Characterization of RbpA – a master regulator of gene expression from Mycobacterium tuberculosis

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Résumé

RbpA, a RNA polymerase binding transcriptional activator protein from Mycobacterium tuberculosis (Mtb), regulates transcription without binding to the double stranded DNA. RbpA is involved in unwinding of the promoter DNA in transcription complexes containing either the housekeeping sigma factor – sigma A (σA) or the stress-response sigma factor – sigma B (σB). RbpA, predominantly found in actinomycetes, increases the tolerance levels to antibiotics including Rifampicin, most commonly used antibiotic against tuberculosis (the second infection causing highest number of deaths). By using the in-vitro transcription system (IVTS) and electrophoretic mobility shift assays (EMSA), we showed that the action of RbpA is sequence specific, as transcription from the housekeeping sigAP promoter of Mtb requires RbpA for activation, but another housekeeping promoter of B. subtilis, sinP3 doesn't require RbpA for the activation. Furthermore, series of mutations of the nucleotides upstream of the sigAP promoter suppressed the promoter dependency on RbpA. Thus, the fact that RbpA is involved in RNA polymerase - σA and σB mediated transcriptional activation and increased tolerance to rifampicin, corroborates its role in global regulation of the antibiotic-induced stress in Mycobacterium. Hence, a new approach for Mtb, known as Run-off microarray (ROMA), has been elaborated using in-vitro transcription on genomic DNA, for studying the genome-wide regulation of the gene expression by RbpA.

Mots-Clés: Mtb – RbpA – Invitro transcription – sigma factors – ROMA

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