxygen species (ROS) such as the singlet oxygen species (‘O₂), the hydroxyl radical (OH⁻), the superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂) are by-products of aerobic metabolism and in photosynthetic organisms, they are mainly produced when the intensity of the light collected by the photosystems is greater than the rate of electron consumption. Cyanobacteria, like all living organisms, have developed several defense systems which reduce and eliminate these ROS before they can react with biomolecules and oxidize them. The state of imbalance where the level of ROS exceeds the amount of antioxidant molecules is called oxidative stress [2,27,28]. Since no enzymatic defense mechanisms exist for these species, the cellular responses to singlet oxygen species and
hydroxyl radicals do not result in any modulation of gene expression. The regulation of oxidative stress therefore consists in the transduction of superoxide and peroxide signals.

Superoxide anions resulting from the photoreduction of oxygen can be converted into H₂O₂ by the metalloenzymes superoxide dismutases (SOD), which are thought to constitute the main antioxidant defense system against O₂⁻ [28]. In the multicellular cyanobacterium Nostoc (Anabaena) PCC 7120, the transcription of the sodB gene encoding FeSOD is under the direct negative control of the transcriptional factor CalA (Cyanobacterial AbrB like) [29]. AbrB homologues, which are present in all the cyanobacterial genomes [30], have been found to regulate several metabolic pathways such as the carbon fixation, nitrogen uptake and hydrogen oxidation pathways [31-35]. Since CalA is essential in Nostoc, obtaining a strain deleted from calA is not possible. The negative effect on the transcription of sodB was therefore analyzed by overexpressing the calA gene [29]. The question as to how CalA perceives the superoxide anion has not yet been answered. Interestingly, in Synechocystis, the transcription of the sodB gene was reported to be repressed by the transcriptional factor PrqR, and this control was found to be indirect [36]. If CalA also represses the transcription of sodB in Synechocystis, the regulatory scheme responsible for superoxide signalling may be based on the fact that PrqR perceives the signal and transduces it via CalA (Figure 3A).

Several studies have converged in designating PerR as the main specific regulator of the response to peroxide in Cyanobacteria [37-40]. PerR is a zinc-metalloprotein in which either Fe²⁺ or Mn²⁺ ligated to two conserved His and Asp residues act as corepressors. The activity of PerR is regulated by a metal-catalyzed oxidation (MCO) process. The metal present in the catalytic center reacts with peroxide in a Fenton-type chemistry which leads to the irreversible inactivation of the repressor and the induction of the target genes, including peroxidase and catalase encoding genes [41]. Based on structural modeling studies, it has been suggested that the binding of PerR to its target genes may occur via multimers of PerR-protein interacting with AT-rich repeats present in the promoters of the repressed genes [42]. The involvement of PerR in the repression of the transcription of peroxide-related genes in cyanobacteria has been described in Synechocystis and Nostoc, and metal oxidation has been found to contribute to the action of PerR in Nostoc [37]. In addition, the overexpression of PerR has been found to affect the composition and the stability of the photosynthetic machinery and the division process in Nostoc [43]. This link between PerR, photosynthesis and cell division might explain why perR is an essential gene in this strain.

In addition to the regulatory effects mentioned above, the response of Synechocystis to oxidative stress resulting from HL or peroxide treatment has also been found to rely on Group-2 sigma factors, namely SigB and SigD factors. A strain lacking all the Group-2 sigma factors was unable to sustain its growth when challenged by oxidative stress, although this ability was rescued by the introduction of the sigB or sigD gene. In addition, RNA polymerase holoenzyme associated with either SigB or SigD accumulate in response to peroxide stress [44]. The signalling of oxidative stress is therefore based on transcriptional regulators (CalA, PrqR, PerR) combined with the programming of the RNA polymerase with dedicated non-essential sigma factors (Figure 3B).
Figure 3: Regulation of oxidative stress responses

A: Superoxide anion stress

In the absence of stress, the PrqR sensor might phosphorylate the response regulator CalA, which therefore represses the gene transcription process. In the presence of superoxide, PrqR is inactivated by a hitherto unknown mechanism. The Group 2 sigma factors SigB and/or SigD associate(s) with the RNA polymerase, resulting in the initiation of the transcription process.

B: Peroxide stress

The metalloregulator PerR represses gene transcription in the absence of peroxide. Under oxidative conditions, metal catalyzed oxidation (MCO) inactivates PerR, thus inducing gene transcription.
4. Salt stress

Salinity, defined as the total inorganic ion concentration in the environment, is one of the main changing abiotic factors that Cyanobacteria have to cope with in both aquatic and terrestrial niches [4,45]. A high ion concentration in the medium results in an osmotic loss of water and a concomitant increase in some inorganic ions which can be deleterious to the macromolecules in the cell. By contrast, at low salinity levels, water largely flows into the cell, resulting in lysis due to high turgor pressure. Even if acclimation to changing salinity levels is of two kinds, depending on the salt concentration in the surrounding medium, salt stress nomenclature is often attributed to high salinity conditions. Cyanobacteria, like many other non-halophilic prokaryotes, respond to salt stress by accumulating small organic solutes (often in the form of sugars) and monitoring an active export of inorganic ions. This strategy has therefore been called "the salt-out-strategy". The organic solutes in question have a low molecular mass and do not interfere with the cell metabolism, which explains why they are referred to as compatible solutes. In cyanobacteria, the strain-specific salt tolerance level is correlated with the nature of the main compatible solute produced [46].

Many studies in which it was proposed to elucidate how *Synechocystis* adapt to salt stress (reviewed in [4,47,48]) have shown that the cellular responses involved are dynamic processes and that they occur at various levels, including the post-translational regulation of the activity of several transporters and enzymes, as well as a global change in the process of gene transcription, in which the molecular mechanisms involved are not totally known. The exceptions here are the regulation of the transcription of genes involved in the synthesis of the organic solutes produced by *Synechocystis*, namely sucrose and glycosylglycerol. The synthesis of sucrose involves two enzymes: the sucrose phosphate phosphatase (Spp) and the sucrose phosphate synthase (SpsA), which is the rate limiting enzyme. Upon exposure to salt stress, the activity of the SpsA enzyme and the transcription level of the *spsA* gene are both enhanced. Under normal salinity conditions, the expression of *spsA* is repressed by the Rre39 response regulator [48,49], but since this is an orphan regulator (i.e., the cognate histidine kinase has not yet been identified), the question as to how the "high salinity" signal is transduced to Rre39 has not yet been elucidated. The synthesis of glycosylglycerol is a two-step reaction involving the glycosylglycerol phosphate synthase (*GgpS*) and the glycosylglycerol phosphate phosphatase (*GgpP*) enzymes [50]. Under salt stress conditions, the production of glycosylglycerol is enhanced by the induction of *ggpS* gene transcription and by an increase in the activity of the GppS enzyme. The Group 2 sigma factor SigF seems to be the specific sigma factor responsible for *ggpS* transcription, as a *sigF* mutant is unable to adapt to salt stress and shows significantly low accumulation rates of *ggpS* transcripts [51,52]. Upstream of the *ggpS* gene, the small regulatory gene *ggpR*, has been identified, the deletion of which increases the *ggpS* mRNA levels under normal salinity conditions, which suggests that this gene may act as a repressor of *ggpS* expression [53]. As the GppR protein does not contain any typical DNA binding motifs, it is questionable whether GppR is acts after binding directly to the *ggpS* promoter. The *ggpR* gene is not conserved in any other cyanobacterial genomes, but interestingly, the synteny between *ggpS* and another small gene is observed in many genomes, which means that the possible involvement of the corresponding protein in the regulation of *ggpS* expression cannot be ruled out (Figure 4).

Sucrose is the only osmolyte produced in *Nostoc* in response to salt stress, and in addition to being a compatible solute, it is involved in the nitrogen fixation process [54,55]. The synthesis of sucrose involves two sucrose phosphate synthases (SpsA and SpsB) and a sucrose phosphate phosphatase...
Sucrose can also be formed by the reversible action of the synthases SusA and SusB but as they are thought to preferentially catalyze the degradation of sucrose in vivo, they will not be further discussed here [54]. The increase observed in the sucrose accumulation rates in response to salt is due to higher Spps activity and the induction of the transcription of spsA gene expression. Salt induction of spsA was abolished in a mutant strain lacking the response regulator OrrA, which indicates that this transcriptional regulator may play a positive role in the process of sucrose synthesis [56]. The orrA gene has been detected in a genetic screen set up for the identification of salt-induced genes in Nostoc [57]; the signalling pathway leading from salt perception to OrrA activation still remains to be determined (Figure 4).

In conclusion, the dynamics of compatible solute accumulation and the regulation of the enzymatic activities involved have both been thoroughly documented in Cyanobacteria, but this is far from being the case as far as the regulatory mechanisms involved in gene expression are concerned. Since the nature of the main solute(s) produced and the ability to adapt to high salt levels vary among Cyanobacteria, the possibility cannot be ruled out that the regulatory mechanisms involved may also differ from one strain to another.

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**Diagram**

- **Optimal salinity**
  - Sucrose synthesis: RpaB
  - Glycerolglycerol synthesis: GgpR

- **Salt stress**
  - Sucrose synthesis: ??
  - Glycerolglycerol synthesis: RNAP, Sigma

**Legend**

- **spsA**: Repression
- **spsA**: Induction
- **ggpS**: Repression
- **ggpS**: Induction
Figure 4: Regulation of salt stress responses

A: the transcription of the \textit{spsA} gene involved in the synthesis of sucrose is repressed by RpaB. The cognate sensor involved has not yet been identified.

B: the transcription of the \textit{ggpS} gene involved in the synthesis of glycosylglycerol is subjected to the negative control of GppR, but the underlying molecular mechanism has not yet been elucidated. Under salt stress conditions, the presence of the SigF sigma factor enables the RNA polymerase to initiate the transcription process.

5. Heat shock

The optimal growth temperatures for cyanobacteria cover a large range, as this phylum includes several strains inhabiting extreme environments, from hotspring to cryosphere environments [58]. However, with the exception of these extremophile members, most mesophilic strains are sensitive to temperature fluctuations and the processes of photosynthesis and nitrogen fixation are both inhibited by heat, for instance [59,60]; the molecular responses to heat shock are therefore crucial. Like many other organisms, Cyanobacteria induce the expression of heat-shock genes (\textit{hsp}) in response to temperature upshifts [60-63]. Many of the HSPs are molecular chaperones or proteases playing a major role in proteostasis, such as the Hsp60 members (mainly GroEL and GroES), which are the most abundant HSPs produced in cyanobacteria after the occurrence of temperature upshifts. In many bacteria, the \textit{hsps} gene promoters contain a highly conserved 9-bp inverted repeat sequence, which is required for the heat-induction process. This sequence, which has been called the Controlling Inverted Repeat of Chaperone Expression (CIRCE), is the binding site of the HcrA repressor, which is also highly conserved in many bacteria. HcrA is a dimeric transcriptional regulator that undergoes denaturation upon being subjected to temperature upshifts. The subsequent synthesis of GroEL, regenerates the HcrA dimer, thus restoring the repression of the \textit{hsps} genes. Chaperone activity therefore acts as the molecular heat shock sensor [64]. The \textit{hcrA} gene is widely conserved in the cyanobacterial genomes, and its role in the repression of \textit{hsps} genes expression has been documented in both \textit{Synechocystis} and \textit{Nostoc} [60,61,65]. In both strains, the deletion of the \textit{hcrA} gene results in the constitutive expression of \textit{hsps} genes, which is consistent with the idea that a negative control may be exerted by HcrA on heat shock genes. Microarray studies in which the global gene response of a \textit{Synechocystis} mutant strain deleted from the \textit{hcrA} gene was compared with that of the wild type strain have shown that HcrA might also regulate some genes devoid of the CIRCE element that do not belong to the Hsp60 family [61,65], which suggests that HcrA might be a global regulator of gene expression, or alternatively that the latter effect might be due to the constitutive expression of \textit{groEL} and \textit{groES} genes in this mutant. Interestingly, in both \textit{Synechocystis} and \textit{Nostoc}, the expression of some \textit{hsps} genes in the \textit{hcrA} deleted strain was not fully derepressed, since a small induction was still observed in response to a temperature upshift, which indicates that their expression is regulated by another mechanism in addition to HcrA [60,61] [60].

A fast response to temperature upshifts is ensured by the mechanism controlling the translation of some \textit{hsps} mRNA. This mechanism involves specific sequences located in the 5’ untranslated region of the mRNA, which change their conformation in response to heat shock. At low temperatures, the secondary structure they generate encompasses the ribosome binding site, which affects the translation efficiency. These riboregulators, which are known as thermometer RNAs (or
thermosensor RNAs) are present in many bacteria [66,67], including Cyanobacteria. The *hsp17* gene of *Synechocystis* harbors a rather small 5′ untranslated region which has been found to act like a typical thermosensor [66,68], and similar cis-acting riboregulatory RNAs have been identified in *hsp* genes in *Anabaena variabilis*, *Nostoc* and the thermophilic cyanobacterium *Thermosynechococcus elongatus* [69].

Another system of regulation of the *hsp* genes which occurs in response to heat shock consists in reprogramming the RNA polymerase core enzyme with the appropriate sigma factor. In *Synechocystis*, the sigma factors SigB and SigD play an important role in high-temperature responses; the growth of a double sigBsigD mutant is much more severely impaired at 43 degrees than that of the simple mutants [61,70]. Interestingly, a protein (SinA) interacting with the principal sigma factor (RpoD1) and playing a role in heat shock responses has been recently identified in *Synechococcus*. The RNA polymerase-RpoD-SinA complex was dissociated after a temperature upshift, a sinA-deletion mutant was unable to sustain its growth at 40°C, and the induction profile of *hspA* gene was affected in the mutant. All in all, these data point to the conclusion that SinA may play a role in the replacement of RpoD1 by the heat-stress specific sigma factor. The finding that homologs of SinA were present in 361 genomes out of the 367 analyzed suggests that the function of this protein may be widely conserved among the members of the cyanobacterial phylum [71] (Figure 5).

**Figure 5: Heat shock response regulation**

Under optimal growth conditions, the transcription of the *hsp* genes is repressed by HcrA, which binds to a conserved sequence called CIRCE. Upon undergoing heat shock, HcrA is inactivated and *hsp* transcription is induced. The GroEL chaperone facilitates the refolding of HcrA at the release of the stress, thus acting as the molecular sensor of the signal. Some of the *hsp* mRNAs carry an untranslated sequence forming a secondary (thermometer) structure sequestering the ribosome.
binding site under normal growth temperature conditions. Upon undergoing heat shock, this secondary structure dissociates, which makes it possible for the translation process to proceed.

6. Cold stress

Mesophilic bacteria are often challenged by temperature downshifts to below their optimum growth temperature, which results in a decrease in the fluidity of the cell membranes and in the efficiency of the transcription and translation processes due to the abnormal stabilization of secondary structures in the DNA and RNA. The activities of the ribosomes and those of protein foldases are also impaired [72]. Bacteria respond by inducing the production of proteins called CSPs (cold shock induced proteins) that serve to enhance the transcription and translation processes by acting on secondary nucleic structures; these are RNA binding proteins which affect the transcription and translation processes at low temperature via their RNA chaperoning function and RNA helicases which stimulate the degradation or translation of RNA at low temperatures. The second main response is the induction of desaturated fatty acids which counterbalance the loss of membrane fluidity. The expression of the csp genes, which has been most closely studied in *Escherichia coli*, is regulated at the transcription, mRNA stabilization, and translation levels. In *Nostoc*, the RNA-helicase encoding gene chrC is specifically induced in response to temperature downshifts, and its regulation was found to occur at several levels including the transcription, mRNA stability and translation levels, but the exact molecular mechanisms involved have not yet been determined [73]. The ribosomal protein S2 has also been found to be continuously phosphorylated in *Nostoc* during exposure to cold stress, resulting in down regulation of the translation process, with the exception of cold stress-induced mRNA [74]. In *Synechocystis*, the expression of about half of the cold-induced genes is controlled by the transmembrane histidine kinase Hik33 [75], and depends on the fluidity of the membrane [76]. Hik33 kinase also controls the responses of the genes to oxidative, osmotic and salt stress (see below), which suggests the possible existence of a common signal triggering gene induction in response to various stresses. It has been suggested that the oxidation status of the quinone pool, which was found to vary depending on the membrane fluidity during cold-stress exposure may be the common response signal to stressors affecting the membrane fluidity [77].

7. Nutrient starvation

Among the multiple environmental stresses that Cyanobacteria encounter, nutrient depletion is often a limiting growth factor for their growth. Like most bacterial species, Cyanobacteria do not form typical dormant spores but are nevertheless able to survive long periods of nutrient starvation. How these starved cells manage to survive and how they resume their metabolic activities once the nutrients are available de novo is one of the most intriguing questions being addressed today (see for examples [78-80]). In vitro, Cyanobacteria survive prolonged nutrient starvation by decreasing their central metabolism and by degrading their photosynthetic apparatus, resulting in a loss of autofluorescence and cell bleaching, a state called chlorosis (Reviewed in [81]). Chlorosis is a highly orchestrated process which starts with the synthesis of the Clp-protease adaptor protein NbLA [82]. Since the regulation of the nbLA gene has recently been reviewed [83], we will not discuss this topic here, but rather focus on specific signalling pathways involved in the transduction of well-defined states of nutritional deficiency.

(i) Phosphate starvation:
Phosphate deficiency affects photosynthetic activity, cell growth, phospholipid and nucleotide synthesis and cell growth. When starved of phosphorus, Cyanobacteria induce the expression of specific genes (known as the Pho regulon), which enhances phosphate uptake [84-86], triggers a process of alkaline phosphatase synthesis releasing phosphorus from several components [87-89], and decreasing the phospholipid levels present in the membrane via a remodeling process [90]. The Pho regulon, has been found in Synechocystis [91,92] and Synechococcus [93,94] to be under the exclusive positive control of the SphS/SphR two-component system. The signal transduction mechanism performed by the SphS/SphR system has been studied in Synechocystis: the fact that deletion of the extended N-terminal extremity of the SphS kinase sensor abolished the activation of the Pho regulon suggests that this sequence is required for sensing the phosphorus levels [95]. The activity of the SphS/SphR system is negatively regulated by the SphU protein, probably by interacting with and inactivating the transmitter domain of SpHS [96]. Gel mobility shift assays have shown the existence of a conserved sequence in the promoter of genes belonging to the Pho regulon, known as the ‘Pho box’, which is required for the activation of transcription by SpHR [92]. The Pho regulon has been predicted to exist in 19 cyanobacterial strains and interestingly, the loss of SphS/SphR was observed in the genome of 3 of them known to inhabit phosphate rich niches [97]. Whether the need to adapt to phosphate deficiency has been lost in the course of evolution or whether a regulator other than SpHR is involved in these strains still remains to be elucidated (Figure 6A).

(ii) iron starvation:
Iron plays the role of cofactor in the case of several essential proteins, but free iron is rarely available in nature, which makes it an important limiting factor for bacterial and phytoplankton growth in various environments [98]. Iron homeostasis is tightly regulated to prevent both starvation and excess, which lead to oxidative stress in cyanobacteria [2]. The ferric uptake regulator (Fur) is the main transcriptional regulator of the genes involved in iron homeostasis in most bacteria [99]. Fur acts as a repressor and an iron sensor: at higher iron concentrations, it binds to Fe²⁺, and dimerizes and binds to target promoters in a conserved sequence termed “Fur-box”. During iron starvation, the release of Fe²⁺ inactivates Fur and cancels the repression exerted by this regulator [100]. Fur homologues are widely distributed in cyanobacterial genomes and the involvement of Fur in their adaptation to iron starvation has been investigated in some model freshwater strains (Nostoc, Synechococcus, Synechocystis). Interestingly, the latter studies have shown that the fur gene is essential in cyanobacteria, which suggests that it is required for some essential processes in addition to iron-response control [101-103]. Studies using a "transcript-depletion" strategy have shown that in Nostoc, Fur controls the expression of genes involved in several processes, including exopolysaccharide biosynthesis, phycobilisome degradation, chlorophyll catabolism, nitrogen fixation, and exopolysaccharide biosynthesis [104]. Several functions must be inhibited in response to iron starvation, but how could this control be exerted since Fur is inactivated under these conditions? A recent study on Synechocystis has yielded a clue to understanding the molecular basis if this homeostasis [105]. The gene expressing the small regulatory RNA IsaR1 (Iron-Stress-Activated RNA 1) is repressed by Fur. IsaR1 accumulates in response to iron starvation and controls at a post-transcriptional level the expression of several genes involved in central cellular processes: photosynthesis, [iron-sulfur] cluster biogenesis, citrate cycle and tetrapyrrole biogenesis [105]. Homologs of IsaR1 are largely conserved in the genomes of Cyanobacteria [105], and the involvement
of this riboregulator in the control of photosynthesis via iron homeostasis might be conserved in the cyanobacterial phylum (Figure 6B).

(iii) nitrogen starvation:

Although Cyanobacteria are able to assimilate a number of combined nitrogen compounds including ammonium, nitrate, nitrite and urea, the preferential is ammonium. The metabolism of various compounds therefore starts with their intracellular assimilation to ammonium, which is then incorporated into the carbon skeleton of 2-oxoglutarate (2-OG) via the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway, giving glutamate. The fact that 2-OG is an intermediate of the TCA cycle means that the processes of nitrogen and carbon assimilation are interconnected. A state of combined nitrogen deficiency therefore leads to inhibition of the GS-GOGAT cycle and ultimately to 2-OG accumulation, which provides us with a useful indicator of the nitrogen status of the cell.

Non-diazotrophic cyanobacterial strains have to cope with nitrogen deficiency, and this adaptative response depends on the ability to perceive the state of starvation and to modulate the pattern of gene expression accordingly in order to use alternative nitrogen sources. The facultatively nitrogen-fixing Cyanobacteria also have to perceive the state of combined nitrogen depletion in order to induce the genetic program enabling them to shift their metabolism towards the reduction of atmospheric nitrogen. In both cases, 2-OG has been found to act as a molecular sensor of nitrogen deficiency. We will focus below on the response to nitrogen starvation in non-fixing Cyanobacteria, as it is only in these organisms that this situation constitutes a stress resulting in chlorosis, and if prolonged, to lysis of the cells and death [81]. The transduction of the 2-OG signal, which has been intensively studied in unicellular freshwater strains (mainly *Synechocystis* and *Synechococcus*) involves several factors, including the transcriptional regulator NtcA, which is thought to be the main one involved. The NtcA protein is a member of the CRP family of transcription regulators, and deletion of the *ntcA* gene impairs the ability of the strains to grow on nitrogen sources other than ammonium, which is consistent with the finding that NtcA activates the transcription of genes required for the assimilation of nitrogen sources such as nitrate. The activity of NtcA is modulated depending on the nitrogen source and its concentration: it is induced in the absence of ammonium and under conditions where the 2-OG level is high (which corresponds to low levels of nitrogen). A dimer of NtcA has the ability to bind to 2-OG, and structural studies have established that the binding of the effector induces a conformational change that enhances the DNA-binding activity [106,107]. PipX, a protein present only in cyanobacteria, acts as a coactivator of NtcA [108]. Biochemical studies have shown that PipX enhances the affinity of NtcA for promoters and the effective affinity of NtcA for 2-OG [109]. In addition to NtcA, the second sensor at work in 2-OG signaling is the protein PII, which is encoded by the *glnB* gene present in all the cyanobacterial genomes. PII proteins constitute one of the largest and most widely distributed family of signal transduction factors, all the members of which are able to bind to ATP/ADP in addition to 2-OG. Via protein-protein interactions, they control the activity of target proteins in response to cellular ATP/ADP levels and the 2-OG status, thus creating a link between the carbon and nitrogen metabolisms (for a recent review on PII, see [110]). When the combined nitrogen source is abundant (and the intracellular 2-OG level is low), PII-ADP binds to PipX and NtcA is mainly present in the apo form; whereas under low combined nitrogen levels (and high 2-OG concentrations) PII interacts with 2-OG and ATP, inhibiting its interactions with PipX. At the same time, the binding of 2-OG to NtcA favors its interactions with PipX, resulting in the
enhancement of the transcriptional activation of the genes involved in nitrogen assimilation [107,108].
The role played by PII and its protein partners in the control of the nitrogen/carbon balance in
Cyanobacteria has been intensively discussed during the last few years. For further information on
this subject, readers can consult the following reviews [111-113] (Figure 6C).

Figure 6: Regulation of nutrient starvation acclimation
**A: Phosphate starvation**

The group of genes induced in response to phosphate starvation is called "the *pho* regulon". In response to phosphate limitation, the transcription of these genes is activated by the TCS formed by the kinase SphS and the response regulator SphR. Under replete phosphate conditions, the activity of SphS is inhibited by the protein SphU, presumably by interacting with the transmitter domain of SphS.

**B: Iron starvation**

The Fur repressor is a metalloprotein in which iron serves as a cofactor. When iron becomes limiting, it is inactivated by the shift of Fur to its apoform, and the transcription process is thus induced. In addition, the translation of several genes is inhibited by the rRNA *isaR1*, which is expressed under iron starvation conditions.

**C: Nitrogen starvation**

The NtcA protein, the main transcriptional factor of nitrogen-induced genes, is activated by PipX. The intracellular level of 2-OG (red circles), which reflects the nitrogen status of the cell, is perceived by PII and NtcA. Under replete nitrogen conditions, the 2-OG level is low, PipX interacts with PII, and NtcA is inactive. When nitrogen is a limiting factor, 2-OG accumulates, PipX changes its partner and interacts with NtcA. The 2-OG-NtcA-PipX complex regulates the gene transcription process.

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8. **Multiple stresses sharing common sensors and/or transducers**

All the environmental stresses discussed above generally decrease the maximum photosynthetic capacity of Cyanobacteria, resulting in hyper-reduction of the electron flow and ultimately in a decrease in the anabolism. Interestingly, genetic screening for mutations impacting the adaptive responses to several stresses at work have led to the identification of the sensor Hik33/NblS(DspA) and the cognate response regulators as leading players in the transduction of the stress signals (reviewed in [5]). Photosynthetic redox stress can be assumed, to be the signal that is actually perceived by Hik33/NblS/(DspA) kinase during exposure to various stresses. As mentioned above, the redox state of the quinone pool reflects the fluidity status of the membrane [77]. The redox-sensitive transcriptional regulator PedR has been found to be reduced by thioredoxin and to be inactivated under HL conditions, which shows the existence of a relationship between gene expression and the photosynthetic activity [114]. Gene regulation through PedR can thus be expected to respond to various stresses that affect the photosynthetic ability of these bacteria. In addition to the redox signal that mediates a pleiotropic transduction pathway, more specific regulatory cross-talk occurs between some stress responses, which will not be discussed here.

**Conclusion:**

Given the ecological role of Cyanobacteria, their wide pattern of distribution and their versatile metabolism, data on the stress responses at work in these bacteria are relevant to many fields, including industrial biotechnological applications. A thorough knowledge of the regulatory networks mediating stress responses is a prerequisite for circumventing inhibitory mechanisms in order to maintain the growth of these microorganisms even under the unfavourable conditions that frequently occur during large-scale production processes.
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References

1. Wiltbank, L.B.; Kehoe, D.M. Diverse light responses of cyanobacteria mediated by phytochrome superfamily photoreceptors. Nature reviews. Microbiology 2019, 17, 37-50, doi:10.1038/s41579-018-0110-4.

2. Latifi, A.; Ruiz, M.; Zhang, C.C. Oxidative stress in cyanobacteria. FEMS microbiology reviews 2009, 33, 258-278, doi:10.1111/j.1574-6976.2008.00134.x.

3. Muramatsu, M.; Hihara, Y. Acclimation to high-light conditions in cyanobacteria: from gene expression to physiological responses. J Plant Res 2012, 125, 11-39, doi:10.1007/s10265-011-0454-6.

4. Hagemann, M. Molecular biology of cyanobacterial salt acclimation. FEMS microbiology reviews 2011, 35, 87-123, doi:10.1111/j.1574-6976.2010.00234.x.

5. Los, D.A.; Zorina, A.; Sinetova, M.; Kryazhov, S.; Mironov, K.; Zinchenko, V.V. Stress sensors and signal transducers in cyanobacteria. Sensors (Basel) 2010, 10, 2386-2415, doi:10.3390/s100302386.

6. Osanai, T.; Ikeuchi, M.; Tanaka, K. Group 2 sigma factors in cyanobacteria. Physiol Plant 2008, 133, 490-506, doi:10.1111/j.1399-3054.2008.01078.x.

7. Montgomery, B.L. Sensing the light: photoreceptive systems and signal transduction in cyanobacteria. Molecular microbiology 2007, 64, 16-27, doi:10.1111/j.1365-2958.2007.05622.x.

8. Sanfilippo, J.E.; Garczarek, L.; Partensky, F.; Kehoe, D.M. Chromatic Acclimation in Cyanobacteria: A Diverse and Widespread Process for Optimizing Photosynthesis. Annual review of microbiology 2019, 73, 407-433, doi:10.1146/annurev-micro-020518-115738.

9. Schuergers, N.; Mullineaux, C.W.; Wilde, A. Cyanobacteria in motion. Curr Opin Plant Biol 2017, 37, 109-115, doi:10.1016/j.pbi.2017.03.018.

10. Hihara, Y.; Kamei, A.; Kanehisa, M.; Kaplan, A.; Ikeuchi, M. DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. Plant Cell 2001, 13, 793-806, doi:10.1105/tpc.13.4.793.

11. Eriksson, J.; Salih, G.F.; Ghebramedhin, H.; Jansson, C. Deletion mutagenesis of the 5' psbA2 region in Synechocystis 6803: identification of a putative cis element involved in photoregulation. Mol Cell Biol Res Commun 2000, 3, 292-298, doi:10.1006/mcbr.2000.0227.

12. Kappell, A.D.; van Waasbergen, L.G. The response regulator RpaB binds the high light regulatory 1 sequence upstream of the high-light-inducible hliB gene from the cyanobacterium Synechocystis PCC 6803. Archives of microbiology 2007, 187, 337-342, doi:10.1007/s00203-007-0213-1.

13. Kappell, A.D.; Bhaya, D.; van Waasbergen, L.G. Negative control of the high light-inducible hliA gene and implications for the activities of the NblS sensor kinase in the cyanobacterium Synechococcus elongatus strain PCC 7942. Archives of microbiology 2006, 186, 403-413, doi:10.1007/s00203-006-0154-0.

14. Hanaoka, M.; Tanaka, K. Dynamics of RpaB-promoter interaction during high light stress, revealed by chromatin immunoprecipitation (ChIP) analysis in Synechococcus elongatus PCC 7942. The Plant journal : for cell and molecular biology 2008, 56, 327-335, doi:10.1111/j.1365-313X.2008.03600.x.

15. Seki, A.; Hanaoka, M.; Akimoto, Y.; Masuda, S.; Iwasaki, H.; Tanaka, K. Induction of a group 2 sigma factor, RPOD3, by high light and the underlying mechanism in Synechococcus elongatus PCC 7942. The Journal of biological chemistry 2007, 282, 36887-36894, doi:10.1074/jbc.M707582200.

16. Kato, H.; Kubo, T.; Hayashi, M.; Kobayashi, I.; Yagasaki, T.; Chibazakura, T.; Watanabe, S.; Yoshikawa, H. Interactions between histidine kinase NblS and the response regulators RpaB and SrrA are involved in the bleaching process of the cyanobacterium Synechococcus elongatus PCC 7942. Plant Cell Physiol 2011, 52, 2115-2122, doi:10.1093/pcp/pcr140.

17. Moronta-Barrios, F.; Espinosa, J.; Contreras, A. In vivo features of signal transduction by the essential response regulator RpaB from Synechococcus elongatus PCC 7942. Microbiology (Reading) 2012, 158, 1229-1237, doi:10.1099/mic.0.057679-0.
18. Lopez-Redondo, M.L.; Moronta, F.; Salinas, P.; Espinosa, J.; Cantos, R.; Dixon, R.; Marina, A.; Contreras, A. Environmental control of phosphorylation pathways in a branched two-component system. *Molecular microbiology* 2010, 78, 475-489, doi:10.1111/j.1365-2958.2010.07348.x.

19. Ashby, M.K.; Houard, J. Cyanobacterial two-component proteins: structure, diversity, distribution, and evolution. *Microbiology and molecular biology reviews* : MMBR 2006, 70, 472-509, doi:10.1128/MMBR.0046-05.

20. van Waasbergen, L.G.; Dolganov, N.; Grossman, A.R. nbIS, a gene involved in controlling photosynthesis-related gene expression during high light and nutrient stress in Synechococcus elongatus PCC 7942. *Journal of bacteriology* 2002, 184, 2481-2490, doi:10.1128/jb.184.9.2481-2490.2002.

21. Hsiao, H.Y.; He, Q.; Van Waasbergen, L.G.; Grossman, A.R. Control of photosynthetic and high-light-responsive genes by the histidine kinase DspA: negative and positive regulation and interactions between signal transduction pathways. *Journal of bacteriology* 2004, 186, 3882-3888, doi:10.1128/JB.186.12.3882-3888.2004.

22. Salinas, P.; Ruiz, D.; Cantos, R.; Lopez-Redondo, M.L.; Marina, A.; Contreras, A. The regulatory factor SipA provides a link between NblS and NblR signal transduction pathways in the cyanobacterium Synechococcus sp. PCC 7942. *Molecular microbiology* 2007, 66, 1607-1619, doi:10.1111/j.1365-2958.2007.06035.x.

23. Kadowaki, T.; Nagayama, R.; Georg, J.; Nishiyama, Y.; Wilde, A.; Hess, W.R.; Hihara, Y. A Feed-Forward Loop Consisting of the Response Regulator RpaB and the Small RNA PsrR1 Controls Light Acclimation of Photosystem I Gene Expression in the Cyanobacterium Synechocystis sp. PCC 6803. *Plant Cell Physiol* 2016, 57, 813-823, doi:10.1093/pcp/pcw028.

24. Georg, J.; Dienst, D.; Schurgers, N.; Wallner, T.; Kopp, D.; Stazic, D.; Kuchmina, E.; Klahn, S.; Lokstein, H.; Hess, W.R., et al. The small regulatory RNA SyR1/PsrR1 controls photosynthetic functions in cyanobacteria. *Plant Cell* 2014, 26, 3661-3679, doi:10.1105/tpc.114.129767.

25. Hu, J.; Li, T.; Xu, W.; Zhan, J.; Chen, H.; He, C.; Wang, Q. Small Antisense RNA RblR Positively Regulates RuBisCo in Synechocystis sp. PCC 6803. *Frontiers in microbiology* 2017, 8, 231, doi:10.3389/fmicb.2017.00231.

26. Gierga, G.; Voss, B.; Hess, W.R. Non-coding RNAs in marine Synechococcus and their regulation under environmentally relevant stress conditions. *The ISME journal* 2012, 6, 1544-1557, doi:10.1038/ismej.2011.215.

27. Imlay, J.A. Pathways of oxidative damage. *Annual review of microbiology* 2003, 57, 395-418, doi:10.1146/annurev.micro.57.030502.090938.

28. Johnson, L.A.; Hug, L.A. Distribution of reactive oxygen species defense mechanisms across domain bacteria. *Free Radic Biol Med* 2019, 140, 93-102, doi:10.1016/j.freeradbiomed.2019.03.032.

29. Agervald, A.; Baebprasert, W.; Zhang, X.; Incharoensakdi, A.; Lindblad, P.; Stensjo, K. The CyAbrB transcription factor CalA regulates the iron superoxide dismutase in Nostoc sp. strain PCC 7120. *Environmental microbiology* 2010, 12, 2826-2837, doi:10.1111/j.1462-2920.2010.02255.x.

30. Larsson, J.; Nylander, J.A.; Bergman, B. Genome fluctuations in cyanobacteria reflect evolutionary, developmental and adaptive traits. *BMC evolutionary biology* 2011, 11, 187, doi:10.1186/1471-2148-11-187.

31. Lieman-Hurwitz, J.; Haimovich, M.; Shalev-Malul, G.; Ishii, A.; Hihara, Y.; Gaathon, A.; Lebendiker, M.; Kaplan, A. A cyanobacterial AbrB-like protein affects the apparent photosynthetic affinity for CO2 by modulating low-CO2-induced gene expression. *Environmental microbiology* 2009, 11, 927-936, doi:10.1111/j.1462-2920.2008.01818.x.

32. Agervald, A.; Zhang, X; Stensjo, K.; Devine, E.; Lindblad, P. CalA, a cyanobacterial AbrB protein, interacts with the upstream region of hypC and acts as a repressor of its transcription in the cyanobacterium Nostoc sp. strain PCC 7120. *Applied and environmental microbiology* 2010, 76, 880-890, doi:10.1128/AEM.02521-09.

33. Dutheil, J.; Saenkham, P.; Sakr, S.; Leplat, C.; Ortega-Ramos, M.; Bottin, H.; Cournac, L.; Cassier-Chauvat, C.; Chauvat, F. The AbrB2 autorepressor, expressed from an atypical promoter, represses the hydrogenase operon to regulate hydrogen production in Synechocystis strain PCC6803. *Journal of bacteriology* 2012, 194, 5423-5433, doi:10.1128/JB.00543-12.

34. Yamashita, Y.; Kaniya, Y.; Kaneko, Y.; Hihara, Y. Physiological roles of the cyAbrB transcriptional regulator pair Sl0822 and Sl0359 in Synechocystis sp. strain PCC 6803. *Journal of bacteriology* 2011, 193, 3702-3709, doi:10.1128/JB.00284-11.

35. Oliveira, P.; Lindblad, P. An AbrB-Like protein regulates the expression of the bidirectional hydrogenase in Synechocystis sp. strain PCC 6803. *Journal of bacteriology* 2008, 190, 1011-1019, doi:10.1128/JB.01605-07.
36. Khan, R.I.; Wang, Y.; Afrin, S.; Wang, B.; Liu, Y.; Zhang, X.; Chen, L.; Zhang, W.; He, L.; Ma, G. Transcriptional regulator PrqR plays a negative role in glucose metabolism and oxidative stress acclimation in Synechocystis sp. PCC 6803. *Scientific reports* 2016, 6, 32507, doi:10.1038/srep32507.

37. Yingping, F.; Lemeille, S.; Talla, E.; Janicki, A.; Denis, Y.; Zhang, C.C.; Latifi, A. Unravelling the cross-talk between iron starvation and oxidative stress responses highlights the key role of PerR (alg0957) in peroxide signalling in the cyanobacterium Nostoc PCC 7120. *Environmental microbiology reports* 2014, 6, 468-475.

38. Li, H.; Singh, A.K.; McIntyre, L.M.; Sherman, L.A. Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of Synechocystis sp. strain PCC 6803. *Journal of bacteriology* 2004, 186, 3331-3345, doi:10.1128/JB.186.11.3331-3345.2004.

39. Kobayashi, M.; Ishizuka, T.; Katayama, M.; Kanehisa, M.; Bhattacharyya-Pakrasi, M.; Pakrasi, H.B.; Ikeuchi, M. Response to oxidative stress involves a novel peroxiredoxin gene in the unicellular cyanobacterium Synechocystis sp. PCC 6803. *Plant Cell Physiol* 2004, 45, 290-299, doi:10.1093/pcp/pcd034.

40. Houot, L.; Floutier, M.; Marteyn, B.; Michaut, M.; Picciocchi, A.; Legrain, P.; Aude, J.C.; Cassier-Chauvat, C.; Chauvat, F. Cadmium triggers an integrated reprogramming of the metabolism of Synechocystis PCC6803, under the control of the Slr1738 regulator. *BMC genomics* 2007, 8, 350, doi:10.1186/1471-2164-8-350.

41. Lee, J.W.; Helmann, J.D. The PerR transcription factor senses H2O2 by metal-catalysed histidine oxidation. *Nature* 2006, 440, 363-367, doi:10.1038/nature04537.

42. Garcin, P.; Delalande, O.; Zhang, J.Y.; Cassier-Chauvat, C.; Chauvat, F.; Boulard, Y. A transcriptional-switch model for Slr1738-controlled gene expression in the cyanobacterium Synechocystis. *BMC Struct Biol* 2012, 12, 1, doi:10.1186/1472-6807-12-1.

43. Sevilla, E.; Sarasa-Buisan, C.; Gonzalez, A.; Cases, R.; Kufryk, G.; Peleato, M.L.; Fillat, M.F. Regulation by FurC in Anabaena Links the Oxidative Stress Response to Photosynthetic Metabolism. *Plant Cell Physiol* 2019, 60, 1778-1789, doi:10.1093/pcp/pcz094.

44. Koskinen, S.; Hakkiia, K.; Kurkela, J.; Tyystjarvi, E.; Tyystjarvi, T. Inactivation of group 2 sigma factors upregulates production of transcription and translation machineries in the cyanobacterium Synechocystis sp. PCC 6803. *Scientific reports* 2018, 8, 10305, doi:10.1038/s41598-018-28736-9.

45. Allakhverdiev, S.I.; Murata, N. Salt stress inhibits photosystems II and I in cyanobacteria. *Photosynthesis research* 2008, 98, 529-539, doi:10.1007/s11120-008-9334-x.

46. Reed R.H., S.W.D.P. Osmotic adjustment and organic solute accumulation in unicellular cyanobacteria from freshwater and marine habitats. *Marine Biology* 1985, 88, 1-9.

47. Pade, N.; Hagemann, M. Salt acclimation of cyanobacteria and their application in biotechnology. *Life (Basel)* 2014, 5, 25-49, doi:10.3390/life5010025.

48. Chen, L.; Wu, L.; Zhu, Y.; Song, Z.; Wang, J.; Zhang, W. An orphan two-component response regulator Slr1588 involves salt tolerance by directly regulating synthesis of compatible solutes in photosynthetic Synechocystis sp. PCC 6803. *Mol BioSynt* 2014, 10, 1765-1774, doi:10.1039/c4mb00095a.

49. Kirsch, F.; Klahn, S.; Hagemann, M. Salt-Regulated Accumulation of the Compatible Solutes Sucrose and Glucosylglycerol in Cyanobacteria and Its Biotechnological Potential. *Frontiers in microbiology* 2019, 10, 2139, doi:10.3389/fmicb.2019.02139.

50. Hagemann, M.; Schoor, A.; Jeanjean, R.; Zuther, E.; Josef, F. The stpA gene form synechocystis sp. strain PCC 6803 encodes the glucosylglycerol-phosphate phosphatase involved in cyanobacterial osmotic response to salt shock. *Journal of bacteriology* 1997, 179, 1727-1733, doi:10.1128/jb.179.5.1727-1733.1997.

51. Huckauf, J.; Nomura, C.; Forchhammer, K.; Hagemann, M. Stress responses of Synechocystis sp. strain PCC 6803 mutants impaired in genes encoding putative alternative sigma factors. *Microbiology (Reading)* 2000, 146 (Pt 11), 2877-2889, doi:10.1099/00221287-146-11-2877.

52. Marin, K.; Huckauf, J.; Fulda, S.; Hagemann, M. Salt-dependent expression of glucosylglycerol-phosphate synthase, involved in osmolyte synthesis in the cyanobacterium Synechocystis sp. strain PCC 6803. *Journal of bacteriology* 2002, 184, 2870-2877, doi:10.1128/JB.184.11.2870-2877.2002.

53. Klahn, S.; Hohne, A.; Simon, E.; Hagemann, M. The gene ssl3076 encodes a protein mediating the salt-induced expression of ggpS for the biosynthesis of the compatible solute glucosylglycerol in Synechocystis sp. strain PCC 6803. *Journal of bacteriology* 2010, 192, 4403-4412, doi:10.1128/JB.00481-10.

54. Curatti, L.; Flores, E.; Salerno, G. Sucrose is involved in the diazotrophic metabolism of the heterocyst-forming cyanobacterium Anabaena sp. *FEBS Lett* 2002, 513, 175-178, doi:10.1016/s0014-5793(02)02283-4.
55. Lopez-Igual, R.; Flores, E.; Herrero, A. Inactivation of a heterocyst-specific invertase indicates a principal role of sucrose catabolism in heterocysts of Anabaena sp. *Journal of bacteriology* **2010**, *192*, 5526-5533, doi:10.1128/JB.00776-10.

56. Ehira, S.; Kimura, S.; Miyazaki, S.; Ohmori, M. Sucrose synthesis in the nitrogen-fixing Cyanobacterium Anabaena sp. strain PCC 7120 is controlled by the two-component response regulator OrrA. *Applied and environmental microbiology* **2014**, *80*, 5672-5679, doi:10.1128/AEM.01501-14.

57. Schwartz, S.H.; Black, T.A.; Jager, K.; Panoff, J.M.; Wolk, C.P. Regulation of an osmoticum-responsive gene in Anabaena sp. strain PCC 7120. *Journal of bacteriology* **1998**, *180*, 6332-6337.

58. Mikami, K.; Kanesaki, Y.; Suzuki, I.; Murata, N. The histidine kinase Hik33 perceives osmotic stress and cold stress in Synechocystis sp PCC 6803. *Molecular microbiology* **2002**, *46*, 905-915, doi:10.1046/j.1365-2958.2002.03202.x.
76. Mironov, K.S.; Sidorov, R.A.; Trofimova, M.S.; Bedbenov, V.S.; Tsydensembaev, V.D.; Allakhverdiev, S.I.; Los, D.A. Light-dependent cold-induced fatty acid unsaturation, changes in membrane fluidity, and alterations in gene expression in Synechocystis. Biochimica et biophysica acta 2012, 1817, 1352-1359, doi:10.1016/j.bbapap.2011.12.011.

77. Maksimov, E.G.; Mironov, K.S.; Trofimova, M.S.; Nechaeva, N.L.; Todorenko, D.A.; Klementiev, K.E.; Tsoravev, G.V.; Tsydensembaev, E.V.; Zorina, A.A.; Feduraev, P.V., et al. Membrane fluidity controls redox-regulated cold stress responses in cyanobacteria. Photosynthesis research 2017, 133, 215-223, doi:10.1007/s11120-017-0337-3.

78. Klotz, A.; Georg, J.; Bucinska, L.; Watanabe, S.; Reimann, V.; Januszewski, W.; Sobotka, R.; Jendrossek, D.; Hess, W.R.; Forchhammer, K. Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. Current biology : CB 2016, 26, 2862-2872, doi:10.1016/j.cub.2016.08.054.

79. Roth-Rosenberg, D.; Aharonovich, D.; Luzzatto-Knaan, T.; Vogts, A.; Zoccarato, L.; Eigemann, F.; Nago, N.; Grossart, H.P.; Voss, M.; Sher, D. Prochlorococcus Cells Rely on Microbial Interactions Rather than on Chlorotic Resting Stages To Survive Long-Term Nutrient Starvation. mBio 2020, 11, doi:10.1128/mBio.01846-20.

80. Sauer, J.; Schreiber, U.; Schmid, R.; Volker, U.; Forchhammer, K. Nitrogen starvation-induced chlorosis in Synechococcus PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. Plant physiology 2001, 126, 233-243, doi:10.1104/pp.126.1.233.

81. Schwarz, R.; Forchhammer, K. Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. Microbiology (Reading) 2005, 151, 2503-2514, doi:10.1099/mic.0.27883-0.

82. Collier, J.L.; Grossman, A.R. A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. The EMBO journal 1994, 13, 1039-1047.

83. Forchhammer, K.; Schwarz, R. Nitrogen chlorosis in unicellular cyanobacteria - a developmental program for surviving nitrogen deprivation. Environmental microbiology 2019, 21, 1173-1184, doi:10.1111/1462-2920.14447.

84. Hudek, L.; Premachandra, D.; Webster, W.A.; Brau, L. Role of Phosphate Transport System Component PstB1 in Phosphate Internalization by Nostoc punctiforme. Applied and environmental microbiology 2016, 82, 6344-6356, doi:10.1128/AEM.01336-16.

85. Pitt, F.D.; Mazard, S.; Humphreys, L.; Scanlan, D.J. Functional characterization of Synechocystis sp. strain PCC 6803 pst1 and pst2 gene clusters reveals a novel strategy for phosphate uptake in a freshwater cyanobacterium. Journal of bacteriology 2010, 192, 3512-3523, doi:10.1128/JB.00258-10.

86. Falkner, R.; Priewasser, M.; Falkner, G. Information Processing by Cyanobacteria during Adaptation to Environmental Phosphate Fluctuations. Plant Signal Behav 2006, 1, 212-220, doi:10.4161/psb.1.4.3242.

87. Ray, J.M.; Bhaya, D.; Block, M.A.; Grossman, A.R. Isolation, transcription, and inactivation of the gene for an atypical alkaline phosphatase of Synechocystis sp. strain PCC 7942. Journal of bacteriology 1991, 173, 4297-4309, doi:10.1128/jb.173.14.4297-4309.1991.

88. Wagner, K.U.; Masepohl, B.; Pistorius, E.K. The cyanobacterium Synechococcus sp. strain PCC 7942 contains a second alkaline phosphatase encoded by phoV. Microbiology (Reading) 1995, 141 ( Pt 12), 3049-3058, doi:10.1099/13500872-141-12-3049.

89. Sebastian, M.; Ammerman, J.W. The alkaline phosphatase PhoX is more widely distributed in marine bacteria than the classical PhoA. The ISME journal 2009, 3, 563-572, doi:10.1038/ismej.2009.10.

90. Peng, Z.; Feng, L.; Wang, X.; Miao, X. Adaptation of Synechococcus sp. PCC 7942 to phosphate starvation by glycolipid accumulation and membrane lipid remodeling. Biochim Biophys Acta Mol Cell Biol Lipids 2019, 1864, 158522, doi:10.1016/j.bbalip.2019.158522.

91. Hirani, T.A.; Suzuki, I.; Murata, N.; Hayashi, H.; Eaton-Rye, J.J. Characterization of a two-component signal transduction system involved in the induction of alkaline phosphatase under phosphate-limiting conditions in Synechocystis sp. PCC 6803. Plant Mol Biol 2001, 45, 133-144, doi:10.1023/a:1006425214168.

92. Suzuki, S.; Ferrani, A.; Suzuki, I.; Murata, N. The SphS-SphR two component system is the exclusive sensor for the induction of gene expression in response to phosphate limitation in synechocystis. The Journal of biological chemistry 2004, 279, 13234-13240, doi:10.1074/jbc.M313358200.

93. Nagaya, M.; Aiba, H.; Mizuno, T. The sphR product, a two-component system response regulator protein, regulates phosphate assimilation in Synechococcus sp. strain PCC 7942 by binding to two sites upstream from the phoA promoter. Journal of bacteriology 1994, 176, 2210-2215, doi:10.1128/jb.176.8.2210-2215.1994.
114. Horiuchi, M.; Nakamura, K.; Kojima, K.; Nishiyama, Y.; Hatakeyama, W.; Hisabori, T.; Hihara, Y. The
113. Labella, J.I.; Cantos, R.; Salinas, P.; Espinosa, J.; Contreras, A. Distinctive Features of PipX, a Unique
112. Forchhammer, K.; Selim, K.A. Carbon/nitrogen homeostasis control in cyanobacteria.
110. Forchhammer, K.; Luddecke, J. Sensory properties of the PII signalling protein family.
109. Forcada-Nadal, A.; Forchhammer, K.; Rubio, V. SPR analysis of promoter binding of Synechocystis
108. Espinosa, J.; Forchhammer, K.; Burillo, S.; Contreras, A. Interaction network in cyanobacterial
106. Zhao, M.X.; Jiang, Y.L.; He, Y.X.; Chen, Y.F.; Teng, Y.B.; Chen, Y.; Zhang, C.C.; Zhou, C.Z. Structural
105. Georg, J.; Kostova, G.; Vuorijoki, L.; Schon, V.; Kadowaki, T.; Huokko, T.; Baumgartner, D.; Muller,
104. Gonzalez, A.; Bes, M.T.; Peleato, M.L.; Fillat, M.F. Expanding the Role of FurA as Essential Global
103. Gonzalez, A.; Bes, M.T.; Valladares, A.; Peleato, M.L.; Fillat, M.F. FurA is the master regulator of iron
102. Kunert, A.; Vinnemeier, J.; Erdmann, N.; Hagemann, M. Repression by Fur is not the main
101. Ghassemian, M.; Straus, N.A. Fur regulates the expression of iron-stress genes in the cyanobacterium
100. Pecqueur, L.; D’Autreaux, B.; Dupuy, J.; Nicolet, Y.; Jacquamet, L.; Brutscher, B.; Michaud-Soret, I.;
99. Hantke, K. Iron and metal regulation in bacteria. Current biology : CB 2018, 31, 1116-1130, doi:10.1016/j.tplants.2018.09.008.
98. Wandersman, C.; Delepelaire, P. Bacterial iron sources: from siderophores to hemophores. FEBS microbiology reviews 2020, 44, 33-53, doi:10.1093/femsms/fuz025.
97. Su, Z.; Olman, V.; Xu, Y. Computational prediction of Pho regulons in cyanobacteria. Current biology : CB 2017, 27, 1425-1436 e1427, doi:10.1016/j.cub.2017.04.010.
96. Juntarajumnong, W.; Hirani, T.A.; Simpson, J.M.; Incharoensakdi, A.; Eaton-Rye, J.J. Phosphate
95. Burut-Archanai, S.; Incharoensakdi, A.; Eaton-Rye, J.J. The extended N-terminal region of SphS is
94. Aiba, H.; Nagaya, M.; Mizuno, T. Sensor and regulator proteins from the cyanobacterium Synechococcus species PCC7942 that belong to the bacterial signal-transduction protein families: implication in the adaptive response to phosphate limitation. Molecular microbiology 1993, 8, 81-91, doi:10.1111/j.1365-2958.1993.tb01205.x.
93. Burut-Archanai, S.; Incharoensakdi, A.; Eaton-Rye, J.J. The extended N-terminal region of SphS is
92. Forchhammer, K.; Selim, K.A. Carbon/nitrogen homeostasis control in cyanobacteria.
91. Hihara, Y. The PedR transcriptional regulator interacts with thioredoxin to connect photosynthesis with gene expression in cyanobacteria. The Biochemical journal 2010, 431, 135-140, doi:10.1042/Bj20100789.