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

Regulation of the Shigella flexneri icsP gene and H-NS dependent repression

Rosa Ojeda
University of Nevada, Las Vegas

Amanda Wigley
University of Nevada, Las Vegas

Dustin Harrison
University of Nevada, Las Vegas

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

Repository Citation
Ojeda, Rosa; Wigley, Amanda; Harrison, Dustin; and Wing, Helen, "Regulation of the Shigella flexneri icsP gene and H-NS dependent repression" (2011). Undergraduate Research Opportunities Program (UROP). 32.
https://digitalscholarship.unlv.edu/cs_urop/2011/aug9/32

This Event is brought to you for free and open access by the Undergraduate Research at Digital Scholarship@UNLV. It has been accepted for inclusion in Undergraduate Research Opportunities Program (UROP) by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.
Regulation of the *Shigella flexneri* icsP gene and H-NS dependent repression

Rosa Ojeda, Amanda Wigley, Dustin Harrison and Helen J. Wing

School of Life Sciences, University of Nevada, Las Vegas

**Introduction**

*Shigella flexneri* is a gram negative bacterium which is known to cause dysentery in humans and primates. *S. flexneri* is the cause of over a million deaths worldwide especially in children and the elderly. When Shigella enters its host it is able to recruit actin from the host’s cell, forming an actin based tail that allows it to move. The outer membrane protein which recruits actin is IcsA, and the protein which regulates IcsA is IcsP, where IcsP cleaves excess IcsA. The icsP gene is regulated by VirB and the histone-like nucleoid structuring protein (H-NS) which are two transcription factors. At 30°C H-NS represses transcription of virulence genes and at 37°C VirB derepresses virulence genes. Our goal is to identify the DNA sequences required for the H-NS dependent repression of the icsP promoter. These studies will improve our understanding of the regulation of the icsP promoter, and this knowledge may be helpful when studying the regulation of other virulence genes in *S. flexneri*.

**Hypothesis 1**

H-NS dependent repression requires sequences between -637 and -351 with respect to TSS.

**Figure 1**

Previous studies tell us H-NS dependent repression of the icsP promoter requires sequences located between -893 and -351 with respect to the transcription start site (TSS) (Fig. 1 & 2).

**Figure 2**

Experiment to identify regions of the icsP promoter required for H-NS dependent repression.

**Figure 3**

Further analyzing the region between -665 and -351 relative to the icsP TSS. Four new truncations between -665 and -351 have been made, shown in red.

**Figure 4**

Promoter fragments were cloned into pMW20 to measure beta galactosidase activity.

**Figure 5**

IcsP promoter activity is less repressed near the -436 truncation.

**Figure 6**

Sequences -893 to -637 and -601 to -436 are required for H-NS dependent repression.

**Conclusion**

2. Insert lambda oop (transcription terminator) was unable to be amplified by PCR, possible hairpin occurring.

**Future Directions**

1. Zone in on the two sequences between -893 to -637 and -601 to -436 to determine if H-NS binds within these regions.
2. Further analyze regions -893 to -637 and -601 to -436 using protein experiments
3. Assay these truncations in the presence of VirB
4. Instead of PCR amplification of transcription terminator, we will isolate the terminator DNA from a plasmid using restriction enzymes.

Acknowledgements

- Rosa Ojeda is recipient of an award from the NSF Research Experience for Undergraduates (REU) program A Broad View of Environmental Microbiology at the University of Nevada, Las Vegas (DBI1005223).
- This work was also supported by NIH R15 AI090573-01
- Dr. Wing, Dr. Zavala, Dr. Medh, Dr. Hogue, Nick Egan, Pashtana Usufzy, Juan Duhart, Holly Martin, Denisse Reyes, Amanda Wigley, Hiro Park and Anthony Daulo

References


**Figure 7**

To test this we will insert a transcription terminator after the gentamycin cassette stopping all transcription before the icsP promoter.

**Figure 8**

Vector for terminator (15075 bps) and insert (277 bps) amplification by PCR.