# Insights into transcriptional repression of the homologous toxin-antitoxin cassettes *yefM-yoeB* and *axe-txe*

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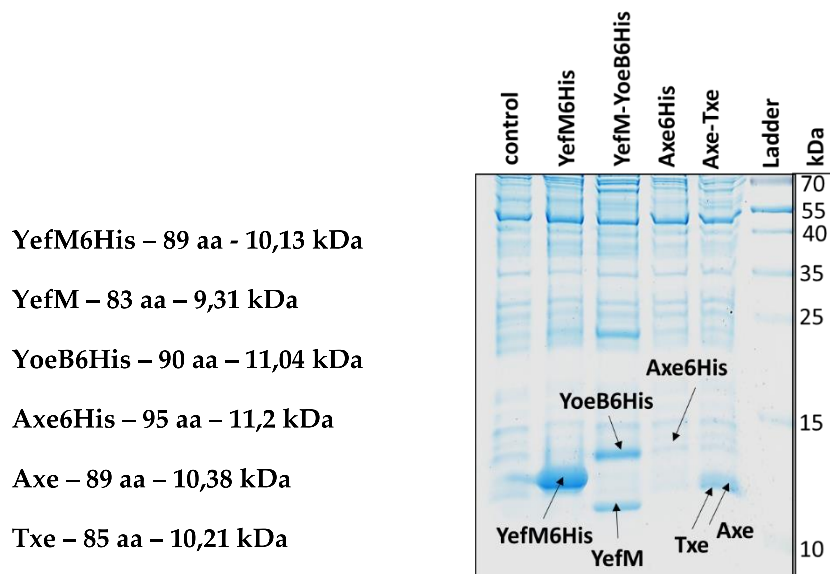


**Figure S1.** 15% SDS-PAGE shows the indicated proteins' overproduction in lysates of BL21(DE3) cells transformed with appropriate pET22(b) derivatives. PageRuler Prestained Protein Ladder (Thermo Scientific) was loaded in the last lane. Appropriate molecular weights of the protein marker are indicated in kDa.

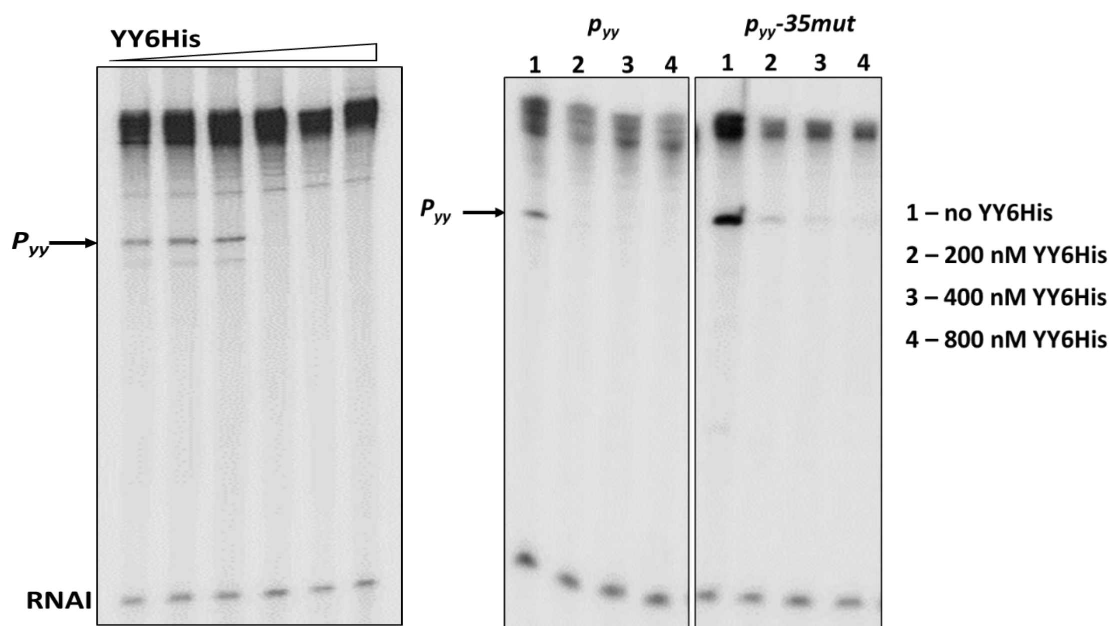


**Figure S2.** Transcriptional repression of $p_{yy}$ by the YefM-YoeB complex *in vitro*. Multiround *in vitro* transcription reactions were performed on 5 nM pTEpyy DNA template, using 25 nM *E. coli* $\sigma^{70}$ RNA polymerase holoenzyme and increasing concentrations of YefM-YoeB6His complex (0, 20, 100, 200, 1000, 2000 nM) – left panel. *In vitro* transcription reactions on pTEpyy and pTEyy-35mut DNA templates with increasing concentrations of YefM-YoeB6His complex (0, 200, 400, 800 nM) – right panels. Reactions were carried out and analyzed as outlined in Materials and Methods.

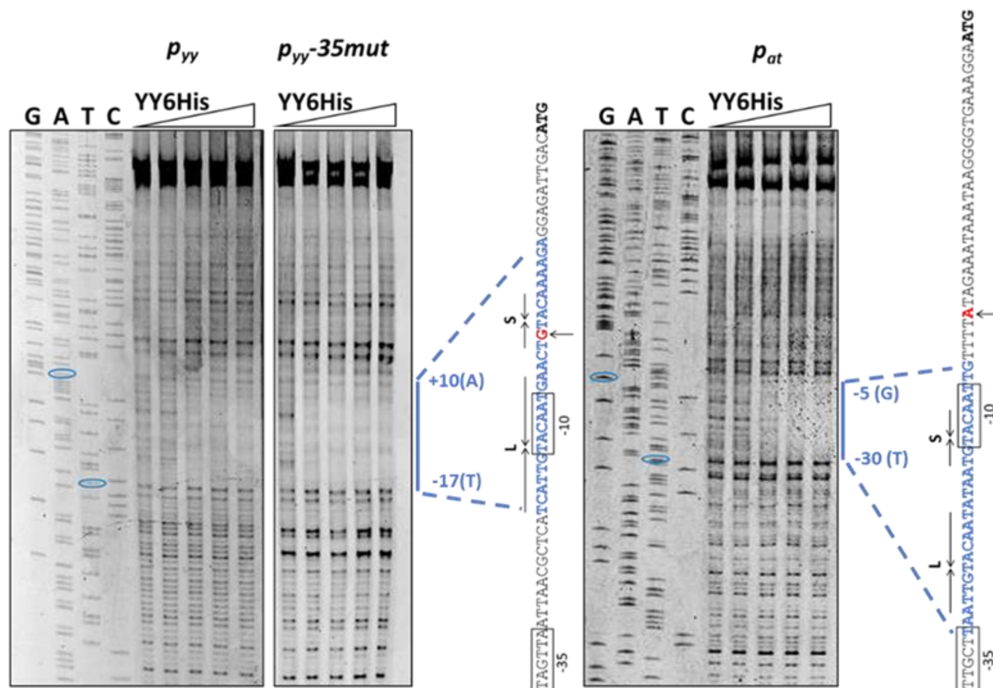


**Figure S3**. DNaseI footprinting of the YefM-YoeB complex on $p_{yy}$ wt, $p_{yy}$-35mut and $p_{at}$ promoter-operator regions. Footprinting reactions were performed as outlined in Materials and Methods using increasing concentrations of YefM-YoeB6His (0, 0.25, 0.5, 1, 2 μM) bound to 20 nM cy5-labeled appropriate DNA fragment for 10 min at 37$^0$C. The samples were then treated with DNaseI and reactions were run on 8% denaturing acrylamide gels along with sequencing reactions (GATC). The relative positions of regions on the upper strand that is protected from DNaseI digestion are illustrated on the right side of the gels.

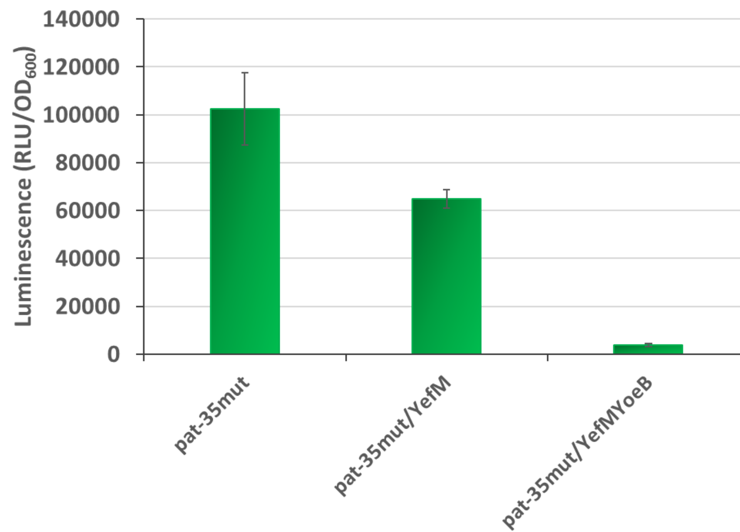


**Figure S4.** Transcription repression of $p_{at}$-35mut mediated by YefM and YefM-YoeB *in vivo.* Transcriptional *lux* gene fusions of $p_{at}$-35mut promoter were made in pBBRlux-amp vector and were introduced into the SC301467 strain together with pBAD33 vector control (for promoter unrepressed conditions – first bars) and pBAD33 derivatives producing YefM or YefM-YoeB complex. Assays were conducted after induction with 0.2% L-arabinose. Luminescence was measured in RLU (relative luminescence units) when cell cultures reached $OD_{600}$~0.5. The basal activity of pBBRlux-amp vector control was ~100 units. These results are average of three independent experiments.

**Table S1.** Plasmids used in this work.

| Plasmid | Description | Source |
|---|---|---|
| pBBRlux-amp | Vector for generating transcriptional fusion to *lux*, *bla* gene was cloned into *EcoR*I site within the *cat* gene, $Amp^R$ | [41] |
| pBBRlux_pat | $p_{at}$ promoter-operator region, digested with *Spe*I-*BamH*I and cloned between equivalent sites in pBBRlux-amp | [41] |
| pBBRlux_pyy | $p_{yy}$ promoter-operator region, amplified with primers 84 and 85, digested with *Spe*I-*BamH*I and cloned between equivalent sites in pBBRlux-amp | This work |
| pBBRlux_pat+1mut | The same as pBBRlux_pat, but $p_{at}$ promoter fragment was amplified with primers 76 and 38 on a pTEpat+1mut template and cloned between *Spe*I and *BamH*I sites | This work |
| pBBRlux_pyy+1mut | The same as pBBRlux_pyy, but $p_{yy}$ promoter fragment was amplified with primers 84 and 85 on a pTE*pyy*+1mut template and cloned between *Spe*I and *BamH*I sites | This work |
| pBBRlux_pat-35mut | The same as pBBRlux_pat, but $p_{at}$ promoter fragment was amplified with primers 76 and 38 on a pTEpat-35mut template and cloned between *Spe*I and *BamH*I sites | This work |
| pBBRlux_pyy-35mut | The same as pBBRlux_pyy, but $p_{yy}$ promoter fragment was amplified with primers 84 and 85 on a pTEpyy-35mut template and cloned between *Spe*I and *BamH*I sites | This work |
| pBAD33 | Arabinose-inducible expression vector, $Cm^R$ | [65] |
| pBADyefM | *yefM* gene cloned into pBAD33 between *Hind*III and *Xho*I restriction sites, under control of L-arabinose inducible promoter | [37] |
| pBADyefMyoeB | *yefM-yoeB* cassette cloned into pBAD33 between *Hind*III and *Xho*I restriction sites, under control of L-arabinose inducible promoter | [37] |
| pTE103 | Vector for generating transcription templates, contains the multicloning site placed upstream from a bacteriophage T7 transcriptional terminator, $Amp^R$ | [66] |
| pTEpat | $p_{at}$ promoter fragment (bearing $p_{at2}$ and $p_{at1}$ sequences) amplified with primers 37 and 38 and cloned between *EcoR*I and *BamH*I restriction sites of pTE103 | This work |
| pTEpyy | $p_{yy}$ promoter fragment amplified with primers 72 and 85 and cloned between *EcoR*I and *BamH*I restriction sites of pTE103 | This work |
| pTEpat+1mut | The same as pTEpat, but transcription start site (+1) of $p_{at}$ was changed from A to G with primers 169 and 170 | This work |
| pTEpyy+1mut | The same as pTEpyy, but transcription start site (+1) of $p_{yy}$ was changed from G to A with primers 167 and 168 | This work |
| pTEpat-35mut | The same as pTEpat, but -35 box of $p_{at}$ was changed from TTGCTT to TAGTTA with primers 165 and 166 | This work |
| pTEpyy-35mut | The same as pTEpyy, but -35 box of $p_{yy}$ was changed from TAGTTA to TTGCTT with primers 163 and 164 | This work |
| pET22b | IPTG-inducible expression vector allowing fusion of C-terminal 6His-tag to the target protein, $Amp^R$ | Novagen |

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|---|---|---|
| pETyefM | *yefM* cloned between *Nde*I and *Xho*I restriction sites in pET22b, resulting YefM with C-terminal 6His-tag | [37] |
| pETyefM-yoeB | *yefM-yoeB* cloned between *Nde*I and *Xho*I restriction sites in pET22b, resulting YefM-YoeB complex with C-terminal 6His-tag on YoeB | [37] |
| pETaxe | *axe* cloned between *Nde*I and *Xho*I restriction sites in pET22b, resulting Axe with C-terminal 6His-tag | [41] |
| pETpat_axe-txe | *axe-txe* (with $p_{at}$ promoter) cloned between *Nde*I and *Xho*I restriction sites in pET22b, resulting Axe-Txe complex (without 6His-tag) | [41] |
| pTEpat1 | Promoter fragment bearing only $p_{at1}$ promoter sequence (without $p_{at2}$ promoter sequence) was amplified with primers 93 and 38 and cloned between *EcoR*I and *BamH*I restriction sites of pTE103 | This work |
| pTEpat2mut-10pat1 | The same as pTEpat, but -10 box of $p_{at2}$ was changed from TAGAAT to CGGAAT with primers 201 and 202 | This work |
| pTCVlac | Broad-host-range low copy number shuttle vector for construction of transcriptional fusion with promoterless *lacZ* gene (bearing Gram+ ribosome binding site) to study regulatory elements in gram positive bacteria, $Kan^R$, $Erm^R$ | [62] |
| pTCVlac_pat1 | Promoter fragment bearing only $p_{at1}$ promoter sequence (without $p_{at2}$ promoter sequence) was amplified with primers 93 and 38 and cloned between *EcoR*I and *BamH*I restriction sites of pTCVlac | This work |
| pTCVlac_pat2pat1 | Fragment bearing $p_{at2}p_{at1}$ promoters sequence was amplified with primers 37 and 38 and cloned between *EcoR*I and *BamH*I restriction sites of pTCVlac | This work |

**Table S2.** Oligonucleotides used in this study. Restriction enzyme sites and mutated positions are underlined.

| Name | Sequence (5′-3′) | Description |
|---|---|---|
| **84** | GAGTACTAGTGGGGAAAGGAGGGGG | Forward primer for amplification of $p_{yy}$ promoter fragment to pBBRlux-amp, *Spe*I restriction site; forward primer for one strand labelled EMSA $p_{yy}$ and $p_{yy}$-35mut promoter fragment |
| **85** | CATTCGGATCCTGACGCGCTTCGCTG | Revers primer for amplification of $p_{yy}$ promoter fragment to pBBRlux-amp and pTE103, *BamH*I restriction site |
| **38** | CCAAGGATCCGAATAAGCTACTGCTTCC | Revers primer for amplification of $p_{at}$ promoter fragment to pBBRlux-amp and pTE103 and pTCVlac, *BamH*I restriction site |
| **76** | GAGTACTAGTGAAAAAGCAGGATTTGAGG | Forward primer for amplification of $p_{at}$ promoter fragment to pBBRlux-amp, *Spe*I restriction site |
| **37** | GAGTGAATTCGAAAAAGCAGGATTTGAGG | Forward primer for amplification of $p_{at}$ promoter fragment to pTE103 and pTCVlac, *EcoR*I restriction site |
| **72** | CGCGGGAATTCGGGGAAAGGAGGGGG | Forward primer for amplification of $p_{yy}$ promoter fragment to pTE103, *EcoR*I restriction site |
| **169** | CAATATAATGTACAATTGTTTTGTAGAAATAAATAAGGG | Forward primer for +1 site mutagenesis of $p_{at}$ promoter |
| **170** | CCCTTATTTATTTCTACAAAACAATTGTACATTATATTG | Reverse primer for +1 site mutagenesis of $p_{at}$ promoter |
| **167** | CATTGTACAATGAACTATACAAAAGAGGAGATTG | Forward primer for +1 site mutagenesis of $p_{yy}$ promoter |
| **168** | CAATCTCCTCTTTTGTATAGTTCATTGTACAATG | Reverse primer for +1 site mutagenesis of $p_{yy}$ promoter |
| **165** | GTTAGAATATTAATCATTAGTTAAATTGTACAATATAATG | Forward primer for -35 box mutagenesis of $p_{at}$ promoter |
| **166** | CATTATATTGTACAATTTAACTAATGATTAATATTCTAAC | Reverse primer for -35 box mutagenesis of $p_{at}$ promoter |
| **163** | CTACAAACTAATTAATAAATTGCTTATTAACGCTCATCAT | Forward primer for -35 box mutagenesis of $p_{yy}$ promoter |
| **164** | ATGATGAGCGTTAATAAGCAATTTATTAATTAGTTTGTAG | Reverse primer for -35 box mutagenesis of $p_{yy}$ promoter |
| **154** | [cy5]AGCTGATGGCACCAAAAATAGCG | Forward primer for cy5 $p_{yy}$ promoter fragment, for DNaseI footprinting and EMSA |
| **155** | [cy5]TTGTTGCCGACAAATTCTGACG | Reverse primer for cy5 $p_{yy}$ promoter fragment for EMSA |
| **156** | [cy5]AGCAGGATTTGAGGTACCAGC | Forward primer for cy5 $p_{at}$ promoter fragment, for DNaseI footprinting and EMSA |
| **157** | [cy5]TCATATAACTACGTAAATTTTGGCGG | Reverse primer for cy5 $p_{at}$ promoter fragment for EMSA |
| **86** | TTTCCCAGTCACGACGTTG | Forward primer for one strand labelled competitive EMSA on $p_{at}$ promoter fragment |
| **181** | ATTAAGGCGCGAGAGATATTCG | Reverse primer for generating $p_{at}$ and $p_{yy}$ fragments for DNaseI footprinting |

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|---|---|---|
| **93** | GATCGAATTCATCATTTGCTTAATTG | Forward primer for amplification of pat1 promoter fragment to clone to pTE103 and pTCVlac vectors, *Eco*RI restriction site |
| **201** | TAATTATAAGAAGTCGGAATATTAATC | Forward primer for mutagenesis of $p_{at2}$ promoter -10 box |
| **202** | GATTAATATTCCGACTTCTTATAATTA | Reverse primer for mutagenesis of $p_{at2}$ promoter -10 box |