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Regulation of hox genes in the cyanobacterium *Synechocystis* PCC 6806

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Hydrogenases are widespread amongst prokaryotes, and they play a central role in microbial energy metabolism. The hydrogenase of the cyanobacterium *Synechocystis* PCC 6803, which is a unicellular oxygenic photoautotroph cyanobacterium, is a NiFe-type bidirectional enzyme, that can reversibly oxidize hydrogen (Houchins 1984). However, its physiological role has not been clarified.

Throughout the present investigation, we studied the regulation of the hox genes encoding the bidirectional enzyme on the transcript level by quantitative RT PCR, which was carried out as described elsewhere (Kós PB et al. 2008).

The bidirectional hydrogenase is an oxygen sensitive enzyme (Eisbrenner 1981). Oxygen may affect not only the enzyme activity, but also the expression of the hox genes. In order to verify this hypothesis we studied the effect of anaerobiosis on the hox transcript levels. Lowering the oxygen content of the media below 1μM caused induction of the hox genes.

One hypothesis about the function of the bidirectional hydrogenase is that it plays a role in adapting to new environmental conditions, predominantly adjusting to changes in the intensity and/or spectral quality of light (Appel et al. 2000). According to this idea, it is probable, that the hydrogenase is regulated by photosynthetic electron transport, in particular, by the redox poise of one of the electron carriers of the electron transport chain. We tested if this plausible regulation occurs at the transcript level. Obstruction of the linear electron transport by inhibitors during anaerobic treatment did not alter the induction pattern of hox genes. However, blocking the cyclic electron transport increased the level of the first two genes in the operon, while the last three genes were slightly repressed. These data indicate the existence of a transcriptional regulatory mechanism connected to the cyclic electron transport.

The hydrogenase of *Synechocystis* 6803 is encoded by the hoxEFUYH gene cluster (Bothe H. et al.1986) which can be transcribed as a single operon (Appel et al. 2005; Oliveira et al. 2005). During anaerobic induction the intensity of the accumulation of the first two genes in the operon (hoxE, and hoxF) differs from the last three genes (hoxU, hoxY and hoxH), implying that there is an additional transcriptional regulatory mechanism acting on the hox operon, which results in an alteration between the transcript levels of the genes within the operon. We supported this assumption by Northern blot analysis.

It has been shown recently that the transcription factor LexA binds to the untranslated region of the hox operon, and suggested to act as a positive regulator of hox gene expression (Appel et al. 2005; Oliveira et al. 2005). During our experiments we monitored the lexA transcript level in parallel with the hox mRNA level. In most of the cases we could not find correlation between the transcript levels of the hox operon, and its putative transcriptional regulator. Furthermore, we frequently observed that changes in their expression levels were opposite to one another. This result shows that lexA is unlikely to act as a direct transcriptional regulator of hox gene expression. Our data is also in agreement with the recent identification of another transcriptional regulator which is also proposed to bind the hox promoter region (Oliveira et al. 2007).

Appel J, Phunpruch S, Steinmüller K, Schulz R (2000) The bidirectional hydrogenase of *Synechocystis* sp. PCC 6803 works as an electron valve during photosynthesis. Arch Microbiol 173(5-6):333-338.

Eisbrenner G, Roos P, Bothe H (1981) The number of hydrogenases in cyanobacteria J Gen Microbiol 125:383-390.

Gutekunst K, Phunpruch S, Schwarz C, Schuchardt S, Schulz-Friedrich R, Appel J (2005) LexA regulates the bidirectional hydrogenase in the cyanobacterium *Synechocystis* sp. PCC 6803 as a transcription activator. Mol Microbiol 58(3):810-823.

Houchins JP (1984) The physiology and biochemistry of hydrogen metabolism in cyanobacteria. Biochim Biophys Acta 768:227-255.

Kós PB, Deák Zs, Cheregi O, Vass I (2008) Differential regulation of psbA and psbD gene expression, and the role of the different D1 protein copies in the cyanobacterium *Thermosynechococcus elongatus* BP-1. Biochim Biophys Acta 1777(1):74-83.

Oliveira P, Lindblad P (2005) LexA, a transcription regulator binding in the promoter region of the bidirectional hydrogenase in the cyanobacterium *Synechocystis* sp. PCC 6803. FEMS Microbiol Lett 251:59-66.

Oliveira P, Lindblad P (2007) An AbrB-Like Protein Regulates the Expression of the Bidirectional Hydrogenase in *Synechocystis* sp. Strain PCC 6803. J Bacteriol 190(3):1011-1019.

Papen H, Kentemich T, Schmulling T, Bothe H (1986) Hydrogenase activities in cyanobacteria. Biochim 68:121-132.

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The role of nitric oxide (NO), as signalling molecule in root development

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In this work the effects of osmotic stress and exogenous auxin (indole-3-butyric acid, IBA) on root morphology and nitric oxide (NO) generation in roots were compared in pea plants. Five-day old plants were treated with 0, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} or 10^{-9} M IBA or with polyethylene glycol (PEG 6000) at concentrations that determined 0, 50, 100, 200 or 400 mOsm in the medium, during 5 days. NO generation was examined by *in situ* and *in vivo* fluorescence method, using a NO-specific dye, 4,5-diaminofluorescein diacetate (DAF-2DA).