Signal transduction cascades controlling the expression of NiFe hydrogenases and photosynthetic apparatus in *Thiocapsa roseopersicina*

Ph.D. thesis

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Introduction

Through the 3,000 million years while bacteria have been colonising the Earth, they have evolved marvellous and various mechanisms for adaptation to various environmental conditions. During adaptation, they had to sense the environmental signals and they had to switch on/off sets of pathways by modulating the expression of selected genes or operons.

Thiocapsa roseopersicina belongs to the group of photosynthetic sulfur purple bacteria, *Chromaticeae* of the γ-subdivision. The members of the *Chromaticeae* family are able to photosynthesize under anoxic conditions, to utilize reduced sulfur compounds, as electron donors, and to fix nitrogen.

In anaerobic, phototrophic, purple sulphur and non-sulphur bacteria, the presence of oxygen is toxic under light because of the reactive oxygen species. Therefore, multilevel and parallel networks have been developed to repress the operons involved in the formation of photosynthetic apparatus either by direct repression of the operons or by abolishing their activation. In other systems, where the energy conservation is maximal in the presence of oxygen (e.g. *Escherichia coli*), the enzymes involved in different anaerobic metabolic pathways can only be expressed in the absence of oxygen. Decreasing the oxygen tension upregulates the expression of genes coding for enzymes involved in the anaerobic metabolisms and represses operons required for the aerobic ones.

The anoxygenic photosynthesis in the bacteria converts the light energy to ATP via a cyclic electron flow. This process produces energy (ATP) for the microbes, but does not provide electrons for the reduction carbon dioxide, or NAD^+ to $NADH+H^+$, which is a basic electron source in many biochemical reactions. Hence, these photosynthetic microorganisms need additional electron source(s), which might be organic compounds, reduced (sulfur) compounds, hydrogen, etc. Hydrogen is utilized by numerous bacteria, and in these cases H_2 might have dual function: it may provide electrons and also energy for the cells.

To uncover the physiological function of a given protein, investigation of the regulation of its expression might be a very powerful approach. Among the most important bioenergetic and redox processes I focus on the enzymes necessary for the light conversion and hydrogen metabolism as well as the control of their expression in *Thiocapsa roseopersicina*.

My final goal was to identify components involved in photosynthesis/pigment biosynthesis and to establish the regulatory mechanisms controlling the expression of the photosynthetic pigments and various hydrogenases.

Methods

DNA manipulation and analysis was done according to standard techniques or the specifications of the manufacturers. Plasmids were transferred into *T. roseopersicina* and *R. capsulatus* recipient strains using conjugation. Site directed and transposon mutagenesis was applyed to create mutants. Primer extension and RT-PCR were done to map the transcriptional initiation sites and to detect the presence or the continuity of a specific mRNA. Carotenoids were characterized by UV spectrometry after aceton-methanol extraction. LacZ enzyme assay was preformed on cultures permeabilized with toluene. Affinity purification of 6His- tagged proteins was done according to the manufacturers instructions. Protein-DNA interaction was assayed in gel mobility retardation assay. The DNA and protein sequence comparisons to the various databases were done with Web-based and local bioinformatic tools.

Results

I can summarize my results in the following points:

I. I isolated a pigment mutant strain of *Thiocapsa roseopersicina* by plasposon mutagenesis. The plasposon was inserted into the *crtD* gene and the carotenoid composition of the mutant strain corresponded to the aborted spirilloxantin pathway.

II. 19 *orf*s, most of which are thought to be genes involved in the biosynthesis of carotenoids, bacteriochlorophyll and photosynthetic reaction centre were identified in a 22 kbp long chromosomal locus. In addition to the *crtCDEF* genes, I demonstrated the presence of *crtI* gene, hereby describing almost every gene involved in spirilloxantin biosynthesis in *T. roseopersicina* BBS.

III. I could restore the spirilloxantin pathway in the mutant strain by introducing the *crtDC* from *T. roseopersicina*. On the basis of heterologous complementation experiments with the *crtDC* from *R. gelatinosus* it was suggested that the selection between the spirilloxantin and spheroidene route found in purple bacteria is determined by the unique properties of the CrtI and CrtC enzymes.

IV. I showed that expression of the *crtE* and *crtD* genes are repressed by oxygen and mobility shift experiments with purified CrtJ from *R. capsulatus* proposed the role of CrtJ/PpsR type transcription factor in this regulation.

V. The genomic context of the *hyn* operon (the presence of *isp*1 and *isp*2 genes between the structural genes, *hynS* and *hynL*) indicated that the putative electron transferring transmembrane Isp dimer was linked to the hydrogenase. RT-PCR results proved that all the four genes were located on a single message confirming that the gene products are likely to have linked function.

VI. Three transcriptional initiation points were determined at 40, 376 and 393 bp from the start codon of the *hynS* gene. A -24/-12 like promoter structure was recognized preceding the proximal initiation site, but no typical promoter sequences could be identified upstream from the distal ones. This may lead to the identification of new type of promoter sequences.

VII. I demonstrated the role of oxygen on the regulation of *hyn* operon in *T. roseopersicina*, and also in heterologous hosts, *E. coli* and *R. capsulatus*. The same upstream region was shown to be important in each case.

VIII. I proved the importance of FNR in the regulation of *hyn* operon in *E. coli* and *R. capsulatus*. Mutation in the *fnr* gene reduced the reporter activity similar to the level of oxygenic repression in heterologous hosts, suggesting the dominant role of FNR in the regulation of the *hyn* operon.

IX. I isolated the *fnrT* gene from *T. roseopersicina* BBS.

X. I demonstrated that the FNR binding half site located in the upstream activating region is important for the anaerobic activation of the *hyn* operon in *E. coli*. This is a quite new and unusual result as regard as of the FNR interaction with its target DNA.

XI. I observed a strict regulation of the *hup* operon by oxygen, and a RegA binding site was recognized in the upstream region of the *hup* promoter, which might be involved in this regulation.

XII. The expression of the *hup* operon was uneffected in the presence or absence of hydrogen, and it was proven that the response regulator *hupR* gene was essential for the expression of the HupSL.

XIII. I identified the components of the hydrogen sensing signal transduction cascade, which was apparently non-functional. I demonstrated, that lack of the *hupTUV* expression caused the hydrogen independent expression of the *hupSL* genes.

XIV. I could restore the H_2 dependent regulation after introduction of the actively expressed *hupTUV* genes from *T. roseopersicina* and *R. capsulatus*.

Publications

Publications related to the thesis

1. Kovács, Á.T., Rákhely, G., & Kovács, K.L. (2003). Genes involved in the biosynthesis of photosynthetic pigments in the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina. Appl Environ Microbiol* **69**, 3093-3102.

2. Kovács, K. L., Fodor, B., Kovács, Á. T., Csanádi, G., Maróti, G., Balogh, J., Arvani, S. & Rákhely, G. (2002). Hydrogenases, accessory genes and the regulation of [NiFe] hydrogenase biosynthesis in *Thiocapsa roseopersicina. Int J Hydrogen Energy* **27,** 1463-1469.

3. Kovács, Á.T., Rákhely, G., & Kovács, K.L. (2003). Anaerobic, FNR linked regulation of the thermophilic hydrogenase operon in *Thiocapsa roseopersicina* and heterologous hosts. *Submitted to Microbiology, UK***.**

Other publications

4. Maróti, G., Fodor, B. D., Rákhely, G., Kovács, Á. T., Arvani, S. & Kovács, K. L. (2003). Accessory proteins functioning selectively and pleiotropically in the biosynthesis of [NiFe] hydrogenases in *Thiocapsa roseopersicina. Eur J Biochem* **270,** 2218-27.

5. Fodor, B., Rákhely, G., Kovács, Á. T. & Kovács, K. L. (2001). Transposon mutagenesis in purple sulfur photosynthetic bacteria: identificationof *hypF*, encoding a protein capable of processing [NiFe] hydrogenases in alpha,beta, and gamma subdivisions of the proteobacteria. *Appl Environ Microbiol* **67,** 2476-83.

6. Dahl, C., Rákhely, G., Pott-Sperling, A. S., Fodor, B., Takács, M., Tóth, A., Kraeling, M.,Győrfi, K., Kovács, Á., Tusz, J. & Kovács, K. L. (1999). Genes involved in hydrogen and sulfur metabolism in phototrophic sulfur bacteria. *FEMS Microbiol Lett* **180,** 317-24.

7. Fodor, B. D., Kovács, Á. T., Csáki, R., Hunyadi-Gulyás, É., Klement, É., Maróti, G., Mészáros, L. S., Medzihradszky, K. F., Rákhely, G., Kovács, K. L. (2003). Modular broad-host-range expression vectors for the purification of proteins and protein complexes and their application. *Submitted to Appl Environ Microbiol.*

8. Rákhely, G., Kovács, Á. T., Maróti, G., Fodor, B. D., Csanádi, Gy., Latinovics, D., Kovács, K. L. (2003). A cyanobacterial type, heteropentameric NAD^+ reducing [NiFe] hydrogenase in the purple sulfur photosynthetic bacterium, *Thiocapsa roseopersicina. Submitted to Appl Environ Microbiol.*

Posters

1. Kovács, Á. T., Rákhely, G., Balogh, J., Maróti, G., Latinovics, D., Kovács, K. L. (2003). Characterization of signal transduction cascades responsible for the control of the expression of NiFe hydrogenases and photosynthetic apparatus in purple sulfur

photosynthetic bacteria. Poster on 11th International Symposium on Phototrophic Prokaryotes. 24-29 August, Tokyo, Japan.

2. Kovács, Á. T., Rákhely, G., Moura, J., Balogh, J., Maróti, G., Kovács, K. L. (2002). Regulatory mechanisms controlling the expression of genes encoding the membrane bound hydrogenases in *Thiocapsa roseopersicina.* Poster on COST 841 Workshop on the Biosynthesis and regulation of hydrogenases. 5-8 October, Cercedilla, Spain.

3. Fodor, B. D., Rákhely, G., Kovács, Á. T., Kovács, K. L. (2002). Genes and gene products of the operon (*hynS-isp1-isp2-hynL*) coding for the stable hydrogenase of *Thiocapsa roseopersicina.* Poster on COST 841 Workshop on the Biosynthesis and regulation of hydrogenases. 5-8 October, Cercedilla, Spain.

4. Maróti, G., Fodor, B. D., Rákhely, G., Kovács, Á. T., Arvani, S., Kovács, K. L. (2002). Unusual features and organization of the [NiFe] hydrogenase accessory genes in *Thiocapsa roseopersicina*. Poster on COST 841 Workshop on the Biosynthesis and regulation of hydrogenases. 5-8 October, Cercedilla, Spain.

5. Rákhely, G., Csanádi, Gy., Fodor, B. D., Kovács, Á. T., Maróti, G., Latinovics, D., Kovács, K. L. (2002). Characterization of the operon coding for the soluble heteropentameric [NiFe] hydrogenase in the photosynthetic bacterium, *Thiocapsa roseopersicina*. Poster on COST Action Workshop on Biosynthesis and regulation of hydrogenases. 5-8 October, Cercedilla, Spain.

6. Kovács Á. T., Rákhely, G., Kovács, K. L. (2002). Global signal transduction cascade coupled to anaerobiosis regulates the expression of the stable hydrogenase in *Thiocapsa roseopersicina*. Poster on Biohydrogen 2002 Conference. 21-24 April, Ede, Holland.

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10. Fodor, B., Rákhely, G., Kovács, Á., Kovács, K. L. (2000). The *hypF* gene of *Thiocapsa roseopersicina* is pleiotropic in its host, and functions universally in other bacteria. (PR8.) Poster on Proc. 6th Int. Conf. on the Molecular Biology of Hydrogenases. 5-10 August, Potsdam, Germany.