SUPPLEMENTARY DATA

Silencing of toxic gene expression by Fis

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Supplementary Figure S1. Factors regulating expression of the mom operon. At the transcriptional level, expression of mom is governed by interplay between host factors Dam and OxyR. Asterisks depict the sites recognised by Dam. Several intrinsic features of mom promoter (see text) make it a weak promoter. The phage protein C acts as a transcriptional activator of mom. Mg\(^{2+}\) is essential for the activity of C protein. Further, at the level of translation, phage protein Com is required to melt the secondary structure in com-mom mRNA that occludes the mom start codon.

Supplementary figure S2. Schematic depiction of mom promoter fragments used in the study. P\(_{\text{mom}}\) (from positions -136 to +79 relative to the +1 start site) (thick line) has been cloned between EcoRI and BamHI sites of pUC19 to generate the plasmid pUW4. The dashed lines on either side denote vector-specific regions. The -35 and -10 promoter elements are boxed and the mom transcription start site indicated. Relevant restriction sites are depicted by arrows. All nucleotide positions are with respect to the +1 start site. Brackets mark the boundaries of various promoter fragments used in the study.
Supplementary Figure S3. Repression of *mom* transcription by Fis. Multiple round transcription of the linear P<sub>mom</sub> fragment (-136 to +79) was carried out in the presence of increasing concentrations of Fis. 40 nM P<sub>mom</sub> template DNA, 100 nM RNAP, 300 nM C and indicated concentrations of Fis were used.

**Supplementary Figure S4. EMSA of P<sub>mom</sub> with purified Fis.** Full length P<sub>mom</sub> (-136 to +79, obtained from EcoRI-BamHI digestion of pUW4) (A) and 60 bp P<sub>mom</sub> fragment (positions -136 to -85) (B) were incubated with increasing concentrations of Fis as indicated. EMSA was performed as described in Materials and Methods.
Supplementary Figure S5. Effect of Fis on in vitro transcription from various \(P_{mom}\) fragments. Multiple round transcription reactions using the full length \(P_{mom}\) transcription template (-136 to +79, lanes 1 and 2) and templates digested with BstUI (-114 to +79, lanes 3 and 4) and Pvul (-66 to +79, lanes 5 and 6) were carried out in the absence or presence Fis. 40 nM template DNA, 100 nM RNAP and 300 nM C were used.
**Supplementary Table 1. Strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> MG1655</td>
<td>$F^{-} \lambda^{-}$ $ilvG^{-}$ $rfb^{-}$ 50 $rph^{-}$ 1</td>
<td>laboratory stock</td>
</tr>
<tr>
<td>BW25113</td>
<td>$rrnB3 \Delta lacZ4787 hsdR514 \Delta (araBAD)567 \Delta (rhaBAD)568 rph^{-}$</td>
<td>30, <em>E. coli</em> Genetic Stock Center</td>
</tr>
<tr>
<td>JW3229-1</td>
<td><em>fis</em> derivative of BW25113</td>
<td>30, <em>E. coli</em> Genetic Stock Center</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>$F^{-}ompT hsdS_{B}(r_{B}^{-} m_{B}^{-}) gal dcm$ (DE3)</td>
<td>laboratory stock</td>
</tr>
<tr>
<td>W3110</td>
<td>$F^{-} \lambda^{-}$ mcrA mcrB</td>
<td>23, a kind gift from Prof. Georgi Muskhelishvili</td>
</tr>
<tr>
<td>DH10B</td>
<td>$\Delta (mrr-hsd rns-mcrBC) mcrA recA1$</td>
<td>laboratory stock</td>
</tr>
<tr>
<td>CSH50</td>
<td>$ara \Delta (lac pro) thi rpsL$</td>
<td>28, a kind gift from Prof. Georgi Muskhelishvili</td>
</tr>
<tr>
<td>CSH50 <em>fis::kan</em></td>
<td><em>fis</em> derivative of CSH50</td>
<td>29, a kind gift from Prof. Georgi Muskhelishvili</td>
</tr>
</tbody>
</table>

**Plasmids**

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUHE25–2<em>fis</em></td>
<td><em>fis</em> gene under the tightly regulated <em>lac</em> promoter pA1–04/03</td>
<td>22</td>
</tr>
<tr>
<td>pVR7</td>
<td>C under T7 promoter in pET11d</td>
<td>20</td>
</tr>
<tr>
<td>pUW4<em>mom</em></td>
<td>220 bp <em>mom</em> promoter fragment cloned between EcoRI-BamHI sites of pUC19</td>
<td>20</td>
</tr>
<tr>
<td>pUW4<em>tin7</em></td>
<td>220 bp <em>tin7</em> promoter fragment cloned between EcoRI-BamHI sites of pUC19</td>
<td>20, 26</td>
</tr>
<tr>
<td>pVN184</td>
<td>C under <em>tet</em> promoter in pACYC184</td>
<td>19</td>
</tr>
<tr>
<td>pLW4<em>tin7</em></td>
<td>Fusion of P$_{tin7}$ (-136 to +79) to <em>lacZ</em></td>
<td>19</td>
</tr>
<tr>
<td>pLW4<em>tin7</em> FBS-49-38</td>
<td>Same as pLW4 <em>tin7</em> except that Fis binding sites centered at -49 and -38 disrupted</td>
<td>This study</td>
</tr>
<tr>
<td>pLW4<em>tin7</em> FBS+3</td>
<td>Same as pLW4 <em>tin7</em> except that Fis binding site centered at +3 disrupted</td>
<td>This study</td>
</tr>
<tr>
<td>pLW4 <em>tin7</em> FBS-49-38+3</td>
<td>Same as pLW4 <em>tin7</em> except that Fis binding sites centered at -49 and -38 and +3 disrupted</td>
<td>This study</td>
</tr>
</tbody>
</table>
Supplementary Table 2. Oligonucleotides used for generating mutagenic megaprimers.

<table>
<thead>
<tr>
<th>Name of the primer</th>
<th>Fis binding site(s) targeted for disruption</th>
<th>Sequence (5’ to 3’)</th>
</tr>
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<tbody>
<tr>
<td>WTFwd</td>
<td>-</td>
<td>CGGTAATACAGATCGATTATGCCCCAATAAACCACACTCAACCCATG</td>
</tr>
<tr>
<td>FBS-49-38Fwd</td>
<td>-49 and -38</td>
<td>CGGTAATACATATCGATTATGCCCCAATAAACCACAGTCAACCCATG</td>
</tr>
<tr>
<td>WTRev</td>
<td>-</td>
<td>TGATTTCATCTCACCTCTTTGCATCAATTGCGCACTATCTTAAC</td>
</tr>
<tr>
<td>FBS+3Rev</td>
<td>+3</td>
<td>TGATTTCATCTCACCTCTTTGCATCAATTGCGCAATATCTTAACA</td>
</tr>
</tbody>
</table>

The G and C residues located at -7 and +7, relative to the center of the core Fis binding site were targeted for mutation to disrupt the Fis binding site. For disrupting Fis binding sites centered at -49 and -38, the primers FBS-49-38Fwd and WTRev were used whereas for disrupting the Fis binding site at +3, WTWd and FBS+3Rev primers were used. All the three Fis binding sites at -49, -38 and +3 were mutated using FBS-49-38Fwd and FBS+3Rev. Mutated residues are highlighted.

Supplementary Table 3. In vivo effect of disrupting Fis binding sites on Fis mediated transcriptional repression

<table>
<thead>
<tr>
<th>pLW4 mutants</th>
<th>Description</th>
<th>Genotype (CSH50 fis/wild-type)</th>
<th>β-galactosidase activity (Miller units)</th>
<th>Fold repression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>All Fis binding sites intact</td>
<td>fis^-</td>
<td>2306.4</td>
<td>4.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wild-type</td>
<td>517.7</td>
<td></td>
</tr>
<tr>
<td>FBS-49-38</td>
<td>Fis binding sites centered at -49 and -38 mutated</td>
<td>fis^-</td>
<td>1309</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wild-type</td>
<td>559.6</td>
<td></td>
</tr>
<tr>
<td>FBS+3</td>
<td>Fis binding site centered +3 mutated</td>
<td>fis^-</td>
<td>2418</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wild-type</td>
<td>981.3</td>
<td></td>
</tr>
<tr>
<td>FBS-49-38+3</td>
<td>Fis binding sites centered at -49 and -38 and +3 mutated</td>
<td>fis^-</td>
<td>691</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wild-type</td>
<td>645.2</td>
<td></td>
</tr>
</tbody>
</table>

*Fold repression is defined as the ratio of β-galactosidase activity produced by a construct (pLW4tin7 wild-type or mutants) in fis^- (CSH50 fis^-) background to that produced in isogenic fis^- (CSH50 wild-type) background strain.