

The Genetic Regulation of The Chromosomal *yefM-yoeB_{Spn}* Toxin-Antitoxin Locus of *Streptococcus pneumoniae*

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Toxin-antitoxin (TA) systems encoded on prokaryotic chromosomes have been found to have effects on genome stability, gene regulation, growth control and programmed cell death [Magnuson (2007) J. Bacteriol. 189: 6089 – 6092]. At least 3 TA loci have been identified in the chromosome of *Streptococcus pneumoniae*. One of these loci, designated *yefM-yoeB_{Spn}*, is homologous to the *yefM-yoeB* TA genes of *E. coli*. Overexpression of the *YoeB_{Spn}* toxin led to cell growth arrest, which could be reversed by expression of its cognate antitoxin *YefM_{Spn}* in both *S. pneumoniae* and *E. coli*. This indicated that *yefM-yoeB_{Spn}* is a functional TA system, [Nieto *et al.* (2007) J. Bacteriol. 189:1266-1278].

In the present study, we demonstrated using reverse-transcriptase PCR of total RNA of *S. pneumoniae* that *yefM-yoeB_{Spn}* were organized in a single operon, which is a norm for TA systems. Experiments using *lacZ* transcriptional fusions in *E. coli* DH5 α showed that the *yefM-yoeB_{Spn}* genes were co-transcribed from two Σ^{70} -type promoters upstream of the *yefM_{Spn}* reading frame, designated *yefMp1* and *yefMp2*. Transcriptional fusion results also indicate that in the presence of the *yefM_{Spn}* reading frame, the promoter activity is increased by up to 3.7-fold. This indicated that the *YefM_{Spn}* antitoxin may possibly function as a transcriptional activator unlike other antitoxins which have been reported to act as transcriptional repressors. However when both the *YefM_{Spn}* antitoxin and the *YoeB_{Spn}* toxin were co-expressed, the *YefM_{Spn}*-mediated activation was negated. Gel shift assays indicate both *YefM_{Spn}* and *YefM-YoeB_{Spn}* complex bind to palindrome 2 (44 bp), which is centered 62 bp upstream of *yefM_{Spn}* and overlapped the *yefMp1* and *yefMp2* promoters whereas no binding was observed for palindrome 1 (46 bp), which is centered 196 bp upstream of the *yefM_{Spn}* start codon. How binding of *YefM_{Spn}* to palindrome 2 activates transcription from the *yefMp1* and *yefMp2* promoters is unknown.