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Transcriptional Slippage in *mxiE* Controls Transcription and Translation of the Downstream *mxiD* Gene, Which Encodes a Component of the *Shigella flexneri* Type III Secretion Apparatus

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The *Shigella flexneri* transcription activator MxiE is produced by transcriptional slippage from two overlapping open reading frames. By using plasmids encoding a *mxiD-lacZ* translational fusion, we showed that transcriptional slippage in *mxiE* influences both transcription and translation of the downstream *mxiD* gene encoding an essential component of the type III secretion apparatus.

Bacteria belonging to *Shigella* spp. are responsible for shigellosis in humans. They use a virulence plasmid-encoded type III secretion (TTS) system to induce their entry into epithelial cells (7). Twenty adjacent *mxi* and *spa* genes encode the TTS apparatus (TTSA) and the transcription activator MxiE, an AraC family member (1). MxiE controls transcription of a set of genes encoding proteins that transit through the TTSA, and its activity is regulated by TTSA activity (3, 8). MxiE production is dependent upon transcriptional slippage in the region of overlap between the 59-codon open reading frame (ORF) *mxiEa* containing the translation start site and the 214-codon ORF *mxiEb* encoding the DNA binding domain (9). Transcriptional slippage involves incorporation, by the RNA polymerase, of nontemplated nucleotides into the mRNA (2, 5, 10). The *mxiE* slippage site consists of a run of 9 A's and its efficiency is ≈30%, i.e., one out of three mRNA molecules contains 10 U's. This additional nucleotide places *mxiEb* in the *mxiEa* reading frame, permitting translation of *mxiEab* and production of MxiE (9). The reason why MxiE is encoded by transcriptional slippage is not known. The *mxiEb* 3′ region overlaps the 5′ region of the downstream *mxiD* gene encoding a TTSA component. Since transcriptional slippage directly controls translation of the mRNA downstream from the slippage site and (i) adjacent genes are subject to translational coupling (6) and (ii) premature translation termination within a gene reduces transcription of downstream genes (4), we investigated the consequences of transcriptional slippage in *mxiE* on translation and transcription of *mxiD*.

Due to the virulence plasmid instability, the slippage site could not be mutagenized at the *mxiE* locus to analyze expression of *mxiD* encoded by the virulence plasmid. Instead, we used low-copy-number plasmids carrying an *mxiD-lacZ* translational fusion. pFS23, pFS24, and pFS25 (Fig. 1) contain a DNA fragment encompassing the last 17 codons of *mxiM*, the entire *mxiEa* and *mxiEb* ORFs, and the first 7 codons of *mxiD* cloned between the HindIII and SalI sites of pFS10, i.e., between a *lac* promoter and codon 15 of *lacZ* (9). These plasmids differ by the slippage site sequence, which affects translation efficiency of *mxiEb* (compared to *mxiEa*). pFS23 contains the wild-type slippage site (*mxiEb* translation at 30% efficiency), pFS24 contains a mutated site such that *mxiEb* is in the *mxiEa* reading frame (*mxiEb* translation at 100% efficiency), and pFS25 contains a mutated site such that *mxiEb* is not translated (*mxiEb* translation at 0% efficiency). Each plasmid was introduced into the *Shigella flexneri* wild-type strain M90T-Sm (which does not contain a *lacZ* gene), and β-galactosidase activity was assayed in bacteria harvested in the exponential phase of growth at 37°C in tryptocasein soy broth.

Strains harboring pFS24 (*mxiEb* always translated) and pFS25 (*mxiEb* not translated) contained 592 and 115 U of β-galactosidase activity, respectively, indicating that translation of *mxiEb* leads to a fivefold increase in the expression of MxiD-LacZ. When *mxiEb* is not translated, decreased expression of MxiD-LacZ might be due to the absence of a translational coupling between *mxiEb* and *mxiD*, decreasing translation of *mxiD-lacZ*, and/or to a premature transcription termination over the 610-bp *mxiEb* ORF, decreasing transcription of *mxiD-lacZ*. To test these hypotheses, we constructed pFS43 by treating pFS24 (*mxiEb* always translated) with NsiI and mung bean nuclease, resulting in the deletion of an 8-bp fragment around the NsiI site that introduced a stop codon in *mxiEb* 60 bp upstream from *mxiD*. In pFS43, translational coupling, if any, between *mxiEb* and *mxiD* should be abolished, and the *mxiEb* 3′ untranslated region should be too short to induce a polar effect on *mxiD-lacZ* transcription. The strain harboring pFS43 produced 246 U of β-galactosidase activity, i.e., 2.4 times less than the strain harboring pFS24. This indicates that reduced translation of the 3′ region of *mxiEb* resulted in a concomitant reduction in expression of MxiD-LacZ, suggesting translational coupling of *mxiEb* and *mxiD* expression. Moreover, the strain harboring pFS43 (carrying *mxiEb* with a 3′ region of 60 nucleotides that cannot be translated) contained 2.1 times more β-galactosidase activity than the

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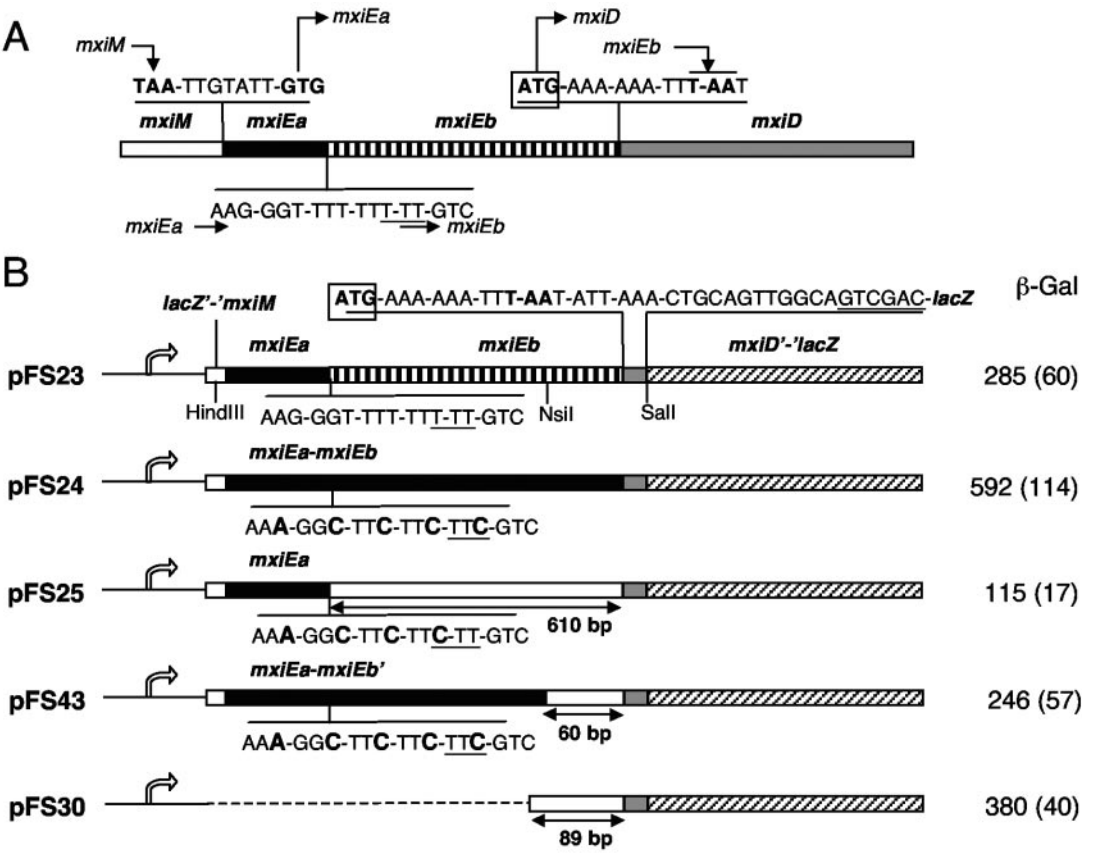

FIG. 1. Inserts carried by plasmids bearing *mxiD-lacZ* fusions and β-galactosidase activities assayed in *S. flexneri* strains harboring these plasmids. (A) The *mxiM-mxiEa* and *mxiEb-mxiD* intergenic regions are indicated above and the *mxiE* slippage site is below a schematic map of the *mxiMED* region (not shown to scale). Start and stop codons are in boldface type, the *mxiD* start codon is boxed, and arrows indicate reading frames. (B) On the left, the arrow corresponds to the *lac* promoter, and the small open box corresponds to the first codons of the α-peptide coding sequence, up to the HindIII site of pSU19, fused in frame to the last 17 codons of *mxiM* (*lacZ'-'mxiM*); on the right, the striped box corresponds to the first 7 codons of *mxiD* fused in frame to codon 15 of *lacZ* (*mxiD'-'lacZ*). The slippage site sequences are shown below the map of each plasmid, with mutated nucleotides indicated in boldface type. Underlined nucleotides indicate the *mxiEb* reading frame. The box corresponding to *mxiEb* is striped to indicate that *mxiEb* is expressed with 30% efficiency compared to *mxiEa*, filled to indicate that *mxiEb* is in the *mxiEa* reading frame, and empty to indicate that *mxiEb* or its 3′ region is not translated. Numbers below double-headed arrows indicate the length of the *mxiEb* region that is not translated. β-Galactosidase activities assayed in derivatives of M90T-Sm harboring each plasmid are indicated on the right. Activities are expressed in Miller units and correspond to mean values of triplicate assays made from at least four independent cultures; standard deviations are indicated in parentheses.

strain harboring pFS25 (carrying the 610-bp *mxiEb* ORF that cannot be translated). Since there is no translational coupling between *mxiEb* and *mxiD* carried by pFS25 and pFS43, this result suggests that lack of translation of *mxiEb* induces transcription termination over the 610-bp *mxiEb* ORF. This polar effect was confirmed by an assay of β-galactosidase activity in the strain harboring pFS30, a plasmid that carries only the last 89 bp (that cannot be translated) of *mxiEb* upstream from *mxiD-lacZ* (Fig. 1). This strain contained 380 U of β-galactosidase activity, i.e., 3.3 times more than the strain harboring pFS25 (carrying the 610-bp *mxiEb* ORF that cannot be translated).

The strain harboring pFS23 (wild-type slippage site) contained 285 U of β-galactosidase activity. Since transcriptional slippage results in the production of one-third of the *mxiE* mRNA population in which *mxiEb* is in the *mxiEa* reading frame (9), the β-galactosidase activity obtained in strain harboring pFS23 is consistent with that calculated by using the slippage efficiency (one-third) and β-galactosidase activities assayed with strains harboring pFS24 (*mxiEb* always translated; 592 U) and pFS25 (*mxiEb* not translated; 115 U), as follows: $(1/3 \times 592) + (2/3 \times 115) = 274$ U. Moreover, the β-galactosidase activity assayed in the strain harboring pFS23 (wild-type slippage site) was half of that assayed in the strain harboring pFS24 (*mxiEb* always translated). This indicates that encoding of MxiE by two ORFs that are placed in the same reading frame by transcriptional slippage, as opposed to encoding of MxiE by a single ORF, leads to a twofold reduction in MxiD expression.

In conclusion, transcriptional slippage in *mxiE* controls production of MxiE, the transcription activator regulated by TTSA activity, and influences both transcription and translation of *mxiD*, encoding a TTSA component. Compared to a situation in which *mxiEa* and *mxiEb* would be in the same reading frame, encoding of MxiE by transcriptional slippage decreases production of MxiE and MxiD by three- and two-

fold, respectively. Paradoxically, the "error" made by the RNA polymerase over the *mxiE* slippage site, i.e., the incorporation of one nontemplated nucleotide, positively controls elongation of the mRNA downstream from the slippage site.

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