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Effect of Hypoxia on AOX2 Expression in Chlamydomonas reinhardtii

Authors : Maria Ostroukhova, Zhanneta Zalutskaya, Elena Ermilova

Abstract : The alternative oxidase (AOX) mediates cyanide-resistant respiration, which bypasses proton-pumping complexes III and IV of the cytochrome pathway to directly transfer electrons from reduced ubiquinone to molecular oxygen. In Chlamydomonas reinhardtii, AOX is a monomeric protein that is encoded by two genes of discrete subfamilies, AOX1 and AOX2. Although AOX has been proposed to play essential roles in stress tolerance of organisms, the role of subfamily AOX2 is largely unknown. In C. reinhardtii, AOX2 was initially identified as one of constitutively low expressed genes. Like other photosynthetic organisms C. reinhardtii cells frequently experience periods of hypoxia. To examine AOX2 transcriptional regulation and role of AOX2 in hypoxia adaptation, real-time PCR analysis and artificial microRNA method were employed. Two experimental approaches have been used to induce the anoxic conditions: dark-anaerobic and light-anaerobic conditions. C. reinhardtii cells exposed to the oxygen deprivation have shown increased AOX2 mRNA levels. By contrast, AOX1 was not an anoxia-responsive gene. In C. reinhardtii, a subset of genes is regulated by transcription factor CRR1 in anaerobic conditions. Notable, the AOX2 promoter region contains the potential motif for CRR1 binding. Therefore, the role of CRR1 in the control of AOX2 transcription was tested. The CRR1-underexpressing strains, that were generated and characterized in this work, exhibited low levels of AOX2 transcripts under anoxic conditions. However, the transformants still slightly induced AOX2 gene expression in the darkness. These confirmed our suggestions that darkness is a regulatory stimulus for AOX genes in C. reinhardtii. Thus, other factors must contribute to AOX2 promoter activity under dark-anoxic conditions. Moreover, knock-down of CRR1 caused a complete reduction of AOX2 expression under light-anoxic conditions. These results indicate that (1) CRR1 is required for AOX2 expression during hypoxia, and (2) AOX2 gene is regulated by CRR1 together with yet-unknown regulatory factor(s). In addition, the AOX2-underexpressing strains were generated. The analysis of amiRNA-AOX2 strains suggested a role of this alternative oxidase in hypoxia adaptation of the alga. In conclusion, the results reported here show that C. reinhardtii AOX2 gene is stress inducible. CRR1 transcriptional factor is involved in the regulation of the AOX2 gene expression in the absence of oxygen. Moreover, AOX2 but not AOX1 functions under oxygen deprivation. This work was supported by Russian Science Foundation (research grant № 16-14-10004).

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