

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 1 μ M 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).

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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).

Increasing concentrations of PEG as well as IBA resulted in shortening of primary root (PR), enhancement of lateral root (LR) number and significant increase of NO generation. Time-dependence investigations revealed that in the case of IBA treatments, the LR number increased in parallel with an intensified NO generation, while elongation of PR was not followed by changes in NO levels. Under osmotic stress, the time curve of NO development was distinct compared to that of IBA-treated roots, since significantly, the appearance of lateral initials was preceded by a transient burst of NO. This early phase of NO generation under osmotic stress was clearly distinguishable from that which accompanied LR initiation. It is concluded that osmotic stress and the presence of exogenous auxin resulted in partly similar root architecture but different time courses of NO synthesis. We suppose that the early phase of NO generation may fulfill a role in the osmotic stress-induced signalization process leading to the modification of root morphology (Kolbert et al. 2008a).

As we already know, NO functions in variable physiological and developmental processes in plants (Bartha et al. 2005; Kolbert et al. 2005) however, the source of this signaling molecule in the diverse plant responses is not well understood. Therefore in our further work we provide genetic and pharmacological evidence that the production of NO is associated with the nitrate reductase (NR) enzyme during IBA-induced lateral root development and under osmotic stress conditions (PEG treatments) in *Arabidopsis thaliana* L. NO production was detected in the NR-deficient *nia1*, *nia2* and *Atnoa1* (former *Atnos1*) mutants of *Arabidopsis thaliana*. As inhibitor for nitric oxide synthase (NOS) N^G -monomethyl-L-arginine (L-NMMA) was applied. Our data clearly show that IBA has increased LR frequency in the wild-type plant and the LR initials emitted intensive NO-dependent fluorescence of the triazol product of NO and DAF-2DA. The presence of increased level of NO was restricted only to the LR initials in contrast to PR sections where it remained at the control level. 200 and 400 mOsm PEG treatments also increased NO fluorescence in roots of *Arabidopsis*. The role of NR in IBA or PEG-induced NO formation in the wild type was shown by the zero effects of the NOS inhibitor L-NMMA. In cases of both treatments the NO synthesis could be inhibited by tungstate treatment, which is a specific inhibitor of NR enzyme. The mutants had different NO levels in their control state (*i.e.* without IBA or PEG treatment), as *nia1*, *nia2* showed lower NO fluorescence than *Atnoa1* or the wild type plant. Finally it was clearly demonstrated that IBA as well as PEG induced NO generation in both the wild type and *Atnoa1* plants, but it totally failed in the NR-deficient mutant. It is concluded that the IBA or osmotic stress-induced NO production is nitrate reductase-associated during lateral root development in *Arabidopsis thaliana* (Kolbert et al. 2008b).

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Comparative anthropological analysis of non-Hungarian skeletal populations from the 16-17th centuries

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The period of the 16th to the 17th centuries was the age of the Turkish occupation of Hungary. Therefore many Hungarians from the southern region of the country escaped to the north, which was not invaded. On the basis of the archaeological and historical data, it is known that appreciable mass of southern Slav populations immigrated from the Balkan-peninsula and settled down mainly to this deserted, empty, southern countryside (Wicker 2006).

The subject of this research is the comparative anthropological examination of these non-Hungarian skeletal populations from the 16-17th centuries. The project has two aims: 1) to describe these populations from an anthropological point of view using osteological age and sex determination, metrical analyses and pathological investigations; 2) to find out the relationship among these non-Hungarian groups and the late medieval Hungarian populations, as well as the origin of the immigrated populations.

The material of this survey is the skeletal population of 6 burial sites (ca. 900 skeletons), which the archaeologists suggest belonged to this immigrated community: Győr-Gabonavásártér, Bácsalmás-Óalmás, Madaras-Bajmoki út, Katymár-Téglagyár, Csávoly-Határ út, Zombor-Repülőtér, (Zombor-Bükkszállás).

To determine the sex and age of death we have used common anthropological methods. The Martin and Saller's (1957) method was applied for measuring the skeletons, and the obtained data have been statistically evaluated with cluster analyses (R Development Core Team 2006). Southern Slav and Romanian series were also involved in the comparison. Paleopathological examinations have been carried out using macromorphological methods, though in certain cases radiographic, histological and molecular biological analyses have been applied as well.