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Evaluation of VirB binding site contribution to the regulation of the icsP promoter in Shigella flexneri

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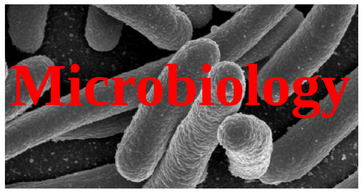

Evaluation of VirB binding site contribution to the regulation of the *icsP* promoter in *Shigella flexneri*

Juan C. Duhart, Maria I. Castellanos, Helen J. Wing, PhD

INTRODUCTION

Shigella species are gram-negative, rod-shaped bacteria that are closely related to *Escherichia coli*. Virulent *Shigella* spp. are intracellular pathogens that invade, replicate and spread through epithelial cells of the lower intestine and cause bacillary dysentery in humans. This disease is characterized by a robust inflammatory response that results in fever, abdominal pain, and bloody diarrhea (3). According to the CDC, approximately 14,000 cases are reported each year in the United States alone. This number however, does not reflect the actual incidence of this disease as many cases go unreported.

The molecular pathogenesis of these bacteria lies in the large virulence plasmid (~230-kb) that is found in all virulent *Shigella* spp. Two key virulence determinants include the ability to invade colonic epithelia (mediated by the *ipa-mxi-spa* gene locus) and the ability to spread to adjacent cells, a process known as actin-based-motility (mediated and controlled by *icsA* and *icsP* respectively*).* These events are largely regulated by VirB, a transcription factor (2, 3).

Canonically, transcription factors are known to bind sequences proximal to the transcriptional start site (within 200-bp). Recent work has focused on the regulation of *icsP* (encodes a protease of the outer membrane) by VirB and has shed light on a novel regulatory strategy, whereby VirB regulates the activation of *icsP* from sites located more than 1-kb upstream of the transcriptional start site (1).

Nine putative VirB binding sites have been found upstream of the *icsP* gene. This work seeks to characterize the contribution made by these putative binding sites to the VirB-dependent regulation of *icsP.*

BACKGROUND

Truncation analysis of the *icsP* promoter region indicates that the two most distal VirB binding sites with respect to the transcriptional start site (between -1144 and -1130) are absolutely required for VirB-dependent regulation of the *icsP* promoter. In the absence of either of these sites, promoter activity is similar to wild-type for both *virB*$^{+}$ *Shigella* strains and *virB*$^{-}$ mutants.

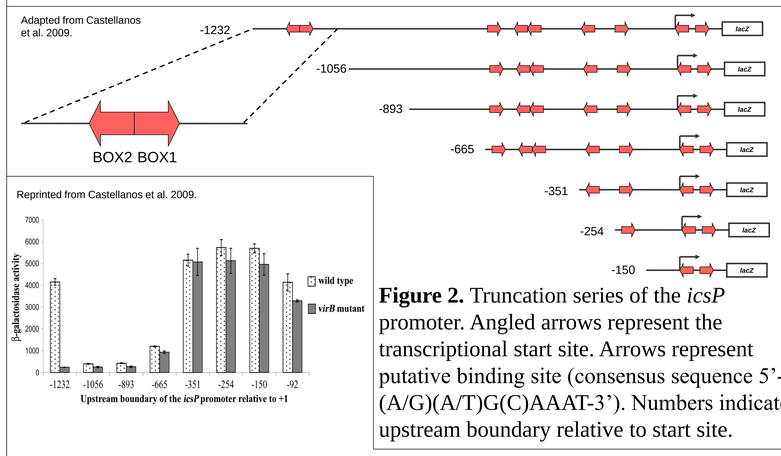

Figure 1. Truncation analysis of *icsP* promoter. Bars indicate promoter activity in Miller units (nmol/min/mg) of P*icsP-lacZ* fusions in a wild-type *Shigella* strain (2457T) and a *virB*$^{-}$ mutant strain (AWY3).

BACKGROUND

Figure and table adapted from Castellanos et. al. 2009.

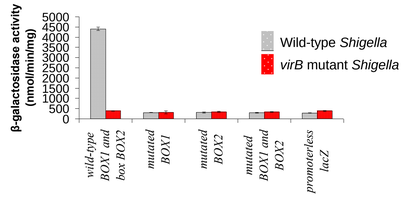

Table 1. Promoter fragment descriptions. Underlined sequences are BOX2 (left) and BOX1 (right)

Fragment	Sequence
Wild-type BOX1 and BOX2	ATTTCAGTATGAAAT
Mutated BOX1	ATTTCAGTCGACCCG
Mutated BOX2	GCCCAGCTATGAAAT
Mutated BOX1 and BOX2	GCCCAGCTCGACCCG
Promoterless *lacZ*	—

Figure 3. Effects of site-directed mutagenesis of BOX 1 and BOX 2 on promoter activity of P*icsP-lacZ* fusions.

HYPOTHESIS

The regulation of the *icsP* promoter is a highly concerted event that is controlled by VirB binding. Elimination of key binding sites via site-directed-mutagenesis of the promoter region will result in loss of VirB-dependent regulation.

METHODS

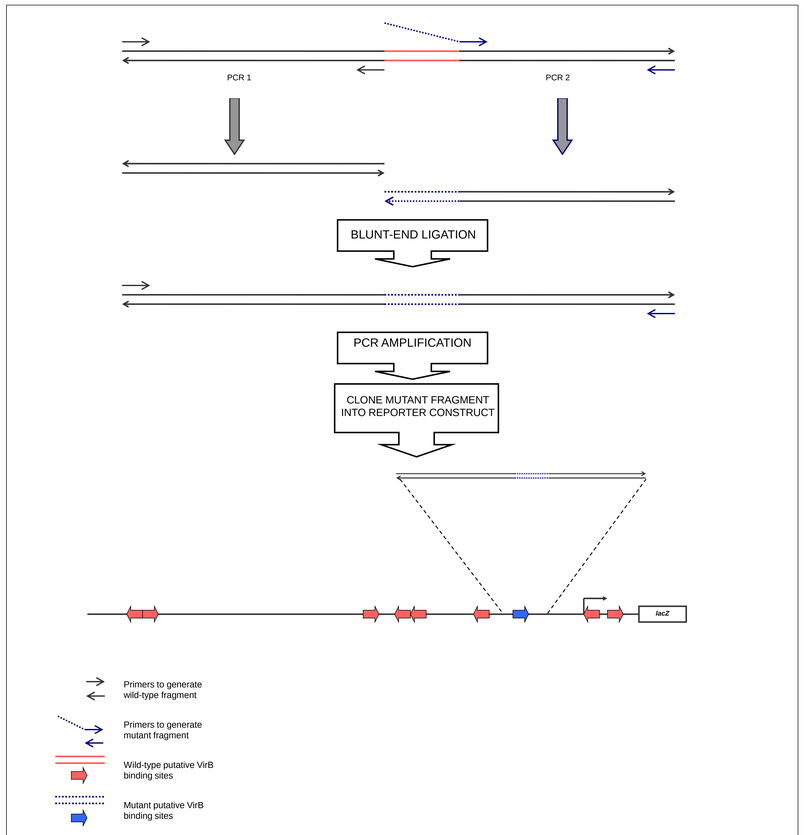

Figure 4. General site-directed mutagenesis strategy using Sow-PCR

RESULTS

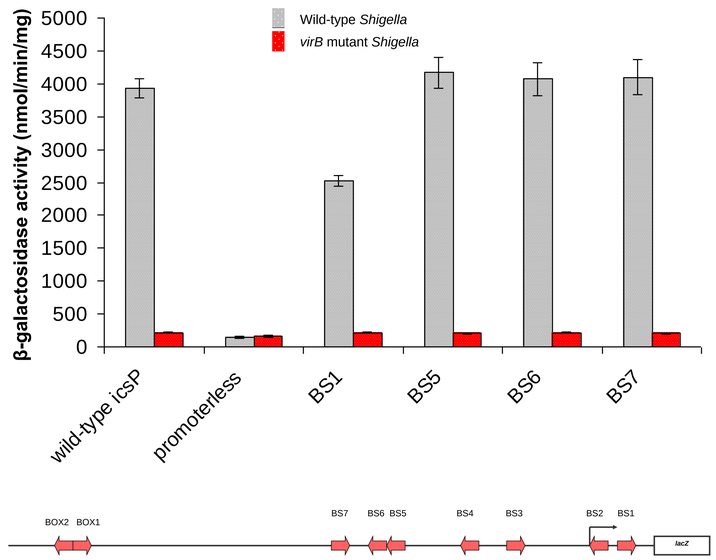

Figure 4. Effects of binding site mutations on VirB-dependent regulation of *icsP.*

CONCLUSION

These data show that mutations at VirB binding sites 5, 6, and 7 do not significantly affect the activity of the *icsP* promoter and are therefore not essential to VirB-dependent regulation. Mutation of VirB binding site 1 however does reduce promoter activity.

This summer I made constructs carrying mutated VirB binding sites 2, 3, and 4 I will conduct a β-galactosidase assay to elucidate their role in the VirB-dependent regulation of *icsP.*

REFERENCES

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