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The title of the doctoral dissertation: Functional analysis of the two-component signal transduction system MSMEG6236/MSMEG6238 in *Mycolicibacterium smegmatis* (*Mycobacterium smegmatis*)

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Summary

Both intracellular pathogen *M. tuberculosis* and saprophytic strain *M. smegmatis* must be able to adapt to changes in the growth environment, such as the availability of nutrients, including carbon and nitrogen sources, the presence of antibiotics, heavy metals or elements of the host's immune system. Adaptive systems that allow bacteria to adapt to unfavorable conditions include, among others, two-component signal transduction systems (TCSs), which are involved in many metabolic processes, including the regulation of catabolic pathways, the development of virulence mechanisms or the transport of nutrients.

The number of TCSs found in bacteria varies. Analysis of the genomes of different species of bacteria showed that these microorganisms have an average of 52 TCSs. Most TCSs contain two basic components: the sensor protein, i.e. histidine kinase (HK) and its target regulatory protein (RR), i.e. the response regulator.

The system presents in *M. smegmatis* cells, having no equivalent in mycobacterium tuberculosis, is the two-component MnoSR signal transduction system, which includes the MnoS histidine kinase (MSMEG_6238) and the MnoR response regulator (MSMEG_6236). The genes encoding the MnoSR system form a common operon (*mnoSR*) and their products are involved in the regulation of methylotrophic metabolism in *M. smegmatis*. The analyzes performed as part of the doctoral thesis were to help determine the function of the MnoS and MnoR proteins, which are part of the MnoSR system, in the metabolism of mycobacteria. For this purpose, targeted, unmarked *M. smegmatis* mutants lacking *msmeg_6236*, *msmeg_6238* or both genes were constructed using homologous recombination. The resulting mutants were also complemented with a functional copy of the genes to obtain control strains. The recombinant strains were then analyzed for growth on minimal media containing defined carbon and nitrogen sources. Growth kinetics analysis showed that inactivation of the MnoSR

system impairs the ability of *M. smegmatis* to use alcohols such as 1,3-propanediol, methanol and ethanol as a carbon source, but does not affect the ability to use most mono- and disaccharides except fructose. However, no impairment of growth of recombinant strains was observed on minimal medium with a range of organic and inorganic nitrogen sources. Inactivation of the MnoSR system also did not change the susceptibility of *M. smegmatis* to aminoglycoside antibiotics, tetracyclines, ethambutol and rifampicin. In order to understand the importance of the MnoSR system in the global responses of mycobacteria to limited carbon access, the total RNA sequencing method (RNASeq) of the control strain and the Δ(*mtrA*/*glnR*) mutant grown on a minimal medium with the addition of 2% glucose, 0.1% glucose (starvation) was used. and 0.1% glucose and 2% 1.3-propanediol. The performed transcriptomic analyzes revealed the global response of mycobacteria to carbon starvation, as well as the direct or indirect role of MnoSR in the metabolism of *M. smegmatis* in the conditions of access (2%) or absence (0.1%) of glucose as the only carbon source. The obtained transcriptomic data indicated a significant role of MnoSR in the metabolism of pyruvate, which acts as an energy substrate and is also a source of precursors for numerous biosynthetic processes, such as the synthesis of amino acids, purines, pyrimidines or glucose. In addition, significant changes were observed in the expression of genes encoding enzymes involved in the metabolism of fructose, methane and participating in the pentose phosphate pathway in a strain lacking a functional MnoSR system.

The aim of the second part of the study was to investigate the role of the MtrA response regulator in the regulation of nitrogen metabolism in mycobacteria. Due to the high similarity of the consensus sequences for the basic regulator of nitrogen metabolism in mycobacteria, the GlnR protein and the MtrA protein, it was hypothesized that these proteins may bind the same regulatory sequences and participate in the regulation of genes whose products determine the transport or utilization of nitrogen sources. In vitro studies have shown that the MtrA and GlnR proteins bind to the promoter sequence of the *amtB* gene encoding a protein involved in ammonium transport. The construction of a double mutant devoid of both tested regulatory proteins and the analysis of the ability to grow Δ*mtrA* and Δ(*mtrA*/*glnR*) strains on a minimal medium containing defined nitrogen sources showed that the lack of the MtrA regulator impairs the ability of mycobacteria to use organic nitrogen sources in the form of certain amino acids (histidine , proline, methionine, glutamic acid). In order to determine the global response of the tested mutants to the deficit of nitrogen

available in the medium, transcriptomic studies of the control strain and the Δ*mtrA* and Δ(*mtrA*/*glnR*) mutants growing on a minimal medium containing ammonium sulfate at a concentration of 1 mM were carried out. Analysis of transcriptomic data item preferred to observe a change in the expression of genes whose products are involved in nitrogen transformations in strains lacking a functional MtrA regulatory protein. The greatest changes in gene expression in the Δ*mtrA* mutant were observed for enzymes involved in the metabolism of arginine, purines, tyrosine, as well as alanine, aspartate and glutamate pathways.

The conducted research allowed for a better understanding of the role of the MnoSR system in the regulation of carbon metabolism in mycobacteria and the MtrA response regulator in the regulation of nitrogen metabolism.

Data Signature