Summary of the Ph.D. thesis

Atypical transcriptional regulation and function of a new toxin-antitoxin-like module in *Bradyrhizobium japonicum*

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Introduction

The recent availability of a high number of prokaryotic genome sequences promoted the identification of a new type of genetic modules in numerous bacterial plasmids and chromosomes. These are the so-called toxin-antitoxin (TA) modules.

The first TA modules were identified on plasmids acting as post-segregational killing systems, preventing the proliferation of plasmid-free progeny. More recently, many homologues of these plasmid-located TA loci were identified on the chromosome of various bacteria and they become of main interest. They are considered to be associated with the modulation of the global level of translation under various stress conditions.

Typically, TA modules are organized into operons in which the first gene encodes an antitoxin protein and the second gene encodes the toxin. These proteins form a complex, inhibiting in this way the damaging effect of the toxin. Both the antitoxin and the toxin-antitoxin complex are capable of binding to their own promoter and repress the

transcription of the module. Under specific conditions, the unstable antitoxin protein is degraded faster by cellular proteases than the stable toxin protein. Cellular targets are then degraded by the free toxin protein.

TA loci have been grouped into seven families based on protein domain structure and module organization. The most abundant family present in Gram-positive and Gram-negative bacteria as well as in Archaea, the *vapBC*, is composed of a toxin carrying a PIN domain, and an antitoxin containing a DNA binding motif.

Previous work demonstrated that the first identified TA system in Rhizobial species, namely the *vapBC*-type *ntrPR* operon of *S. meliloti*, is involved in the adjustment of bacterial metabolism under symbiotic conditions. Toxin-antitoxin systems of symbiotic bacteria may be important regulators of metabolic rates required for bacterial survival under stressful conditions and/or for the transition from free-living to symbiotic life style.

Objectives

Our aim was to identify and structurally and functionally characterize *vapBC*-type TA modules in the symbiotic nitrogen-fixing bacterium *Bradyrhizobium japonicum*, the microsymbiont of soybean (*Glycine max*).

Results

Based on similar organization, size and sequence homology to the *vapBC*-type TA family, one TA-like module (designated as *bat/bto* operon) was identified in *Bradyrhizobium japonicum*. The *bat/bto* locus consists of an upstream 285 bp gene (*bat*) that encodes a putative antitoxin containing a Phd/YefM like domain and a downstream 426 bp gene (*bto*) that encodes a putative toxin presenting a PIN protein domain. The translational start codon for *bto* overlaps with the last base of the translational stop codon of *bat*, strongly indicating a translational coupling.

Further sequence analysis revealed that the *bat/bto* operon may be composed of two evolutionarily independent

modules coupled by a transition region. The *bat/bto* operon seems to be the product of a recombination event between the *vapBC* (SpoVT/AbrB domains) and *phd/doc* families of TA systems, presenting a mixture of Phd and SpoVT-type antitoxin and a PIN domain-type toxin.

The negative autoregulation is characteristic for most of the TA systems. In contrast, the expression level determined from a *bat/bto* promoter-*lacZ* fusion was higher in the wild-type background than in the mutant strain, suggesting a positive role for the toxin-antitoxin complex. The putative promoter region of the *bat/bto* operon in *B. japonicum* overlaps the putative promoter region of the neighbouring *glpD* gene. We identified two putative *glpD*-repressor binding regions in this intergenic sequence, and one of them partially overlapped with a direct repeat sequence which may represent potential binding site for the toxin-antitoxin complex. We supposed that the regulation of the *bat/bto* system may be influenced by a competition of the toxin-antitoxin complex and *glpD*-repressor for the overlapping binding sites. Indeed, we demonstrated that the expression of the *bat/bto* module was positively influenced by the inhibition of the *glpD*-repressor binding.

The expression of Bto toxin in *E. coli* cells did not produce any damaging effect, while the ectopic expression of

either the Bat antitoxin or the complete module resulted in a remarkable loss of cell viability. This suggests that the Bto protein may have lost its toxic function characteristic for the TA modules.

Deletion of the *bat/bto* operon resulted in the alteration of various metabolic pathways in the mutant bacteria. The generation time of the mutant was considerably decreased in media containing complex sources of carbon and nitrogen, but the mutant cells were unable to grow in minimal medium. Different imaging techniques (AFM, LSM) revealed the altered (shorter and wider) shape of mutant cells. Force measurements with the help of AFM indicated a softer cell surface for the mutant, suggesting considerable changes of the mutant membrane.

The lipopolysaccharide production of the mutant was four-fold lower than that of the wild type cells, and resulted in the synthesis of mainly incomplete molecules. By determining the fatty acid and phospholipid components, remarkable differences were observed in the membrane composition of the wild type and mutant strains, which may explain the observed phenotypic properties of the mutant bacteria. In the wild type strain, 80% of the total fatty acids were represented by *cis*-vaccenic acid. In contrast, a variety of fatty acids were present in the mutant membranes. The analysis of

phospholipid content revealed the absence of phosphatidylcholine, and the increased amount of cardiolipin and phosphatidylethanolamine in mutant membranes. The higher amount of cardiolipin domains may greatly contribute to the increased division rate. Changes in the division rate and cell shape may also be provoked by the alterations in the cell wall elongation and in the membrane composition.

Today, the generally accepted idea about the function of these chromosomally located systems is that they act as bacterial metabolic stress managers, being associated with the modulation of the global level of translation under conditions of nutrient limitation, or under various stress conditions. In contrast to this hypothesis, *bat/bto* operon of *B. japonicum* seems to be involved in the maintenance of normal physiological state of the cell, modulating the metabolic rates to a level which will assure a longer survival and a better adaptation to environmental conditions.

List of publications

Bodogai M., Ferenczi Sz., Bashtovyy D., **Miclea P**., Papp P., Dusha I. 2006. The *ntrPR* operon of *Sinorhizobium meliloti* is organized and functions as a toxin-antitoxin module. Mol. Plant-Microbe Interact. 19(7): 811-822. **IF: 3.936**

Miclea S. P., and Dusha I. 2007. Toxin-antitoxin modules affect the stress response and metabolism in Rhizobia, Acta Biologica Szegediensis. 51 (2): 137-160.

Bodogai M., Ferenczi Sz., **Miclea S.P.**, Papp P., Dusha I. 2008. Toxin-antitoxin modules and symbiosis. ED: Dakora FD, Chimphango SBM, Valentine AJ, Elmerich C, Newton WE. Sustainable agriculture. SPRINGER, 2008. pp. 237-238.

Miclea S. P., Dusha I. Mutant *Bradyrhizobium* bacteria for the improvement of nitrogen fixation at various leguminous plants, especially at soybean. Submited and registred to Hungarian Patent Office (Magyar Szabadalmi Hivatal) on 15.04.2008 under the number P0700337.

Miclea, S. P., Péter, M., Végh, G., Cinege, G., Kiss, E., Váró, G., Horváth, I., and Dusha, I. 2010. Atypical

transcriptional regulation and role of a new toxin-antitoxin-like module and its effect on the lipid composition of *Bradyrhizobium japonicum*. Mol. Plant-Microbe Interact. 23:638-50. **IF: 4.136**

Posters

Bodogai M., **Miclea S. P**., Becker A., Puskas L., Dusha I.: Az ntrR gen transzkripcios szintet modulalo hatasa *Sinorhizobium melilot*iban, 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 S*inorhizobium 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.

Miclea S.P., Horvath I., Dusha I.,: Toxin-antitoxin like module in *Bradyrhizobium japonicum*. 8th European Nitrogen Fixation Conference, Ghent, Belgium, September 2008.

Lectures

Paul S. Miclea, Dusha Ilona,: Toxin-antitoxin like module in the soybean microsymbiont, 6th Hungarian Genetics Miniconference, Szeged, Hungary, September, 2007.

Miclea, S. P., Péter, M., Végh, G., Cinege, G., Kiss, E., Váró, G., Horváth, I., and Dusha, I.: "Newcomer" gets a leading role in *Bradyrhizobium japonicum*. Straub-days, Szeged, Hungary, 2010.