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DISSERTATION SUMMARY

A toxin-antitoxin module in *Sinorhizobium meliloti*

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The chromosomal *ntrPR* operon of *Sinorhizobium meliloti* encodes a protein pair, which forms a toxin-antitoxin (TA) module; the first characterized functional TA system in *Rhizobiaceae*. 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 toxins and antitoxins of different TA pairs may belong to unrelated superfamilies. Seven TA gene families were described (Gerdes et. al. 2005); one of them is the most abundant *vapBC* family present in Gram-positive and Gram-negative bacteria as well as in Archaea. The operon organization and domain architecture of the TA modules from this family resemble those of the NtrPR proteins: the antitoxin is an AbrB/MazE homolog and the toxin belongs to the PIN domain family.

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. In further experiments TA loci were also identified on chromosomes and were considered to be associated with the modulation of the global level of translation under conditions of nutrient limitation, or under various stress conditions.

We have shown that the autoregulatory functions of *ntrPR* operon are in accordance with other TA systems: the antitoxin NtrP is able to recognize a DNA segment in the promoter region of the *ntrPR* operon, but its binding is weak, resulting in an unstable DNA-protein complex. 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. The NtrR protein plays a role in the stabilization of the complex, but it has no promoter binding activity.

We determined that the binding site of the NtrPR complex is a direct repeat sequence that partially overlaps the transcription site.

Experiments focusing on the possible function of this operon revealed that a Tn5 insertion in the ntrR gene resulted in increased transcription of *nod* and *nif* genes as compared to that of the wild type strain, and this effect was more pronounced in the presence of an external ammonium source (Dusha et al. 1989; Oláh et al. 2001). When the gene expression patterns of the entire genomes of the wild type and *ntrR* mutant strains were compared under oxic and microoxic conditions, an unexpectedly large number of genes exhibited altered expression in the mutant strain (Puskás et al. 2004).

In order to examine a possible toxic function of NtrR, we tested the growth and viability of *E. coli* derivatives carrying plasmids with *ntrR*, *ntrP*, or both genes controlled by the arabinose inducible promoter. NtrR overexpression resulted in the inhibition of cell growth and colony formation, but this effect was counteracted by the presence of the antitoxin NtrP.

These results and our earlier observations demonstrating a less effective down-regulation of a wide range of symbiotic and metabolic functions in the *ntrR* mutant under microoxic conditions and an increased symbiotic efficiency with the host plant alfalfa suggest that the *ntrPR* module contributes to adjusting metabolic levels under symbiosis and other stressful conditions (Bodogai et al. 2006)

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