SUPPLEMENTARY DATA

Silencing of toxic gene expression by Fis

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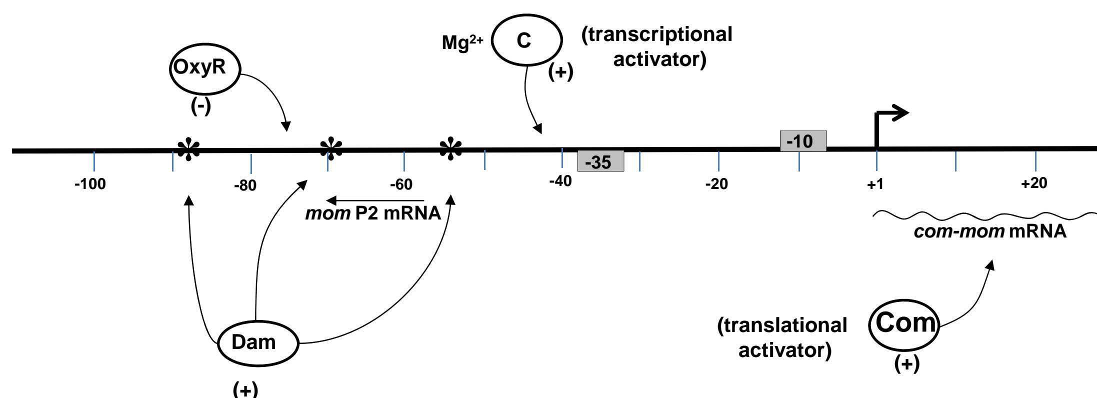
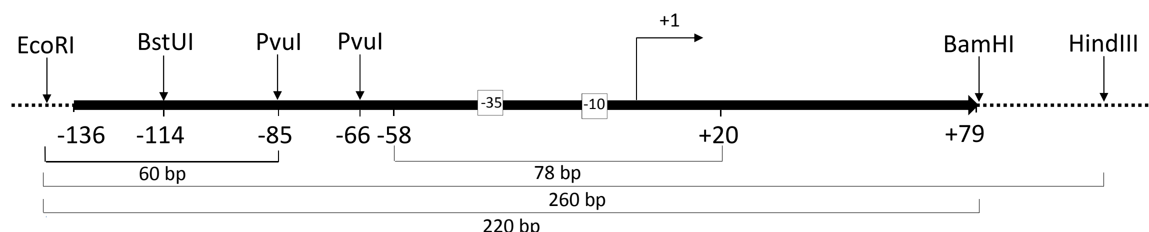
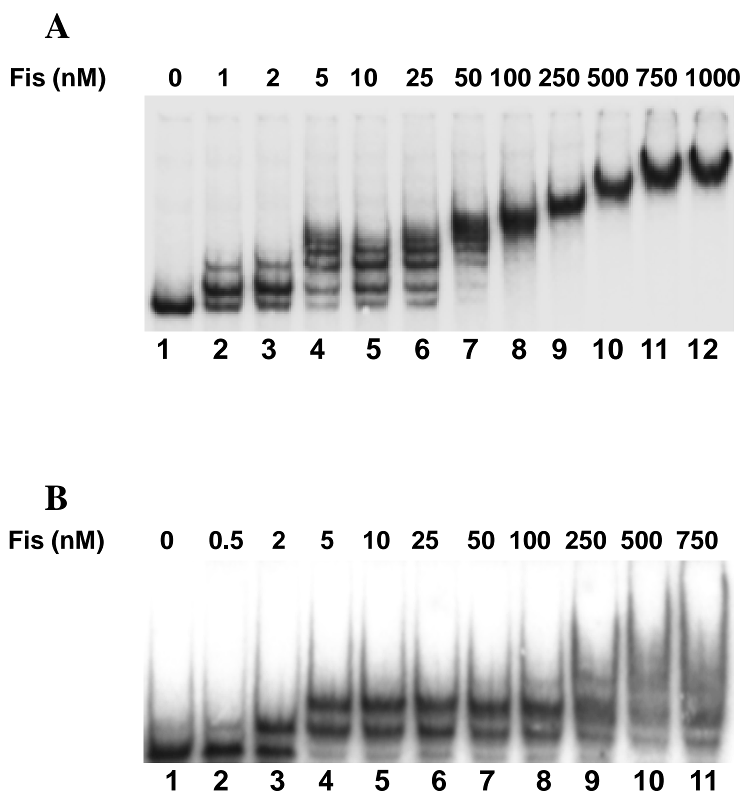


Supplementary Figure S1. Factors regulating expression of the *mom* operon. At the transcriptional level, expression of *mom* is governed by interplay between host factors Dam and OxyR. Asterisks depict the sites recognised by Dam. Several intrinsic features of *mom* promoter (see text) make it a weak 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.

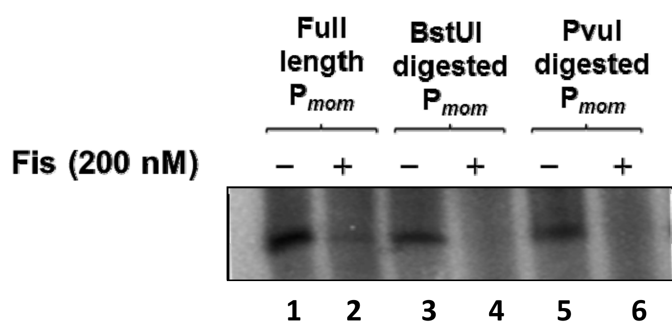


Supplementary figure S2. Schematic depiction of *mom* promoter fragments used in the study. P_{mom} (from positions -136 to +79 relative to the +1 start site) (thick line) has been cloned between EcoRI and BamHI sites of pUC19 to generate the plasmid pUW4. The dashed lines on either side denote vector-specific 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_{mom} fragment (-136 to +79) was carried out in the presence of increasing concentrations of Fis. 40 nM P_{mom} template DNA, 100 nM RNAP, 300 nM C and indicated concentrations of Fis were used.



Supplementary Figure S4. EMSA of P_{mom} with purified Fis. Full length P_{mom} (-136 to +79, obtained from EcoRI-BamHI digestion of pUW4) **(A)** and 60 bp P_{mom} 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 PvuI (-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

Strains	Characteristics	Reference
E. coli MG1655	$F^- \lambda^-$ *ilvG*$^-$ *rfb*$^-$ 50 *rph*$^-$ 1	laboratory stock
BW25113	*rrnB3* Δ*lacZ4787 hsdR514* Δ(*araBAD*)*567* Δ(*rhaBAD*)*568 rph-1*	30, *E. coli* Genetic Stock Center
JW3229-1	*fis*$^-$ derivative of BW25113	30, *E. coli* Genetic Stock Center
BL21(DE3)	F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3)	laboratory stock
W3110	$F^- \lambda^-$ *mcrA mcrB*	23, a kind gift from Prof. Georgi Muskhelishvili
DH10B	Δ(*mrr-hsd rms-mcrBC) mcrA recA1*	laboratory stock
CSH50	*ara* Δ(*lac pro*) *thi rpsL*	28, a kind gift from Prof. Georgi Muskhelishvili
CSH50 *fis::kan*	*fis*$^-$ derivative of CSH50	29, a kind gift from Prof. Georgi Muskhelishvili
Plasmids		
pUHE25–2*fis*	*fis* gene under the tightly regulated *lac* promoter pA1–04/03	22
pVR7	C under T7 promoter in pET11d	20
pUW4*mom*	220 bp *mom* promoter fragment cloned between EcoRI-BamHI sites of pUC19	20
pUW4*tin7*	220 bp *tin7* promoter fragment cloned between EcoRI-BamHI sites of pUC19	20, 26
pVN184	*C* under *tet* promoter in pACYC184	19
pLW4*tin7*	Fusion of P_{tin7} (-136 to +79) to *lacZ*	19
pLW4*tin7* FBS-49-38	Same as pLW4 *tin7* except that Fis binding sites centered at -49 and -38 disrupted	This study
pLW4*tin7* FBS+3	Same as pLW4 *tin7* except that Fis binding site centered at +3 disrupted	This study
pLW4 *tin7* FBS-49-38+3	Same as pLW4 *tin7* except that Fis binding sites centered at -49 and -38 and +3 disrupted	This study

Supplementary Table 2. Oligonucleotides used for generating mutagenic megaprimers.

Name of the primer	Fis binding site(s) targeted for disruption	Sequence (5' to 3')
WTFwd	-	CGGTAATACAGATCGATTATGCCCCAATAACCACACTCAACCCATG
FBS-49-38Fwd	-49 and -38	CGGTAATACATATCGATTATGCCCAAATAACCACAGTCAACCCATG
WTRev	-	TGATTTCATCTCACCTCCTTTGCATCAATTCGCCACTATCTTAACA
FBS+3Rev	+3	TGATTTCATCTCACCTCCTTTCCATCAATTCGCCAATATCTTAACA

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, WTFwd 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

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

*Fold repression is defined as the ratio of β-galactosidase activity produced by a construct (pLW4*tin7* wild-type or mutants) in *fis*$^-$ (CSH50 *fis*$^-$) background to that produced in isogenic *fis*$^+$ (CSH50 wild-type) background strain.