The overall processes of transcription and its regulation have advanced significantly in the last years, making our understanding of prokaryotic biology more complex and detailed. In fact, a systematic study of different aspects of transcriptional regulation opens up outstanding opportunities to improve and develop the perception of complex reaction mechanisms, genetic processes and cell functions. In close connection to the cyanobacterial bidirectional hydrogenase, the main hydrogen-evolving enzyme in non-nitrogen fixing strains, two novel transcription factors have received increasing attention over the past five years: a LexA-related protein and the AbrB-like family members. Recent work on these regulators has produced new insights and advances towards the understanding (and possible interconnection) of several regulatory networks in cyanobacteria, namely nitrogen metabolism, redox response, toxin production, CO₂ concentrating mechanisms and hydrogen metabolism. The fact that a LexA-related protein and AbrB-like family members have been co-purified in independent laboratories studying different sets of cyanobacterial genes suggests a possible common and/or complementary function of these regulators. In this review, we summarize the knowledge gained thus far regarding the transcriptional regulation of the cyanobacterial bidirectional hydrogenase, with special focus on the above mentioned transcription factors. Moreover, we discuss several additional points that warrants further investigation to increase our knowledge in this fast evolving research field.

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Peter Lindblad received his PhD 1987 in physiological botany at Uppsala University (Sweden). After postdoctoral work at University of Western Australia and RPI, Troy (USA) he returned to Uppsala University where he currently is professor in microbial chemistry and a group leader in the Swedish Consortium for Artificial Photosynthesis. Prof. Lindblad's research explores the conversion of solar energy into a fuel, focusing on microalgal based H₂-production and hydrogenases at applied, physiological, biochemical, and molecular levels. In addition, he has a strong interest in the interactions between symbiotic cyanobacteria and higher plants. The combined goal is to engineer photosynthetic microorganisms to produce renewable biofuels from solar energy and water.

Introduction

Green algae and cyanobacteria are the only organisms able to combine oxygenic photosynthesis with the production of molecular hydrogen (H₂), an attractive pathway for renewable production of H₂ from solar energy and water. Cyanobacteria is a large and very diverse group of bacteria, with the capacity to use sunlight as an energy source, water as an electron source, and air as carbon (CO₂) and nitrogen (N₂) sources. Therefore, minimal and inexpensive growth media can be used for the cultivation of cyanobacteria, and, theoretically, the overall energy conversion efficiency may become very high. Here, we restrict our discussion to cyanobacteria and specifically the transcriptional regulation of the bidirectional hydrogenase.

The enzymatic complex (HoxEFUYH)₂, Hox meaning hydrogen oxidation, from the unicellular cyanobacterium *Synechocystis* sp. PCC 6803, and corresponding genes, constitute the most studied and well characterized cyanobacterial bidirectional hydrogenase. In this organism, the structural *hox* genes are clustered in the genome together with three open reading frames of unknown function. On a molecular level, these genes have been shown to be part of the same transcriptional unit, forming an operon that initiates −169 bp upstream of the *hoxE* start codon (Fig. 1). Co-transcription of the five *hox* genes in such a unit suggests that one possible way to maintain the stoichiometric balance of the Hox subunits is achieved by simply regulating the *hox* operon transcription. However, Kiss *et al.* recently reported that an increased accumulation of *hoxEF* transcripts can be observed in particular conditions (e.g. when cells are kept in the dark, or in the presence of the electron transport inhibitor dibromothymoquinone) when compared to *hoxUYH*. As suggested by the authors, this differential transcription could be related to an increased requirement for HoxEF production due to enhanced degradation or additional unknown roles of these subunits. Interestingly, this was not the first indication of differential *hox* gene expressions reported in the literature. In *Synechococcus* sp. PCC 7942, *hoxH* transcription, still in the same range as *hoxEF* expression, was found to be substantially lower compared with *hoxU* expression, on account of different promoter strengths and partial termination of transcription or mRNA instability within the region between *hoxU* and *hoxH*. In addition, the structural genes encoding the cyanobacterial bidirectional hydrogenase are organized on the chromosome in a dissimilar way in different strains. As in *Synechocystis* sp. PCC 6803, the *hox* genes in e.g. *Anabaena variabilis* ATCC 29413 are localized in one cluster, and have been shown to be transcribed as a polycistronic unit. In other strains (e.g. *Synechococcus* sp. PCC 6301, *Synechococcus* sp. PCC 7942 and *Anabaena* sp. PCC 7120), the *hox* genes are organized in two separate clusters in the genome (*hoxEF* and *hoxUYH*). For these, a transcript for each locus was found, in addition to the above mentioned differential expression. This complexity in transcript formation may inherit a control mechanism for the expression of the bidirectional hydrogenase, which is known for its high variability of specific activity levels under different culture conditions.
**Fig. 1** Schematic representation of the hox locus in the genome of *Synechocystis* sp. PCC 6803. (A) Black arrows represent the structural hox genes, while grey arrows illustrate open reading frames that have been shown to be transcribed together with the hox genes. White arrows correspond to neighboring genes. Gene identification tags are shown in accordance to CyanoBase nomenclature (http://genome.kazusa.or.jp/cyanobase/). (B) Illustration of the intergenic region between the genes ssr2227 and sll1220 (hoxE) of *Synechocystis* sp. PCC 6803, harbouring the promoter of the hox operon. In addition, reported and putative regulators of the hox operon expression are equally drawn. Structural aspects of the promoter structure, like TSP, ribosomal binding site, −10 and −35 boxes, are illustrated in their relative positions. CyLexA is represented as a dimer in direct contact with the DNA, since defined regions in the promoter have been identified as targets (black bars). In its turn, the CyAbrB regulator is illustrated as an oligomer, in agreement with the protein-protein interactions reported by Sato *et al.*, but not in direct contact with the DNA. This is because only a broad region of the promoter (grey bar) was identified as the target of its binding. Its specific binding region remains to be shown. The bent arrow represents the putative DNA loop suggested by Gutekunst *et al.* Further regulators are shown to account for reported signals that have been demonstrated to control the hox operon expression.
The transcription of the cyanobacterial hox genes has been studied by various techniques, like RT-PCR, Northern blotting and reporter gene analysis. Additionally, the increasing availability and continuous technical development of microarray technology has opened up the possibility to collect much more information than ever before. Therefore, various environmental factors have been shown to control the expression of hox genes. In *A. variabilis* ATCC 29413, hox transcripts could be detected in nitrogen-fixing conditions, in isolated heterocysts, as well as in ammonia grown filaments. Moreover, the relative amount of hoxH was induced when cells were transferred to a low level of oxygen. In *Nostoc muscorum*, anaerobiosis and nickel were shown to up regulate hox transcription. In *Anabaena* sp. PCC 7120, dark/anaerobic conditions induce the expression of the hox genes in the same line as a temperature shift from 32 to 22 °C, either in light or in dark, and a transfer from light to dark also strongly induce the hox genes expression. In *Lyngbya majuscula* CCAP1446/4, the transcription of hoxH did not vary notably under the tested conditions, neither in nitrogen-fixing nor in non-nitrogen-fixing conditions, even though an increase in the transcript levels could be observed during the dark periods. In *Synechococcus* sp. PCC 7942, the hox genes transcription was found to be under the control of a circadian clock. In *Synechocystis* sp. PCC 6803, the hoxE transcription was equally shown to be controlled by a circadian rhythm as well as by iron availability and combined nitrogen starvation. In addition, light and oxygen dependent regulation of the hox genes was also demonstrated in *Synechocystis* sp. PCC 6803, whilst inorganic carbon down shift was shown to significantly repress hoxH transcripts.

The knowledge around the transcription regulation of the genes encoding the bidirectional hydrogenase in cyanobacteria is quite fragmented and difficult to integrate. Despite the many conditions tested thus far, the mechanisms by which a particular factor unleashes the hox specific response have been poorly investigated. In an attempt to understand the intermediate steps of the signal transduction pathways inherent to the hox genes transcription regulation and, at the same time, to understand how this transcription regulation is operated, the promoter of the hox genes in *Synechocystis* sp. PCC 6803 has been carefully investigated. Reporter gene analysis revealed that three regions of this promoter are essential for the promoter activity. One is situated in the untranslated 5′ leader region (−97 to +10, relative to the hoxE ATG); another is found −569 to −690 bp upstream the hoxE start codon, and the third is located at the positions −690 to −962 bp upstream the translational start point. In addition, the *Synechocystis* sp. PCC 6803 hox promoter has also been subject of intense investigation and characterization concerning the key players interacting and regulating its activity. Two independent studies demonstrated an interaction between a LexA-related protein (hereafter named CyLexA, referring to the cyanobacterial LexA—see discussion below) and the hox promoter.

**CyLexA transcription regulator**

Two distinct regions of the hox promoter from *Synechocystis* sp. PCC 6803 were analyzed and both were further demonstrated to be targets of this interaction. Oliveira and Lindblad demonstrated that CyLexA binds to a region located between the nucleotides −198 and −338 bp in relation to the hoxE start codon, while Gutekunst et al. found that CyLexA interacts further upstream on the hox promoter, at the positions −592 to −690 bp, respective to the translational start point. Furthermore, a CyLexA-depleted mutant showed a reduced hydrogenase activity compared with the wild-type, suggesting that CyLexA works as a transcription activator of the hox genes in *Synechocystis* sp. PCC 6803. The fact that two distant CyLexA binding regions are found in the hox promoter was subsequently interpreted and suggested to be involved in the formation of a DNA loop, a feature that has been long described as being an important event in gene transcription. Preliminary results seem to support this hypothesis, although more detailed and consistent results are needed.

Interestingly, the role of LexA in other well studied bacteria (e.g. in *Escherichia coli* LexA is part of the SOS response) seems to be significantly different from the one found in *Synechocystis* sp. PCC 6803. The SOS response was one of the first clear networks of transcriptional regulation identified in bacteria. In *E. coli*, it comprises a set of coordinated physiological responses induced by DNA damage. The expression of up to 40 genes can be induced when DNA damage occurs. Most of these genes are involved in different mechanisms of DNA repair, like mismatch repair, recombinational repair, excision repair or mutagenesis (error-prone repair). Expression of these genes involves two regulatory proteins, LexA and RecA. LexA is a
typical transcriptional repressor, consisting of two domains connected by a flexible hinge. A palindromic DNA sequence, termed the SOS box, is recognized by the dimeric repressor and a consensus sequence has been determined. The SOS boxes are found upstream of the subordinate genes and, for different genes, the number of operator boxes can vary between one and three. The second protein involved in the regulation of the SOS response is RecA, although this protein serves several other functions in the cell (e.g. important for homologous recombination). Within the frame of the SOS response, however, RecA mediates the induction of the genes coordinately repressed by LexA. In the presence of DNA damaging agents or UV irradiation, single-stranded DNA fragments appear in the cell. The activity of RecA is changed upon binding to such single-stranded DNA sites. The activated RecA form further facilitates a latent capacity of LexA to autodigest. Upon the LexA proteolytic cleavage, the repression imposed by LexA is released, resulting in the expression of the genes involved in the DNA repair. Activation of RecA is reversible and prevails as long as the single-stranded DNA is abundant. Once DNA lesions have been repaired, RecA ceases to be activated and non-cleaved LexA protein returns to its normal levels, repressing again the transcription of SOS genes.

However, it has been shown in other organisms that there are deviations from the classical E. coli-type SOS regulation. In Deinococcus radiodurans, a well known bacterium for its extreme resistance to the lethal effects of ionizing radiation, microarray analyses showed that lexA expression is not significantly induced in cells exposed to acute irradiation, in Bdellovibrio bacteriovorus, a bacterial predator belonging to the δ-proteobacteria, Campoy et al. showed that the canonical SOS genes are not repressed by LexA; many of the obligate parasitic bacteria that thrive inside eukaryotic cells, in which endogenous DNA-damaging agents abound, have lost their lexA gene and maintain most of their DNA repair genes under constitutive expression. These data reveal the existence of different regulatory mechanisms for DNA damage-inducible genes in bacteria belonging to different phyla.

In addition, Domain et al. and Patterson-Fortin et al. independently showed strong evidence that cyanobacteria do not have an E. coli-type SOS regulon. Using DNA microarrays, Domain et al. identified 57 genes with expression that is altered in response to CyLexA depletion in Synechocystis sp. PCC 6803, none of them being predicted to operate in DNA metabolism. On the other hand, Patterson-Fortin et al. observed that lexA is not induced upon UV-irradiation, suggesting that CyLexA is not required for survival following DNA damage. These facts argue against the involvement of CyLexA in the regulation of DNA repair. Instead, most of the CyLexA-responsive genes were known to be involved in carbon assimilation or controlled by carbon availability. Therefore we suggest the term CyLexA, referring to the alternative function of this transcription factor in Synechocystis sp. PCC 6803, and possibly in cyanobacteria.

Lieman-Hurwitz et al. recently identified CyLexA as one of the proteins binding to the promoter of sbtA in Synechocystis sp. PCC 6803, which is believed to encode a high affinity Na+/HCO₃⁻ transporter and is, therefore, highly inducible during the acclimation from high to low CO₂. Interestingly, Domain et al. demonstrated by microarray that sbtA is strongly repressed in a CyLexA depleted mutant, even though Lieman-Hurwitz et al. showed that CyLexA elutes from protein-DNA interaction experiments in approximately similar amounts, when proteins extracted from either high CO₂ or low CO₂ grown cells were used.

Further results are supporting the hypothesis that CyLexA in Synechocystis sp. PCC 6803 is indeed involved in other transcriptional networks. Patterson-Fortin et al. found that the expression of the DEAD-box RNA helicase, crhR, which is regulated in response to conditions that elicit reduction of the photosynthetic electron transport chain, is under the direct control of CyLexA. CrhR has been proposed to regulate gene expression at the translational level through its ability to rearrange RNA secondary structures of RNA substrates, potentially of redox-regulated gene transcripts. Transcript analysis indicated that lexA and crhR are divergently expressed, with the respective transcripts accumulating differently under conditions, which, oxidize and reduce the electron transport chain, respectively. These results, together with data collected from in vitro transcription/translation assays, suggest that CyLexA works as a repressor of the crhR transcription. More recently, the same group has showed, by footprinting, that Synechocystis sp. PCC 6803 CyLexA binds as a dimer to 15 bp direct repeats containing a CTA-N9-CTA sequence conserved in two target genes, lexA and crhR. In addition, the analysis of sequences within the hox promoter region, previously shown to interact with the CyLexA protein, revealed several CTA–N9–CT(A/T) repeats further suggesting their importance in CyLexA recognition of its DNA targets. The ability of dimerization in CyLexA–DNA interactions was also found to be essential, by comparison of the binding constants for the full-length and the carboxyl terminal truncated CyLexA proteins.

Based on the observations reported by Patterson-Fortin et al. on the fact that the bidirectional
hydrogenase activity is directly affected by the redox status of the cell, and that the regulation of the hox gene expression can be operated by CyLexA, a hypothesis was put forward on the direct involvement of the transcription regulator CyLexA as a mediator of the redox-responsive regulation of the hox gene expression in Synechocystis sp. PCC 6803.23 Interestingly, lexA has been shown to be repressed in high-light conditions,34,38 which are known to increase the excitation energy and induce profound changes in the cells to keep the redox homeostasis, as well as in iron deficient conditions and under peroxide stress,35 conditions that greatly alter the regulatory apparatus upon the onset of oxidative stress. However, the signal transduction pathways directly or indirectly involved in the regulation of CyLexA, and consequently its downstream targets, definitely require further investigation.

Synechocystis sp. PCC 6803 CyLexA has been detected in several proteomic studies37–45 and its transcript has also been identified in microarray experiments.20,26,29,30,46 In some proteomic studies, CyLexA was identified in association with thylakoid membrane fractions, which represents an unexpected location for a transcription regulator. Furthermore, it is also relevant to mention that the isoelectric point of CyLexA in some of these reports differs significantly from the predicted pI.29,44,45 In fact, in some studies where 2D-electrophoresis was used, CyLexA could be identified on the same gel in more than one spot,37,39,41,42 strongly suggesting that this regulator undergoes post-translational modifications. The nature of these possible modifications and whether these may be in any way connected to its localization on the membrane remains elusive. However, what is clear from proteomic data is that the levels of CyLexA significantly increase under high pH stress23 during heat shock treatment,24 in heterotrophic growth conditions40 and under prolonged exposure (3–4 d) to UV-B irradiation.22

The singular role found for CyLexA in Synechocystis sp. PCC 6803 became of increasing interest after looking closer at the deduced CyLexA amino acid sequence.6,31,50 In E. coli, LexA self-cleavage and derepression of the SOS regulon requires a catalytic serine/lysine dyad and an alanine–glycine cleavage bond.22 In the absence of these residues, as indicated by mutational studies, LexA self-cleavage in E. coli is defective.24 In Synechocystis sp. PCC 6803, the alanine and serine residues are substituted by glycine and aspartate.5,31,50 Mazón et al.25 suggested that these modifications may negatively affect the autocatalytic cleavage of this transcription factor and thus affect the DNA recognition, further implying a unique cellular function for the CyLexA protein.

Therefore, the study of CyLexA in other cyanobacteria comprises an interesting and important research area, which will help to elucidate the function of this transcription factor. Lyngbya majuscula CCAP 1446/4 and Anabaena sp. PCC 7120 were used to investigate the possible connection between CyLexA and, in particular, both of their hydrogen metabolisms. In Lyngbya majuscula CCAP 1446/4, Synechocystis sp. PCC 6803 recombinant LexA was found to interact in vitro with the promoter of the hyp operon.35 In Anabaena sp. PCC 7120, LexA was shown to bind to the promoters of both hox operons.41 The combination of these results gives the first indication that CyLexA is broadly involved in other transcriptional networks in cyanobacteria, exemplified here by the hydrogen metabolism. However, more research is needed to support this hypothesis.

CyAbrB transcription regulators

The analysis of the hox genes expression in Synechocystis sp. PCC 6803 suggested that other possible factors, in addition to CyLexA, are involved in their regulation.7,23 In fact, the study of the hox operon promoter and possible interacting proteins uncovered an additional transcription factor Sll0359,23 an AbrB-like protein from hereon CyAbrB, see discussion below (Fig. 1). This was further shown in electrophoretic mobility shift assays to specifically interact both with part of the hox promoter and with its own promoter (sll0359), even when an excess of unrelated DNA was present in the reaction. Moreover, the attempt to produce a fully segregated mutant of Sll0359 revealed to be unsuccessful in three different and independent laboratories32,53,54—see below, suggesting that it may be essential for the viability of Synechocystis sp. PCC 6803. Even so, making use of this non-fully segregated deletion mutant of Sll0359 and of a strain overexpressing the same CyAbrB, S110359 was demonstrated to positively regulate the hox genes expression.23

The transcription factor AbrB (antibiotic resistance) has been carefully investigated in representatives of the genus Bacillus (e.g. B. subtilis, B. anthracis) upon entering the stationary phase due to nutrient deprivation. It was shown to be in association with the transcription factor Spo0A, of an elaborate signal transduction network, the phosphorelay.58 Over 40 different genes are subject to regulation due to direct AbrB binding to their promoters or regulatory regions. Many other genes are indirectly controlled by AbrB since it
is a regulator of other regulatory proteins. AbrB-controlled genes function in a wide variety of metabolic and physiological processes, including production of extracellular degradative enzymes, nitrogen utilization and amino acid metabolism, motility, synthesis of antibiotics and their resistant determinants, development of competence, transport systems, oxidative stress response, phosphate metabolism, cell surface components and sporulation. Although not usually considered essential for viability, growth or sporulation in the laboratory, AbrB probably has an essential function for fitness, adaptation and survival in the wild. Multimerization between identical monomeric subunits is absolutely required to form the active surface and is believed to be a key factor in the ability of AbrB to differentially regulate various target genes. Examination of over 40 chromosomal sites of AbrB binding, and over 80 high-affinity binding sites selected using in vitro methods, failed to derive a consensus base sequence that could adequately explain AbrB site selection and recognition. It has been hypothesized that AbrB recognizes a three-dimensional DNA architecture that is shared by a finite subset of base sequences.\textsuperscript{53}

During active growth transcription of the \textit{abrB} gene is auto-regulated. This maintains the intracellular concentration of AbrB at a narrow threshold range of regulatory effectiveness. In response to nutrient deprivation and the onset of the transition state, a regulatory cascade is activated, which increases the intracellular concentration of the phosphorylated form of a critical regulatory protein, Spo0A. Phosphorylated Spo0A is a potent repressor of \textit{abrB} transcription, acting independently of AbrB concentration. The concentration of AbrB drops below its threshold of effectiveness, and the AbrB dependant regulatory effects are lifted (for a review, see ref. \textsuperscript{55} and references therein).

Only fragmented information is available about CyAbrB, although a detailed analysis of sequenced genomes reveals a wide distribution of genes putatively encoding proteins belonging to the AbrB family of transcription regulators in many cyanobacteria.\textsuperscript{53,54} In fact, every cyanobacterial strain fully sequenced this far possesses at least two genes encoding CyAbrB distinct from those conserved among other bacterial species.\textsuperscript{54} A phylogenetic analysis of a few selected CyAbrB homologues identified different clades within this protein family.\textsuperscript{54} However, their function(s) is yet unknown, but the first reports addressing their existence and possible role are now emerging. Shalev-Malul \textit{et al.}\textsuperscript{56} recently suggested that in the filamentous cyanobacterium \textit{Aphanizomenon ovalisporum}, the production of the hepatotoxin cylindrospermopsin might be regulated by CyAbrB. This transcription factor was found to bind to the intergenic region of the genes \textit{aoaA} (amidinotransferase) and \textit{aoaC} (polyketide-synthase), whose gene products are likely to be involved in cylindrospermopsin biosynthesis. In the same work, the comparison of the native CyAbrB isolated from \textit{A. ovalisporum} with that obtained after cloning and over-expression of \textit{abrB} in \textit{E. coli} identified specific post-translational modifications in the native cyanobacterial protein.\textsuperscript{56} These modifications, which are missing in the protein expressed in \textit{E. coli}, include N-acetylation and methylation of specific residues and were suggested to play a role in DNA affinity.\textsuperscript{56} Interestingly, the difference between the theoretical isoelectric point of the \textit{Synechocystis} sp. PCC 6803 CyAbrB (6.9 and 7.1, according to the calculation tool used) and the one identified in proteomic experiments\textsuperscript{52} (5.4) also seems to indicate a post-translational modification of the protein that possibly can affect its activity as a transcription factor.

A recent large-scale protein–protein interaction analysis in \textit{Synechocystis} sp. PCC 6803 revealed interesting new findings: Sl10359 was found to interact with a high degree of confidence with itself (supporting the idea that the functional protein must be an oligomer), and with SlI0822.\textsuperscript{58} The latter protein shows similarity to SlI0359 and it also belongs to the CyAbrB family. Furthermore, when the thylakoid membrane-bound ferredoxin-NADP\textsuperscript+ oxidoreductase (FNR—which catalyzes the terminal step of the linear photosynthetic electron transport) was purified from the cyanobacterium \textit{Spirulina platensis} IAM M-135, a CyAbrB homologue could be identified in association with the FNR complex.\textsuperscript{58} However, its overall function in the complex was not addressed and remains unknown.

Ishii and Hihara reported the production of a fully segregated disrupted mutant of \textit{sll0822} (\textit{Δsll0822} mutant) that exhibits a phenotype of decreased growth rate and pigment content.\textsuperscript{55} Interestingly, the growth rate of the \textit{Δsll0822} mutant was low under any condition, but the low pigment content could be partially recovered by nitrate supplementation of the medium. In the same work, DNA microarray and RNA-blot analyses revealed that the level of expression of a part of the NtcA regulon was significantly decreased in the mutant. In addition, the \textit{hox} genes expression was also analyzed and found to be significantly up regulated in the deletion mutant.\textsuperscript{54} This result suggests that SlI0822 works as a repressor of the \textit{hox} genes transcription, exerting its function either by directly interacting with the \textit{hox} promoter or via an unknown intermediary factor.

Ishii and Hihara proposed that SlI0822 works in parallel with NtcA (global nitrogen regulator in
cyanobacteria) to achieve flexible regulation of the nitrogen uptake system. The Sll0822 protein exists mainly in a dimeric form in vivo, and the amount of the protein was not affected by nitrogen availability, nor amount of CO₂ supplied to the cultures. These observations, together with the low binding specificity of the purified histidine-tagged Sll0822 protein, implies that the activity of Sll0822 may be post-translationally modulated in Synechocystis sp. PCC 6803 cells.

Furthermore, Lieman-Hurwitz et al. identified two CyAbrBs, Sll0359 and Sll0822, additionally to CyLexA (see above) binding to a fragment of the sbtA promoter in Synechocystis sp. PCC 6803. However, Sll0822 did not bind in vitro to the same promoter when using extracts prepared from cells grown under low levels of CO₂, despite its presence in the cells, as examined by Western blotting. Remarkably, genes normally expressed only under low levels of CO₂ in the wild-type were shown to be transcribed independently from the amount of CO₂ supplied in a sll0822 deletion mutant. This strongly suggests that the CyAbrB Sll0822 works as a repressor of genes induced under low levels of CO₂ in Synechocystis sp. PCC 6803. More, Sll0822 grown under high levels of CO₂ clearly has an elevated photosynthetic affinity for extracellular inorganic carbon. These levels of affinity are close to those typically observed only in wild-type cells grown under low levels of CO₂.

As Sll0822 seems to be involved in nitrogen uptake and CO₂ concentrating mechanisms, it is possible that it helps the cells modulate their metabolism in accordance with the availability of CO₂ and nitrogen sources. However, the connection with the classic regulation mechanisms operated by the master player NtcA remains to be clarified. Therefore, since the cyanobacterial closer homologues to the Bacillus AbrB transcription factor do not seem to play a similar role in cyanobacteria as in Bacillus, we suggest the term CyAbrB in agreement with Ishii and Hihara’s terminology.

A point that surely deserves close attention has to due with the general function(s) of CyLexA and CyAbrB-family members in cyanobacteria. The discussion presented here is based on reports studying exclusively Synechocystis sp. PCC 6803. Given the extraordinary diversity of these organisms and their very long evolutionary history, the question arises whether these transcription factors may have the same function(s) in other cyanobacteria.

The hox genes transcription seems to be under the control of both CyAbrBs, Sll0359 and Sll0822. However, while Sll0359 seems to up regulate hox expression, Sll0822 has been shown to repress hox transcription. This situation sounds paradoxical. However, it is worth mentioning that Sll0822 was found to repress sbtA transcription and, at the same time, Sll0359 was demonstrated to bind to the same sbtA promoter, along side Sll0822. Nevertheless, the actual role of Sll0359 in the regulation of sbtA expression is yet unclear. One possible explanation to the dual role of CyAbrBs in hox regulation could be that the relative amounts of Sll0822 and Sll0359 in the cells, associated with probable specific post-translation modifications (see above), sets the fine regulation of the hox genes. Another point that deserves better attention has to due with the possible crosstalk between CyLexA and CyAbrB family members, and even between the latter ones (see discussion above). Preliminary experiments using combinations of various amounts of CyLexA, Sll0822 and Sll0359 produced changes in the extent of mobility shift of the promoter sbtA. However, it remains to be analyzed how these interactions control the DNA recognition and the subsequent regulation of the target genes.

Despite the strong steps towards the understanding of the hox genes transcription associated to the findings of CyLexA and CyAbrB (see Fig. 1 for a general summary of the factors involved in the regulation) it remains to be shown what environmental factors regulate the hox genes via these transcription factors. More, recent results published by Voß et al. may give a novel dimension into the transcription regulation of the hox genes. In the mentioned work, SyR1, a non-coding RNA, was found to accumulate in relatively large amounts, being transcribed from a gene in the fabF-hoxH intergenic spacer in the forward direction as the preceding gene fabF. Its function is yet unknown, and its possible connection to the hox genes regulation through any anti-sense mechanisms remains to be demonstrated.

Finally, it is imperative to study if the regulation of the hox genes in different cyanobacteria is operated in a similar way. If differences are found, could this mean that the enzyme is performing distinct functions in different strains? The topic of transcription regulation has gone through significant advances in the past years and therefore has deserved the full attention in the present review. However, additional forms of regulation should not be neglected: post-transcriptional control, post-translation processing, regulation of the maturation proteins involved in the assembly of the hydrogenase's active site, interaction with other proteins, etc., represent other means of regulation that surely play a key role in the overall process. Consequently, more
emphasis should be put in future studies into these poorly characterized mechanisms. For all this, we consider that the bidirectional hydrogenase represents a unique model system for studying and integrating multiple core metabolic processes in cyanobacteria.

**Conclusion**

The information available towards the understanding of the regulation of the *hox* genes in cyanobacteria is still fragmentary. In *Synecochystis* sp. PCC 6803, in order to gain a consistent overview of the *hox* regulation, double-knockout mutants, in addition to over-expressing strains, are needed to understand how CyLexA and the CyAbrB proteins operate, alone and in combination, to control the activity of the bidirectional hydrogenase. Furthermore, one cannot exclude the possibility that additional factors are involved. The molecular mechanisms determining this expression pattern and their connections to CyLexA and CyAbrB definitely deserve better attention.

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