Summary of Ph.D. thesis

Functional and regulatory characterization of the *ntrPR* operon of *Sinorhizobium meliloti*

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Introduction

The recent expansion of the microbial DNA and protein databases that followed the sequencing of a high number of prokaryotic genomes, promoted the identification of a large number of toxin-antitoxin (TA) modules present in bacterial plasmids and chromosomes.

The first TA modules were identified on plasmids acting as post-segregational killing systems. Their function was to prevent the proliferation of plasmid-free progeny. Subsequently, TA loci were also found on chromosomes and were considered to be associated with the modulation of the global level of translation under various stress conditions. A typical TA module consists of two small genes that form an operon, in which the first gene determines an unstable antitoxin and the second gene, a stable toxin protein. The two proteins form a complex, thus the antitoxin prevents the lethal effect of the toxin. Under stress conditions, the antitoxin is degraded by proteases, and the activity of the free toxin results in the inhibition of translation.

Seven TA gene families were described; one of them is the most abundant *vapBC* family, present in Gram-positive and Gram-negative bacteria as well as in Archaea. The antitoxin protein of this family is an AbrB/MazE homolog and the toxin partner belongs to the PIN domain family.

Previously we have identified the *ntrPR* operon in *Sinorhizobium meliloti* and showed that the organization and domain structure of this operon resembled those of the TA modules belonging to the *vapBC* family. As was demonstrated under symbiotic conditions, a Tn5 insertion in the *ntrR* gene resulted in increased transcription of nodulation and nitrogen fixation genes as compared to that of the wild type strain, and this effect was more pronounced in the presence of an external ammonium source. We supposed that the NtrR protein has a role in the nitrogen regulation of the *nod* and *nif* genes. However, when the gene expression pattern of the entire genomes of the wild type and *ntrR* mutant strains were compared, an unexpectedly large number of genes exhibited altered

expression in the mutant strain, suggesting a more general function for NtrR.

Objectives

Our aim was to examine whether or not, the NtrP and NtrR proteins represent an active TA system, based on their autoregulatory properties, complex formation and function; and to determine the possible physiological role of this operon in *Sinorhizobium meliloti*.

Results

The autoregulatory functions of NtrPR have been investigated by using different methods: measurements of the *ntrPR* promoter activity, electrophoretic mobility shift assays and DNase I footprinting.

We have shown that the the *ntrPR* operon exhibits the characteristic regulatory circuit of bacterial toxin-antitoxin modules: the antitoxin NtrP is able to recognize a DNA segment in the promoter region of the *ntrPR* operon, but its binding is weak. The toxin component alone is not

able to bind to the same DNA region, but the complex of NtrP and NtrR strongly binds to the promoter region resulting in the negative autoregulation. The N-terminal part of NtrP is responsible for the interaction with the promoter DNA, whereas the C-terminal part is required for protein-protein interactions.

NtrR toxicity assays revealed that the expression of this protein results in the inhibition of cell growth and colony formation.

Experiments were performed to determine the induction of the *ntrPR* module in *Sinorhizobium meliloti*. ppGpp, an inducer of the *mazEF* TA family had no effect on the *ntrPR* operon, whereas antibiotics that inhibit transcription or translation were shown to have a weak effect on this module.

If the toxin-antitoxin modules are considered to function as metabolic stress managers, *Sinorhizobium meliloti* can be a valuable test organism for studying such systems. The metabolism of this symbiotic nitrogen fixing bacterium is influenced by a wide-range of conditions under which it is capable to survive: it can be found in the

soil as a free-living bacterium, or it occupies the nodules developed on the roots of leguminous plant, it is able to live under aerobic conditions or function under microoxic conditions as a bacteroid, the differentiated form of the bacterium. Another distinct feature of *Sinorhizobium meliloti* is the special metabolism in the symbiotic state: the carbon source utilized by the bacteroids in the form of dicarboxylic acids is supplied by the plant metabolism to fuel nitrogen fixation, while in exchange, fixed nitrogen is transferred from the bacteroid to the plant cell and the ammonium assimilation is inactive in the bacteroids.

Toxin-antitoxin systems present on the chromosome of the bacterium may have an important role during the transition from one way of life to the other, and may determine the adaptation to the varying environmental conditions.

Previous experiments that demonstrated the higher expression of *nod*, *nif* and *fix* genes in the *ntrPR* mutant compared with the *Sinorhizobium meliloti* 1021 wild type under symbiotic conditions and the wide range of transcriptional changes in the genome of this bacterium,

suggest that the *ntrPR* operon may help this organism to cope with the metabolic stress induced by the transition from free-living to symbiotic state, since this transition involves drastic metabolic changes.

Based on protein homologies, domain architectures and gene neighborhood analysis we could identify 17 TA modules belonging to different gene families in the genome of *Sinorhizobium meliloti* strain 1021.

Ten of the 17 modules are members of the *vabBC* gene family and all of them are located on the bacterial chromosome. What is the reason for encoding such a high number of *vapBC* modules and why only the chromosome carries complete modules, whereas the plasmids encode only solitary and probably inactive toxins?

Taking into account the possible physiological role of these modules in stress management, their presence in high number in the genome of *Sinorhizobium meliloti* may not be surprising. The TA systems may be involved in helping these bacteria to cope with metabolic transitions.

Summarizing our results, in these experiments we characterized the first TA module in a symbiotic bacterium. In addition, we presented new information on the *vapBC* gene family, by determining the DNA binding ability of proteins containing an AbrB/SpoVTdomain. Data on the involvement of the *ntrPR* module in the adaptation to symbiotic state opens new perspectives on how Rhizobia manage to adjust their metabolic processes in accordance with the variable environmental requirements.

Publications

Bodogai M, Ferenczi S, Bashtovyy D, Miclea P, Papp P, Dusha I., The *ntrPR* operon of *Sinorhizobium meliloti* is organized and functions as a toxin-antitoxin module. Mol Plant Microbe Interact. 2006 Jul;19(7):811-22

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Posters

Bodogai, M., Cinege, Gy., Dusha, I., 2003: The regulatory cascade of *nif-fix* genes is under the control of *ntrR* in *Sinorhizobium meliloti*, The 5th Hungarian Genetic Congress, Siofok, 2003

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Bodogai M., Miclea S. P., Becker A., Puskas L., Dusha I.: Az *ntrR* gén transzkripciós szintet modulaló

hatása *Sinorhizobium meliloti*ban, The 6th Hungarian Cell and Development Biology Congress, Eger, Hungary, 2005

Bodogai M., Ferenczi Sz., Miclea S.P., Papp P., Dusha I.: A toxin-antitoxin module in *Sinorhizobium meliloti*, 7th European Nitrogen Fixation Conference. Aarhus, Denmark, 2006

Oral presentations

Bodogai M., Ferenczi Sz., Miclea P., Papp P., Dusha I.: The *ntrPR* operon of *Sinorhizobium meliloti* is organized and functions as a toxin-antitoxin module, Straub-days, Szeged, Hungary, 2005

Bodogai M., Ferenczi Sz., Miclea S.P., Papp P., Dusha I.: A toxin-antitoxin module in *Sinorhizobium meliloti*, 7th European Nitrogen Fixation Conference. Aarhus, Denmark, 2006

Bodogai M., Ferenczi Sz., Miclea S.P., Papp P., Dusha I.: Toxin-antitoxin modules and symbiosis, 15th International Conference on Nitrogen Fixation, Capetown, South Africa, January 2007