Aug 9th, 10:15 AM - 12:00 PM

Combination of VirB binding site mutations to evaluate collective impact on icsP promoter activity in Shigella flexneri

Pashtana Usufuzy
University of Nevada, Las Vegas

Juan C. Duhart
University of Nevada, Las Vegas

Maria I. Castellanos
University of Nevada, Las Vegas

Helen Wing
University of Nevada, Las Vegas, helen.wing@unlv.edu

Combination of VirB binding site mutations to evaluate collective impact on *icsP* promoter activity in *Shigella flexneri*

Pashtana Usufzy, Juan C. Duhart, Maria I. Castellanos, and Helen J. Wing
School of Life Sciences, University of Nevada, Las Vegas

INTRODUCTION

Shigella flexneri is a gram-negative, invasive bacterial pathogen that afflicts the human colonic epithelium, causing shigellosis, an illness triggering severe dysentery. The World Health Organization cites the disease burden of shigellosis near 90 million episodes and 108,000 deaths per year [1].

The motility and spread of *Shigella* is modulated by *icsP*, a virulence gene. The transcription factor VirB positively regulates many virulence genes encoded by the *Shigella* virulence plasmid. Two distal binding sites of VirB have been shown to regulate the promoter activity of *icsP*, despite their location of more than 1 kb upstream of the transcription start site [2]. Five VirB binding sites are located between these two sites and the transcription start site, and two are located in close proximity downstream of the transcription start site [2].

Investigation into the impact of the VirB binding sites is part of a larger effort to understand the workings of VirB, which is the major switch that controls virulence gene expression in *Shigella*.

BACKGROUND

-Previous truncation analysis has shown that two distal binding sites of VirB more than 1 kb upstream play a role in regulating *icsP* promoter activity [2].
- Five other sites are located between the transcription start site and the promoter. Two are located downstream, within 30 base pairs of the start site [2].
- Individual mutations have been inserted in each binding site to measure promoter activity. No single mutated binding site showed great impact upon activity (unpublished work, see Fig. 1 & 2).

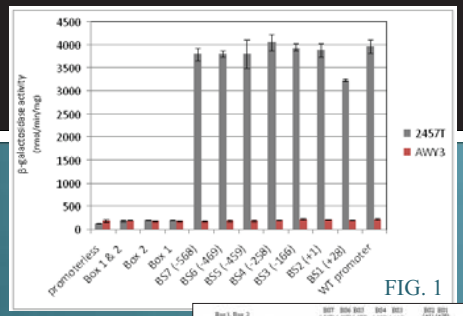

FIG. 1

FIG. 2

HYPOTHESIS

The combination of mutated VirB binding sites into a single plasmid construct will result in decreased activity of the *icsP* promoter.

OVERALL GOAL AND REASONING

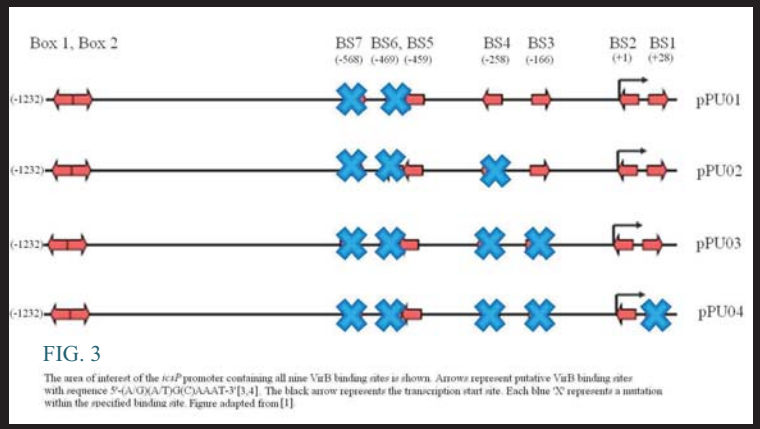

FIG. 3

The area of interest of the *icsP* promoter containing all nine VirB binding sites is shown. Arrows represent putative VirB binding sites with sequence 5'-(A/G)(A/T)G(C)AAAT-3'[3,4]. The black arrow represents the transcription start site. Each blue 'X' represents a mutation within the specified binding site. Figure adapted from [1].

- This project involves combining the individual mutations of VirB binding sites into a single construct (see Fig. 3 for plan).
- Decreased promoter activity would suggest that BS1 and the four VirB binding sites between the transcription start site and the two distal binding sites play a role in *icsP* promoter activity regulation.

MATERIALS AND METHODS

- Beginning with previously created plasmids carrying mutations, I used restriction digests, as seen in Fig. 4, to cut specific segments of the promoter carrying the desired VirB binding sites.
- I used blunt-end ligation techniques by setting my ligation at 16 degrees Celsius overnight. After dialyzing a ligation, I transferred it into viable cells of DH10B *E. coli* through bacterial transformation.
- After a diagnostic digest to confirm that my construct has been created, I cloned the segment carrying the desired binding sites into a pHJW20 reporter construct, which allows usage of a β-galactosidase assay to measure promoter activity through color produced.

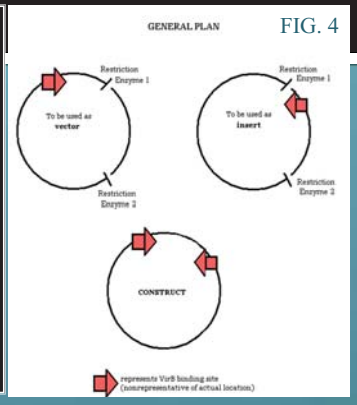

FIG. 4

RESULTS

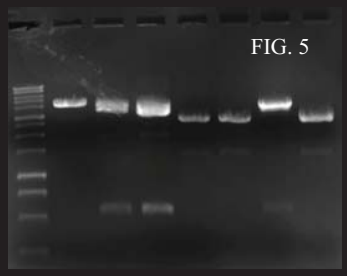

FIG. 5

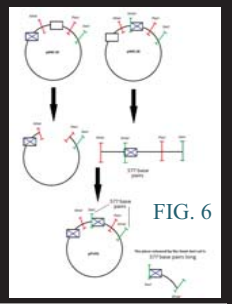

FIG. 6

Fig. 5. The agarose gel image to the left shows a diagnostic digest of pPU01 candidates 1-5 cut with *Sma*I and *Sac*I. Left to right: ladder, vector, insert, candidates 1-5. Candidates 1 and 4 display the expected pattern.
Fig. 6. This scheme shows the construction of pPU01, illustrating why cutting with *Sma*I and *Sac*I would release a 579 base pair piece.

CONCLUSIONS AND CURRENT WORK

The diagnostic digest done on my first construct shows that the desired construct is present. Cutting with *Sma*I and *Sac*I reveals that pPU01 releases a piece approximately 579 base pairs long, the same size piece released by pMIC28, the desired insert. This construct is now being moved into pHJW20, a reporter plasmid that will allow me to test its effect upon promoter activity. Construction of my second construct, using the same process as seen in Fig. 4, is under way.

FUTURE DIRECTIONS

- Combination of VirB mutations will continue. My vector, pPU01, and insert, pJCD03, will form the basis for a construct including sites 4, 6 and 7.
- Sites 3 and 1 will be added individually to form pPU03-pPU04.
- Each combination of binding sites will be utilized as an insert to the reporter plasmid pHJW20 to measure promoter activity.

ACKNOWLEDGEMENTS

Thank you to the Wing Lab.
This project was supported by NIH grant P20 RR-016464 from the INBRE program of the NCRR and by NIH grant R15 AI090573-01.

BIBLIOGRAPHY

1. World Health Organization. http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index6.html
2. Castellanos M.I., D.J. Harrison, J.M. Smith, S.K. Labahn, K.M. Levy, and H.J. Wing. 2009. VirB alleviates H-NS repression of the *icsP* promoter in *Shigella flexneri* from sites more than one kilobase upstream of the transcription start site. J. Bacteriol. 191:4047-4050.
3. Turner E.C. & Dorman C.J. 2007. H-NS antagonism in *Shigella flexneri* by VirB, a virulence gene transcription regulator that is closely related to plasmid partition factors. J.Bacteriol. 189:3403-3413.
4. Taniya T., J. Mitobe, S. Nakayama, Q. Mingshan, K. Okuda , and H. Watanabe. 2003. Determination of the InvE binding site required for expression of IpaB of the *Shigella sonnei* virulence plasmid: Involvement of a ParB boxA-like sequence. J.Bacteriol. 185:5158-5165.