Rv3134c/*dos*R/*dos*S OPERON OF *MYCOBACTERIUM BOVIS* BCG IS DEFERENTIALLY TRANSCRIBED UNDER IN VITRO STRESS CONDITIONS

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DosR is a transcriptional regulator that mediates the genetic response of *Mycobacterium tuberculosis* and *Mycobacterium bovis* to oxygen limitation and nitric oxide exposure. The *dos*R operon includes *dos*S, which codes for an oxygen sensor protein, and Rv3134c which contains a universal stress protein domain. Here, we report the transcriptional analysis and quantitative expression of Rv3134c/*dos*R/*dos*S under *in vitro* stress conditions which included *o*xygen limitation, low nutrients and *ex vivo* macrophage infection.

Methods: Aerobic, oxygen-limited and starvation cultures were established and total RNA was isolated along the growth curves. Transcriptional start points (TSPs) were identified using RACE 5' technology. Transcriptional coupling was detected by amplification of the intergenic regions. Real Time PCR was used for quantitation of gene expression.

Results: The operon was overexpressed in hypoxia, with two TSPs identified, one at 120 bp upstream of *dos*R and the other located 40 bp upstream of Rv3134c. In addition, only one transcript was identified from Rv3134c to *dos*S. Under aerobic conditions, only the -120 TSP was detected and *dos*R/*dos*S were co-transcribed while Rv3134c was transcribed from its own promoter. In starvation, another TSP was identified 102 bp upstream *dos*R; no TSP could be detected upstream Rv3134c. Again, *dos*R/*dos*S were cotranscribed independently of Rv3134c. Although RT-qPCR also showed an increase in expression under starvation, this was two orders of magnitude lower than in hypoxia. Finally, DosR was not expressed during the first days of macrophage infection.

Conclusions: The *dos*S/*dos*R/Rv3134c operon is finely regulated and contains several TSPs with upstream putative binding motifs for DosR that suggest a positive transcriptional regulation. The use of different promoters, such as those identified here, probably facilitate adaptation under different environmental stimuli.