The Genetic Regulation of The Chromosomal \textit{yefM}-\textit{yoeB}\textsubscript{Spn} Toxin-Antitoxin Locus of \textit{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 \textit{Streptococcus pneumoniae}. One of these loci, designated \textit{yefM}-\textit{yoeB}\textsubscript{Spn}, is homologous to the \textit{yefM}-\textit{yoeB} TA genes of \textit{E. coli}. Overexpression of the \textit{YoeB}\textsubscript{Spn} toxin led to cell growth arrest, which could be reversed by expression of its cognate antitoxin \textit{YefM}\textsubscript{Spn} in both \textit{S. pneumoniae} and \textit{E. coli}. This indicated that \textit{yefM}-\textit{yoeB}\textsubscript{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 \textit{S. pneumoniae} that \textit{yefM}-\textit{yoeB}\textsubscript{Spn} were organized in a single operon, which is a norm for TA systems. Experiments using \textit{lacZ} transcriptional fusions in \textit{E. coli} DH5\textordmasculine{a} showed that the \textit{yefM}-\textit{yoeB}\textsubscript{Spn} genes were co-transcribed from two $\Sigma^{70}$-type promoters upstream of the \textit{yefM}\textsubscript{Spn} reading frame, designated \textit{yefM}\textsubscript{p1} and \textit{yefM}\textsubscript{p2}. Transcriptional fusion results also indicate that in the presence of the \textit{yefM}\textsubscript{Spn} reading frame, the promoter activity is increased by up to 3.7-fold. This indicated that the \textit{YefM}\textsubscript{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 \textit{YefM}\textsubscript{Spn} antitoxin and the \textit{YoeB}\textsubscript{Spn} toxin were co-expressed, the \textit{YefM}\textsubscript{Spn}-mediated activation was negated. Gel shift assays indicate both \textit{YefM}\textsubscript{Spn} and \textit{YefM-YoeB}\textsubscript{Spn} complex bind to palindrome 2 (44 bp), which is centered 62 bp upstream of \textit{yefM}\textsubscript{Spn} and overlapped the \textit{yefM}\textsubscript{p1} and \textit{yefM}\textsubscript{p2} promoters whereas no binding was observed for palindrome 1 (46 bp), which is centered 196 bp upstream of the \textit{yefM}\textsubscript{Spn} start codon. How binding of \textit{YefM}\textsubscript{Spn} to palindrome 2 activates transcription from the \textit{yefM}\textsubscript{p1} and \textit{yefM}\textsubscript{p2} promoters is unknown.