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*

By

Chan Wai Ting

Abstract

The genome of *Streptococcus pneumoniae* harbours at least eight putative toxin-antitoxin (TA) loci. Two of these TA loci, *pezAT* and *yefM-yoeB*$_{Spn}$, 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*$_{Spn}$ loci were co-transcribed from σ^{70}-type promoters. Transcriptional fusion assays demonstrated that the PezA and YefM$_{Spn}$ antitoxins served as repressors whereas their respective cognate toxins, PezT and YoeB$_{Spn}$, served as co-repressors to further repress the activities of their promoters. DNAse I footprinting results indicated that the PezA and YefM$_{Spn}$ 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$_{Spn}$ TA protein complexes were able to bind to their respective operator sites with lesser amounts, which indicated that the PezT and YoeB$_{Spn}$ 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*$_{Spn}$ appeared to be more complex than *pezAT* or other common TA loci. A BOX mobile element was found within the intergenic region between *yefM*$_{Spn}$ 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$_{yefM1}$, upstream of its original promoter, P$_{yefM2}$. Transcriptional fusion assays indicated that P$_{yefM1}$ is a much weaker promoter compared to P$_{yefM2}$. Footprinting assays showed that either the YefM$_{Spn}$ antitoxin or the YefM-YoeB$_{Spn}$ TA complex binds only to a palindromic sequence that overlapped the –35 region of the P$_{yefM2}$ promoter but not to any regions overlapping or surrounding the P$_{yefM1}$ promoter. With just the P$_{yefM2}$ promoter alone, the YefM$_{Spn}$ antitoxin repressed transcription from P$_{yefM2}$ both in *trans* and in *cis* and this repression was augmented by YoeB$_{Spn}$. However, in the presence of the entire upstream regulatory region, which included boxA-C, P$_{yefM1}$ and P$_{yefM2}$, slight repression was observed when *yefM*$_{Spn}$ was expressed in *trans*, but no further repression was observed when *yefM*$_{Spn}$ and *yoeB*$_{Spn}$ were expressed in *trans*. Interestingly, when *yefM*$_{Spn}$ 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*$_{Spn}$ reading frames in *cis*. This indicated that the regulation of *yefM-yoeB*$_{Spn}$ may involve *cis*-acting elements which include the entire promoter region along with the *yefM*$_{Spn}$ 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$_{yefM1}$ is a constitutive promoter that provided a basal level of transcription to the *yefM-yoeB*$_{Spn}$ locus to enable a faster response to any drastic changes in the environment.

Regulasi Genetik untuk Lokus Toksin-Antitoksin*yefM-yoeB* dan *pezAT* daripada *Streptococcus pneumoniae*

oleh

Chan Wai Ting

Abstrak

Genom *Streptococcus pneumoniae* dijangka mengandungi sekurang-kurangnya lapan lokus toksin-antitoksin (TA). Dua lokus daripada jangkaan ini, iaitu *pezAT* dan *yefM-yoeB*$_{Spn}$, telah dibukti 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 promotor jenis σ^{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 kuantiti 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 daripada 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 *S. pneumoniae* R6. Penyisipan unsur BOX, yang dinamakan boxA-C dalam kajian ini, menyebabkan penambahan 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 dibandingkan 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 *trans* dan juga di *cis*, dan pengurangan aktiviti promotor ini diperkuatkan lagi oleh YoeB$_{Spn}$. Akan tetapi, kehadiran keseluruhan kawasan regulasi yang meliputi boxA-C, P$_{yefM1}$ dan P$_{yefM2}$, mengakibatkan hanya sedikit pengurangan aktiviti promoter diperhatikan ketika *yefM*$_{Spn}$ diekspres di *trans*, namun tidak ada pengurangan aktiviti promotor yang selanjutnya diperhatikan ketika*yefM*$_{Spn}$ dan *yoeB*$_{Spn}$ diekspres di *trans*. Dengan hairannya, ketika *yefM*$_{Spn}$ berada bersama-sama dengan seluruh promotor di *cis*, pengaktifan transkripsi diperhatikan, dan pengaktifan ini berterusan walaupun dengan kehadiran *yefM-yoeB*$_{Spn}$ di *cis*. Ini menunjukkan bahawa regulasi *yefM-yoeB*$_{Spn}$ mungkin melibatkan unsur *cis* yang meliputi keseluruhan 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 *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-galactopyraniside
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