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

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**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 ips-mxi-esp 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 of their downstream targets. Nine putative VirB binding sites have been found upstream of the icsP promoter and are therefore not essential to VirB-dependent regulation of the icsP promoter. In the absence of either of these sites, promoter activity is similar to wild-type icsP promoter activity. Bars indicate promoter activity in Miller β-galactosidase assay (nmol/min/mg) (Figure 1). The work seeks to characterize the contribution made by these putative binding sites to icsP transcription.

**METHODS**

Truncation analysis of the icsP promoter region indicates that the two most distal VirB binding sites with respect to the transcriptional start site (-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 2). Primers to generate type fragment putative VirB binding sites with respect to the transcriptional start site. J. Bacteriol. 191: 4047–4050.

**RESULTS**

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

**CONCLUSION**

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.

**REFERENCES**


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