The *Mycobacterium smegmatis* MSMEG3765 (TetR-like) protein is involved in stress response

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Mycobacterium tuberculosis is one of the most important human pathogens, being spread in about 1/3 of the whole world population. The pathogenesis of *Mtb* is complex, and involves an elaborate interaction with the host. Pathogenic mechanisms, including the ability to survive in macrophages, within which the bacteria are exposed to various physical and chemical insults, such as acid stress and reactive oxygen (ROI) and nitrogen (RNI) intermediates, are poorly understood.

In a recent study conducted under acid-nitrosative multi-stress conditions that simulated the phagosomal environment (1) two genes, *MSMEG_3765* in *M. smegmatis* and its ortholog *Rv1685c* in *Mtb,* were induced. These genes are annotated as TetR transcriptional regulators. Members of this protein family regulate a wide range of cellular activities, including osmotic stress, biosynthesis of antibiotics, multidrug resistance, virulence and pathogenicity of bacteria.

We have characterized the *MSMEG_3765* transcriptional unit, and partially studied the role of its gene product. *MSMEG_3765* is conserved among mycobacteria, including the pathogens, its deduced amino acid sequence shares an identity of 74% with Rv1685c and 76% with *M. avium paratuberculosis* MAP1391c (Fig. 1)

In the 3 species the TetR coding gene is preceded by two genes with the same transcriptional orientation, annotated as an ABC transporter, and sharing high percentage of identities.

The analysis of the locus in *M. smegmatis* shows that *MSMEG_3760* and *MSMEG_3761,* annotated respectively as hypothetical protein and Clp protease subunit, are transcribed as monocistronic unit, while MSMEG_3765 is the last a of three genes operon (*MSMEG_3762*, *MSMEG_3763* and *MSMEG_3765*) (Fig. 1).

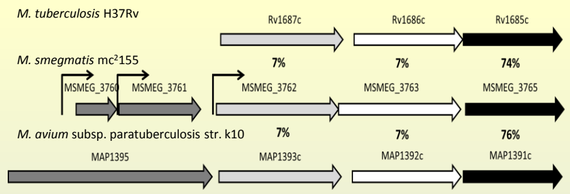
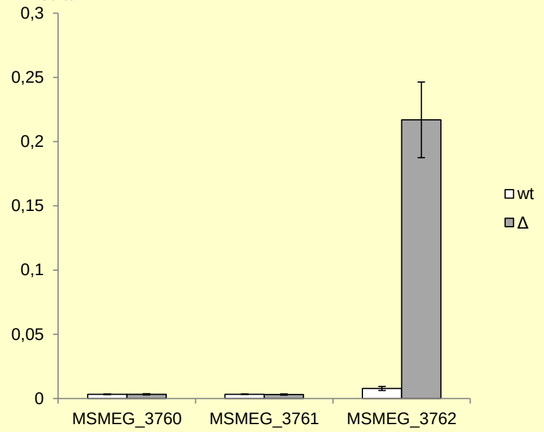


Fig. 1: Organization of the *MSMEG_3765* gene and of its orthologs in *M. tuberculosis* and *M. avium paratuberculosis*.

We constructed a *ΔMSMEG_3765* mutant with a deletion of 453 bp in the coding region, by a two step homologous recombination strategy (2). We performed RT-qPCR experiments on *MSMEG_3762* of wt and *ΔMSMEG_3765* mutant strain under standard growth conditions.

The data indicate that *MSMEG_3762* is up-regulated about 40 fold in the mutant strain while the expression of *MSMEG_3760* and *MSMEG_3761* is not affected by the deletion (Fig. 2).

Fig. 2: transcriptional analysis of *MSMEG_3760*, *MSMEG_61* and *MSMEG_3762* on wt and mutant strain.



A complemented strain was constructed, in which the *MSMEG_3765* coding sequence was cloned in the pMV306hsp vector, expressed under the control of the hsp60 promoter, and introduced in *M. smegmatis* (*ΔMSMEG_3765*). RT-qPCR analysis was performed on wt, mutant and complemented strains.

The data indicate that the level of expression of *MSMEG_3762* are comparable in wt and complemented strain, confirming the role of the MSMEG-3765 protein as repressor of its own operon (Fig. 3).

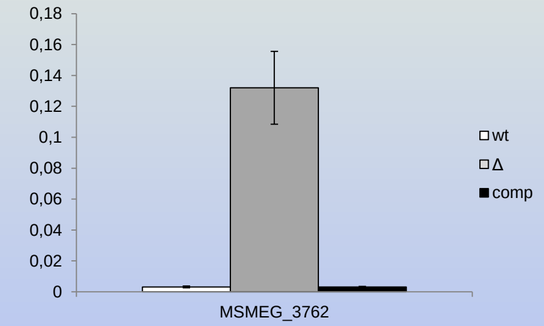


Fig. 3: transcriptional analysis of *MSMEG_3760* on wt, mutant and complemented strain.

In order to perform biochemical and functional analysis, the *MSMEG_3765* gene was cloned in the pet22b+ vector. The C-terminal His-tagged *MSMEG_3765* gene was expressed in *E. coli* BL21(DE3) and purified. Circular dichroism spectroscopy shows that the purified protein is folded (Fig. 4).

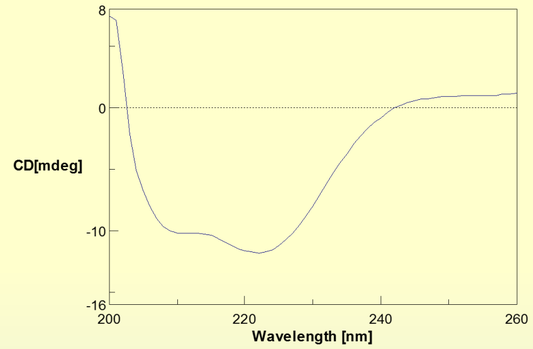


Fig. 4: the spectra obtained by circular dichroism spectroscopy of the purified protein

Conclusion

The *MSMEG_3765* gene product, annotated as a TetR transcriptional regulator, shares 74% identity with the *M. tuberculosis Rv1685c* gene product and 76% identity with the *M. avium subsp. paratuberculosis MAP1391c.* It is reported as upregulated during acid-nitrosative multi-stress conditions. Here we show that *MSMEG_3765* is the last of a three genes operon and codes for a repressor of its own operon.

Future objectives include the identification of the the *MSMEG-3765* DNA-binding site, that may be helpful to clarify if there are other genes regulated by this transcriptional regulator, and the characterization of its putative role in stress response.

References:

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2 Kendall S.L. and Frida R., Construction of a targeted mycobacterial mutants by homologous recomination, Chapter 20, *Mycobacterial Protocols*, T. Parish, A.C. Brown (2009)