Talk

A new element involved in the regulation of tetralin degradation genes in *Sphingopyxis granuli* strain TFA

Inmaculada García-Romero (*), Eduardo Santero and Belén Floriano

Centro Andaluz de Biología del Desarrollo (CABD). Universidad Pablo de Olavide. Departamento de Biología Molecular e Ingeniería Bioquímica. Ctra. de Utrera Km 1, 41013. Sevilla (*igarrom@upo.es)

Keywords: small RNA; translational regulation; tetralin degradation

ABSTRACT

Motivation: *Sphingopyxis granuli* strain TFA is a gram-negative bacterium able to grow on the organic solvent tetralin as the sole carbon and energy source. Tetralin is a bicyclic molecule, composed of an aromatic and an alicyclic moiety, which is toxic to bacterial cells as it makes the membrane permeable for ions (protons) and inhibits the respiratory enzymes (Sikkema *et al.*, 1992). In our lab, the metabolic pathway and the specific regulation of genes involved on tetralin degradation (*thn* genes) has been deeply characterized (López-Sánchez *et al.*, 2010 and references therein, Rivas-Marín *et al.*, 2016 and references therein). Regarding the regulation, it is known that structural *thn* genes are induced in the presence of tetralin by ThnR, a LysR-like transcriptional regulator, and ThnY, a ThnR co-activator. Besides, the expression of *thn* genes is under carbon catabolite repression (CCR) by preferential carbon sources, such as β-hydroxybutyrate (β-HB) or sebacic acid. However, not very much is known about the regulatory elements involved in this repression. Synthesis of the carbon storage granule PHB is indirectly involved in CCR on *thn* genes.

Methods: Comparison of the global gene expression in tetralin- and β-HB-grown cells revealed the presence of a small non-coding RNA (sRNA), annotated by Infernal Software 1.1, preferentially expressed in β-HB. Northern Blot analysis and β-galactosidase assays of a chromosomally integrated *suhB::lacZ* transcriptional fusion confirmed the differential expression of this sRNA. Expression of *thn* genes under CCR conditions in a mutant lacking the sRNA was evaluated using a chromosomally integrated *thnC::lacZ* translational fusion. Furthermore, putative targets of the sRNA were detected in vitro by IntaRNA software and the predicted interaction was experimentally validated by RNA-RNA EMSA.

Results: A differentially expressed sRNA has been identified in TFA as belonging to the Rfam family RF00519 (SuhB) (García-Romero *et al.*, 2016). It is a highly conserved sRNA in α-proteobacteria. Under CCR conditions, *thn* genes are partially de-repressed in a mutant lacking SuhB. Furthermore, the 5' UTR of *thnR* mRNA has been identified *in silico* as a target of SuhB. Direct interaction of SuhB at the *thnR* ribosomal binding site has been demonstrated. The high level of ThnR in the SuhB mutant indicates a negative role of SuhB on ThnR translation.

Conclusions: The available data so far indicate that SuhB is one of the elements involved in CCR of *thn* genes in *Sphingopyxis granuli* strain TFA, by blocking the translation of the regulator ThnR.

REFERENCES

Sikkema, J. Poolman, B. Konings, W. N. and De Bont, A. M. (1992). Effects of the membrane action of tetralin on the functional and structural properties of artificial and bacterial membranes. Journal of Bacteriology, 174(9):2986-2992

López-Sánchez, A. Rivas-Marín, E. Martínez-Pérez, O. Floriano, B. Santero, E. (2009). Co-ordinated regulation of two divergent promoters through higher-order complex formation by the LysR-type regulator ThnR. Molecular Microbiology, 73(6):1086-1100

Rivas-Marín, E. Floriano, B. Santero, E. (2016). Genetic dissection of independent and cooperative transcriptional activation by the LysR-type activator ThnR at close divergent promoters. Scientific Reports, 18;6:24538

García-Romero, I. Pérez-Pulido, A.J. González-Flores, Y.E. Reyes-Ramírez, F. Santero, E. and Floriano, B. (2016). Genomic analysis of the nitrate-respiring *Sphingopyxis granuli* (formerly *Sphingomonas macrogoltabida*) strain TFA. BMC genomics, 4;17:93.