Cell-wall synthesis and ribosome maturation are co-regulated by an RNA switch in *Mycobacterium tuberculosis*

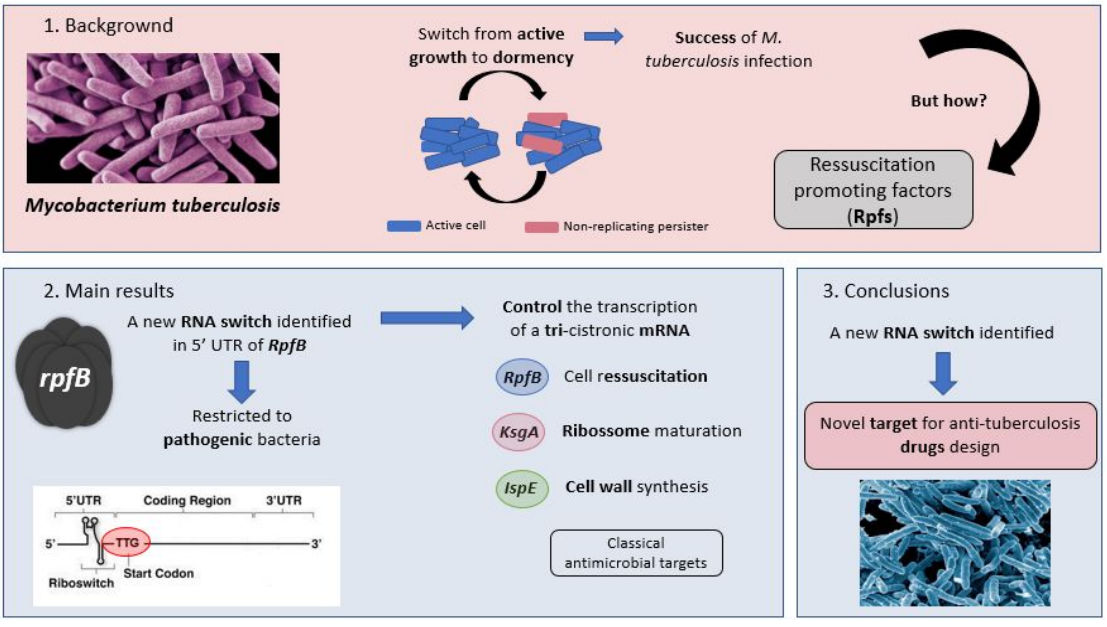
Mycobacterium tuberculosis pathogenesis is related to the bacterial capacity of changing between active replication and persistent state. This pathogen expresses resuscitation-promoting factors (Rpf), which play a role in reactivation of tuberculosis and cell wall remodelling. These mechanisms are highly risky, since they could lead to cellular lysis, so a strict regulation is mandatory. Therefore this work aimed to understand the regulation of the RpfB expression.

To characterize the *rpfB* locus, the authors resorted to RNA-seq, identifying two promoters, P1 and P2, and an antisense RNA expressed from a P_{as} promoter. Additional cloning of mutated promoters into *M. tuberculosis* confirmed that P1 and P2 drive *rpfB* expression.

The authors re-annotated the translation start site (TSS) to a TTG codon upstream, following the detection of an incongruity in the previous annotation. The sequence analysis revealed a potential RNA switch function to the RpfB 5' UTR, which is described for several bacterial gene expression systems. Northern blot showed stronger signal in exponential phase cultures of *M. tuberculosis*, consistent with the possibility of a terminated transcript. In addition, a 3' RACE revealed that a significant part of the 3' termini of the 5' UTR fell within a poly-U tract, strongly favouring the presence of a functional intrinsic terminator. The authors modelled the RNA using *mfold* software, unraveling two conformations for the 5' UTR, one of which contains an intrinsic terminator. Translational reporter fusion assays confirmed that the 5' UTR of RpfB is a RNA switch capable of alternating between two different conformations, providing an additional layer of regulation to RpfB expression.

Finally, RT-PCR analysis revealed that *ksgA* (methyl transferase involved in ribosome maturation) and *ispE* (kinase involved in the cell wall synthesis) are co-transcribed with *rpfB* and belong to the same operon, indicating that the RNA switch regulates the expression of these three genes, therefore linking the regulation of resuscitation, cell wall synthesis and ribosome maturation in *M. tuberculosis.*

In summary, this work disclosed a novel regulatory RNA element, whose switch enables/inhibits the RNA transcription of cell-wall synthesis and ribosome function encoding genes in *M. tuberculosis*. This RNA switch, only present in pathogenic mycobacteria, regulates the expression of essential genes, representing a potential target for anti-tuberculosis drug development. In the future, it remains to be confirmed if this is a riboswitch, and if so, what are its ligands. In addition, since the aim of this work is to find a possible drug target, one other perspective can be to find a way to control the regulation of RpfB so it can be directed to the lysis and death of the *M. tuberculosis* itself.



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