GENETIC REGULATION OF THE *YEFM-YOEB* AND *PEZAT* TOXIN-ANTITOXIN LOCI OF *STREPTOCOCCUS PNEUMONIAE*

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GENETIC REGULATION OF THE YEFM-YOEB AND PEZAT TOXIN-ANTITOXIN LOCI OF STREPTOCOCCUS PNEUMONIAE

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Abstract

The genome of *Streptococcus pneumoniae* harbours at least eight putative toxin-antitoxin (TA) loci. Two of these TA loci, *pezAT* and *yefM-yoeB*<sub>Spn</sub>, have previously been shown to be functional and the regulation mechanism of both TA loci was investigated in this study. Like most TA loci investigated, both the *pezAT* and *yefM-yoeB*<sub>Spn</sub> loci were co-transcribed from σ<sup>70</sup>-type promoters. Transcriptional fusion assays demonstrated that the PezA and YefM<sub>Spn</sub> antitoxins served as repressors whereas their respective cognate toxins, PezT and YoeB<sub>Spn</sub>, served as co-repressors to further repress the activities of their promoters. DNAse I footprinting results indicated that the PezA and YefM<sub>Spn</sub> antitoxins bind to palindromic operator sites which overlap their respective promoter regions where they may hinder the binding of RNA polymerase thus resulting in the observed transcriptional repression. On the other hand, the PezA-PezT and YefM-YoeB<sub>Spn</sub> TA protein complexes were able to bind to their respective operator sites with lesser amounts, which indicated that the PezT and YoeB<sub>Spn</sub> toxins served as co-repressors to enhance the binding affinity of their cognate antitoxins, in agreement with the results from the transcription fusion assays and findings from investigations of other TA systems. However, the regulation of *yefM-yoeB*<sub>Spn</sub> appeared to be more complex than *pezAT* or other common TA loci. A BOX mobile element was found within the intergenic region between *yefM*<sub>Spn</sub> and the upstream gene in the *S. pneumoniae* R6 genome. The insertion of the BOX element, termed boxA-C, led to the incorporation of an additional promoter, P<sub>yefM1</sub>, upstream of its original promoter, P<sub>yefM2</sub>. Transcriptional fusion assays indicated that P<sub>yefM1</sub> is a much weaker promoter compared to P<sub>yefM2</sub>. Footprinting assays showed that either the YefM<sub>Spn</sub> antitoxin or the YefM-YoeB<sub>Spn</sub> TA complex binds only to a palindromic sequence that overlapped the –35 region of the P<sub>yefM2</sub> promoter but not to any regions overlapping or surrounding the P<sub>yefM1</sub> promoter. With just the P<sub>yefM2</sub> promoter alone, the YefM<sub>Spn</sub> antitoxin repressed transcription from P<sub>yefM2</sub> both in trans and in cis and this repression was augmented by YoeB<sub>Spn</sub>. However, in the presence of the entire upstream regulatory region, which included boxA-C, P<sub>yefM1</sub> and P<sub>yefM2</sub>, slight repression was observed when yefM<sub>Spn</sub> was expressed in trans, but no further repression was observed when yefM<sub>Spn</sub> and yoeB<sub>Spn</sub> were expressed in trans. Interestingly, when yefM<sub>Spn</sub> was constructed in cis along with the entire promoter region, transcriptional activation was observed, and the activation persisted even in the presence of the entire *yefM-yoeB*<sub>Spn</sub> reading frames in cis. This indicated that the regulation of yefM-yoeB<sub>Spn</sub> may involve cis-acting elements which include the entire promoter region along with the yefM<sub>Spn</sub> reading frame and/or host factors that have yet to be determined. As the boxA-C element is conserved in the genome of all sequenced *S. pneumoniae* strains in the database, it is suggested that the boxA-C element may provide a selective advantage to the host. It is also postulated that P<sub>yefM1</sub> is a constitutive promoter that provided a basal level of transcription to the yefM-yoeB<sub>Spn</sub> locus to enable a faster response to any drastic changes in the environment.
Regulasi Genetik untuk Lokus Toksin-Antitoksin \( yefM-yoeB \) dan \( pezAT \) daripada 
\textit{Streptococcus pneumoniae}

oleh

Chan Wai Ting

\textbf{Abstrak}

Genom \textit{Streptococcus pneumoniae} dijangka mengandungi sekurang-kurangnya lapan lokus toksin-antitoksin (TA). Dua lokus daripada jangkaan ini, iaitu \( pezAT \) dan \( yefM-yoeB_{Spn} \), telah dibuktikan berfungsi and regulasi mekanisme kedua-dua lokus TA ini telah diselidik dalam kajian ini. Seperti kebanyakan lokus TA yang diselidiki, \( pezAT \) dan \( yefM-yoeB_{Spn} \) adalah turut ditranskrip melalui promoter jenis \( \sigma^{70} \). Kajian gabungan transkripsi menunjukkan bahawa antitoksin PezA dan YoeB\(_{Spn}\) berfungsi sebagai represor manakala toksin berkaitan mereka masing-masing, yakni PezT dan YoeB\(_{Spn}\), berfungsi sebagai ko-represor untuk mengurangkan dengan selanjutnya aktiviti promotor masing-masing. Eksperimen DNAse I footprinting menunjukkan bahawa antitoksin PezA dan YefM\(_{Spn}\) mengikat kepada operator palindrom yang menindih dengan promotor mereka masing-masing. Ini kemungkinannya akan menghalang RNA polimerase daripada mengikat kepada promotor tersebut dan oleh yang demikian, pengurangan aktiviti promotor diperhatikan. Sementelahan pula, komplek protin TA PezA-PezT dan YefM-YoeB\(_{Spn}\) berkeupayaan untuk mengikat kepada operator masing-masing dengan kuanti yang lebih kurang. Ini menunjukkan bahawa toksin PezT dan YoeB\(_{Spn}\) berfungsi sebagai ko-represor untuk meningkatkan afiniti pengikatan antitoksin berkaitan mereka terhadap promotor masing-masing. Ini adalah bersetujuan dengan penemuan diselidik dalam kajian gabungan transkripsi serta penyelidikan sistem TA yang lain. Namun demikian, regulasi \( yefM-yoeB_{Spn} \) adalah lebih kompleks daripada \( pezAT \) atau lokus TA yang umum. Unsur mobil BOX telah ditemui di antara \( yefM_{Spn} \) dan gen di hulunya dalam genom \textit{S. pneumoniae} R6. Penyisipan unsur BOX, yang dinamakan boxA-C dalam kajian ini, menyebabkan penambah satu promotor baru, \( P_{yefM1} \), di hulu promotor asalnya, \( P_{yefM2} \). Kajian gabungan transkripsi menunjukkan bahawa \( P_{yefM1} \) adalah promotor yang jauh lebih lemah apabila dibanding dengan \( P_{yefM2} \). Kajian footprinting menunjukkan bahawa antitoksin YefM\(_{Spn}\) atau komplek TA YefM-YoeB\(_{Spn}\) hanya mengikat pada palindrom yang menindih dengan elemen -35 daripada promotor \( P_{yefM2} \) tetapi tidak mengikat kepada mana-mana tempat di sekitar promotor \( P_{yefM1} \). Jika dengan promotor \( P_{yefM2} \) sahaja, antitoksin YefM\(_{Spn}\) didapati mengurangkan transkripsi daripada \( P_{yefM2} \) di \textit{trans} dan juga di \textit{cis}, dan pengurangan aktiviti promotor ini diperkuatkan lagi oleh YoeB\(_{Spn}\). Akan tetapi, kehadiran keseluruh kawasan regulasi yang meliputi boxA-C, \( P_{yefM1} \) dan \( P_{yefM2} \), mengakibatkan hanya sedikit pengurangan aktiviti promoter diperhatikan ketika \( yefM_{Spn} \) diekspress di \textit{trans}, namun tidak ada pengurangan aktiviti promotor yang selanjutnya diperhatikan ketika \( yefM_{Spn} \) di \textit{cis}. Dengan hairannya, ketika \( yefM_{Spn} \) berada bersama-sama dengan seluruh promotor di \textit{cis}, pengaktifan transkripsi diperhatikan, dan pengaktifan ini berterusan walaupun dengan kehadiran \( yefM-yoeB_{Spn} \) di \textit{cis}. Ini menunjukkan bahawa regulasi \( yefM-yoeB_{Spn} \) mungkin melibatkan unsur \textit{cis} yang meliputi keseluruh kawasan promotor bersama-sama dengan \( yefM_{Spn} \) dan/atau faktor lain dalam bakteria yang belum ditentukan. Unsur boxA-C didapati kekal dalam genom di semua strain \textit{S. pneumoniae} dalam pengkalan data. Dengan sedemikian, adalah disarankan bahawa
unsur boxA-C dapat memberi kelebihan selektif kepada bakteria tersebut. Ini juga mencadangkan bahawa $P_{yefM1}$ adalah promotor konstitutif yang memberi transkripsi tahap basal kepada lokus $yefM$-$yoeB_{Spn}$ supaya lokus ini boleh bertindak balas dengan lebih pantas terhadap perubahan kawasan sekitar yang drastik.
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Abbreviations

5'-RACE  5'-Rapid Amplification of cDNA Ends
bp  base pair(s)
cDNA  complementary deoxyribonucleic acid
CSP  competence stimulating peptide
DNA  deoxyribonucleic acid
EDF  extracellular death factor
EMSA  electrophoretic mobility shift assays
FIS  factor for inversion stimulation
FitIS  Fit interaction sequence
FitPP  Fit perfect palindrome
h  hour(s)
IHF  integration host factor
IPTG  isopropyl-β-D-1-thiogalactopyranoside
min  minute(s)
mRNA  messenger ribonucleic acid
OD  optical density
ω-ε-ζ  omega-epsilon-zeta
ONPG  o-nitrophenyl-β-D-galactopyranoside
ORF  open reading frames
PCD  programmed cell death
PIN  PilT N-terminus
ppGpp  guanosine 5′-diphosphate 3′-diphosphate
PPI1  pathogenicity island I
PSK  post-segregational killing
RNA  ribonucleic acid
rRNA  ribosomal ribonucleic acid
RT-PCR  reverse transcriptase polymerase chain reaction
s  second(s)
SDS-PAGE  sodium dodecyl sulfate polyacrylamide gel electrophoresis
SI  superintegron(s)
TA  toxin-antitoxin
tmRNA  transfer-messenger ribonucleic acid
tRNA  transfer ribonucleic acid
UNICEF  The United Nations Children's Fund
UV  Ultraviolet
WHO  World Health Organization
X-gal  5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside