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# Transcriptional regulation of the bidirectional hydrogenase in the cyanobacterium *Synechocystis* 6803

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ABSTRACT

To identify optimal conditions for renewable hydrogen production from sunlight and water we have studied transcriptional changes of the *hoxEFUYH* genes encoding the bidirectional hydrogenase in the cyanobacterium *Synechocystis* PCC 6803. Transcript abundance detection by real time polymerase chain reaction was supplemented with variable chlorophyll fluorescence measurements to monitor redox changes of the photosynthetic electron transport chain. Our main observations are: (i) abundance of *hox* transcripts decreases in the dark and recovers in the light. (ii) Inhibition of the Calvin cycle by glycolaldehyde suppresses *hox* gene transcription, which can be restored by the addition of electron transport inhibitors 3-(3,4-dichlorophenyl)-1,1-dimethylurea and dibromothymoquinone. (iii) The transcript levels of all *hox* genes are increased in anoxia, with additional induction of *hoxEF* in darkness or in the presence of dibromothymoquinone. (iv) Plastoquinone pool redox changes are not correlated with *hox* transcript level changes. (v) Changes in the transcript levels of *lexA* and *sll0359* genes, encoding putative regulators of *hox* genes, are only partly correlated with transcript changes of *hox* genes under different conditions. Our data demonstrate a previously unrecognized light- and oxygen-dependent regulation of *hox* gene transcription in *Synechocystis* PCC 6803, which is related to photosynthetic electron transport and to unidentified oxygen and redox sensors. We also conclude that neither LexA nor Sll0359 are likely to be exclusive regulators of *hox* gene transcription.

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## 1. Introduction

In the recent years considerable attention has been focused on $H_2$-producing cyanobacteria in the hope that photosynthetic water splitting could be coupled to reduction of protons to generate molecular hydrogen at the expense of solar energy. The cyanobacterium *Synechocystis* sp. PCC 6803 (referred as *Synechocystis* throughout the text), which is a favourite organism of research due to its available genomic sequence (Kaneko et al., 1996), and easy transformation, contains a NiFe-type reversible hydrogenase (Appel and Schulz, 1996; Kaneko et al., 1996), that catalyses the simple $H_2 \leftrightarrow 2H^+ + 2e^-$ redox reaction. Different lines of suggestions are available for possible functions of the bidirectional hydrogenase in *Synechocystis*; i.e. functioning as an electron valve during the light reaction of photosynthesis (Appel et al., 2000), involvement in fermentation (Antal and Lindblad, 2005; Troshina et al., 2002), and being part of the respiratory complex (Appel and Schulz, 1996). However, in situ physiological function for this enzyme in *Synechocystis* is still unclear.

The bidirectional hydrogenase is considered to be a heteropentameric enzyme and encoded by the *hoxEFUYH* genes (Schmitz et al., 2002). Of the five subunits HoxYH constitute the hydrogenase part, whereas HoxEFU constitute the diaphorase part. In *Synechocystis* the *hox* genes together with three additional ORFs are localized in one cluster (Kaneko et al., 1996), which has been suggested to form one transcriptional unit (Oliveira and Lindblad, 2005; Gutekunst et al., 2005). The hydrogen metabolism in cyanobacteria has been shown to be under the control of various environmental factors, such as oxygen, hydrogen, nickel availability (Axelsson and Lindblad, 2002; Oxelfelt et al., 1995), nitrate and sulfur limitations (Antal and Lindblad, 2005; Sheremetieva et al., 2002; Schütz et al., 2004; Zhang et al., 2008), and NAD(P)/NAD(P)H ratio (Cournac et al., 2004; Vignais et al., 2002).

The activity of the bidirectional hydrogenase is correlated with the transcript level of its coding *hox* genes (Antal et al., 2006). Therefore, changes in the *hox* transcript level may provide important information for understanding the function of the bidirectional hydrogenase under various environmental conditions. However, literature data regarding the regulation of *hox* gene transcription in *Synechocystis* are rather complex and only partly clarified. Up to

*Abbreviations:* DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, dibromothymoquinone; GA, glycolaldehyde; PSI, photosystem I; PSII, photosystem II.

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now, two putative regulators have been shown to bind to the promoter region of *hox* operon. Interaction of the LexA protein with two different regions of the *hox* promoter has been demonstrated by two independent studies (Oliveira and Lindblad, 2005; Gutekunst et al., 2005). On the basis of these data LexA has been proposed as a direct transcriptional regulator of *hox* gene expression. However, recent data show that LexA cannot account exclusively for the regulation of the *hox* genes, and another protein, encoded by the *sll0359* gene, has been shown to interact with the *hox* promoter region and was suggested to act as a positive regulator of *hox* gene expression (Oliveira and Lindblad, 2008).

In the present work we show light- and oxygen-dependent regulation of the expression of *hox* genes, as well as the possible involvement of photosynthetic electron transport in these regulatory events. Our experiments suggest that neither *lexA* nor *sll0359* are exclusive regulators of *hox* gene expression, and we propose the existence of independent regulatory pathways acting on the transcription of the bidirectional hydrogenase of *Synechocystis*.

## 2. Materials and methods

### 2.1. Strain and growth conditions

*Synechocystis* sp. PCC 6803 cells were propagated in BG-11 growth medium in a rotary shaker at 30 °C under 3% $CO_2$-enriched atmosphere. The intensity of white light during growth was 40 μE $m^{-2}$ $s^{-1}$. Cells in the exponential growth phase ($A_{580} \sim 1.7$, ~10 μg Chl $mL^{-1}$) were used.

Microaerobic conditions were achieved by incubating the cell suspension in closed conical flasks in the presence of an oxygen scavenging enzyme mix containing 5 mM glucose, 200 U glucose oxidase and 2000 U catalase. Under our experimental conditions the oxygen content in the cultures was below 1 μmol/L as monitored with an immersible oxygen electrode (Presens, Fibox 3).

### 2.2. Variable fluorescence induction

The so-called PEA-type variable chlorophyll fluorescence was measured by a fast fluorimeter (FL 3500/F, PSI, Czech Republic) in the 10 μs to 1 s time region using a logarithmic time scale. The measurements were performed in a 1 cm cuvette at 10 μg Chl $mL^{-1}$.

### 2.3. Gene expression analysis

10 mL of samples were harvested by centrifugation and total RNA was isolated by hot phenol method (Mohamed and Jansson, 1989) with minor modifications. The crude RNA was further purified and freed from DNA contamination using NucleoSpinRNA kit (Macherei Nagel, Düren, Germany). 2 μg of the RNA was reverse transcribed using H-MuLV (Fermentas) and random hexamer primers. Concentration of total RNA was determined by NanoDrop (ND-1000 Spectrophotometer) measurements. Aliquots of the resulted cDNA were used in the RT-PCR reaction as template. Quantitative RT-PCR was carried out on an ABI 7000 Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) using SYBR green PCR Master mix of the same manufacturer. Primer pairs for the individual sequences were designed using Primer Express 2.0 program (ABI) as follows:

*hoxF*-F: CTGCAATGCTGACGAAGGC, *hoxF*-R: AACACACTGCGGTCCATGAA, *hoxE*-F: CACCATGAAGCGCAACCA, *hoxE*-R: TGCAGGATTTCAATGAGGGC, *hoxH*-F: AAATTACCCAATCCCACGCC, *hoxH*-R: AATCAGGACTGCTGAGATGGAAA, *hoxU*-F: TGTGCGAGTTTGCGATGAAA, *hoxU*-R: TAAGCCACATCCCAAACGTG, *hoxY*-F: GTGGTTTTCAGTCCCGTTGG, *hoxY*-R: CCACATTGTCCGGGTATTCCT, *lexA*-F: CCGTCATTGGCGAACTCAA, *lexA*-R: TTTCCACTTCCTCTGCGTCC, *sll0359*-F: ACGGCAAAAGCCTGTGGTT, *sll0359*-R: GAAATGTCGGTTCACGACCC.

## 3. Results

### 3.1. Role of light in hox gene expression

Light is an important regulator of cyanobacterial gene expression as shown by a large number of genes which undergo light-induced transcriptional changes (Hihara et al., 2001). It was previously proposed that the *hox* genes encoding the bidirectional hydrogenase in *Synechocystis* are continuously expressed in aerobic conditions (Appel et al., 2000). However, it has not been studied whether light acts as a regulatory factor for *hox* gene expression. In order to assess this question the *mRNA* level of the five *hox* genes, as well as of the genes of their putative regulators (LexA and Sll0359) was followed in parallel with changes of the redox state of photosynthetic electron transport components in *Synechocystis* cells. When light adapted cells were transferred to darkness the expression of *hoxE* and *hoxF* was only slightly affected, while the transcript levels of *hoxU*, *hoxY* and *hoxH* were significantly (3–5-fold) decreased (Fig. 1). Transfer of cells back to growth light resulted in a reversal of dark-induced changes in the mRNA levels in 30 min roughly to the same level as observed in the original light adapted state (Fig. 1). Of the two putative regulators of *hox* gene expression *sll0359* was suppressed in dark and induced in light to a similar extent as *hoxU*, *hoxY* and *hoxH*, however, the *lexA* gene showed the opposite expression pattern than that of the *hox* genes, since it was induced in darkness by up to 3-fold, followed by a decrease in the light (Fig. 1).

Since light-induced transcriptional changes are often related to changes in the redox state of electron transport components the possible role of redox regulation of *hox* gene expression was further studied by using electron transport inhibitors. Glycolaldehyde (GA) inhibits the phosphoribulokinase that synthesizes ribulose-1,5-bisphosphate, therefore acts as an effective inhibitor of the Calvin cycle (Scheme 1). Illumination of cells in the presence of GA leads to highly reduced electron transport chain (see below). Under these conditions the transcript levels of all studied genes dropped significantly within 20 min (Fig. 2). It is important to note, that the suppressed transcription of the *hox* genes was reversed when elec-

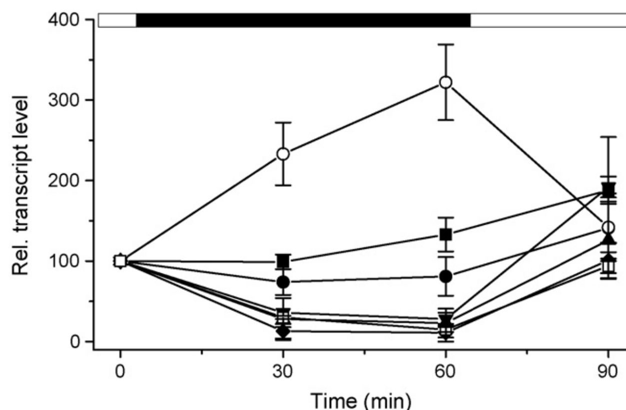


**Fig. 1.** Light-dependent changes of *hox* transcript levels. *Synechocystis* cells after adaptation to growth light intensity (40 μmol $m^{-2}$ $s^{-1}$) were incubated in the dark, and then transferred back to growth light as indicated by the white and black bars, respectively. Transcript abundance changes for *hoxE* (solid squares), *hoxF* (solid circles), *hoxU* (solid up triangles), *hoxY* (diamonds), *hoxH* (solid down triangles), *lexA* (open circles), and *sll0359* (open squares) were determined by RT-PCR and plotted after normalization to their values obtained in the light adapted controls. The data represent mean values and their standard error obtained from 3 to 5 independent experiments.

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