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The Small regulatory RNA RyhB regulates icsA expression in Shigella flexneri

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The small regulatory RNA RyhB regulates icsA expression in *Shigella flexneri*

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### Background Information

*Shigella flexneri* is a gram negative non-motile, non-spore forming, rod-shaped bacterium responsible for bacillary dysentery in humans. The master regulator, VirF, initiates a cascade of virulence gene activation by acting as a transcription factor for the gene encoding the global regulator, VirB (1). Production of VirB is also negatively regulated by the regulatory small RNA (sRNA), RyhB (2). Regulatory sRNAs are untranslated RNA molecules involved in the regulation of both transcription and translation. RyhB, a 90 nt sRNA, was first identified in *E. coli* and subsequently found in all *Shigella* species. In Shigella this sRNA is maximally expressed in response to iron depletion and is responsible for the reduced expression of many virulence genes in *Shigella flexneri* by downregulating virB (2).

A key feature of *Shigella flexneri* virulence is the actin-based mobility of the bacterium which allows bacterial dissemination from one infected cell to another. This process is mediated by the outer membrane protein, IcsA, which polymerizes the host cell actin into a propulsive tail on the bacterial pole (3). IcsA is directly activated by VirF and therefore is not expected to be affected by RyhB, which is predicted to solely modulate VirB levels. Using beta-galactosidase assays to measure icsA promoter activity and Western blot analyses to measure IcsA protein production, we have demonstrated that RyhB does indeed reduce icsA transcription, which also contributes to a reduction in the formation of the IcsA protein. This work raises the possibility that RyhB may contribute to the regulation of other virulence genes and not just through the reduction of virB transcription.

### Objective

The objective of this study is to determine whether the small regulatory RNA RyhB influences the expression of icsA.

### Hypothesis

Because icsA is directly activated by VirF, we hypothesize that RyhB, which is predicted to solely modulate virB transcription levels will have no effect on icsA.

### Results

#### Western blot analysis:

- **Figure 1a.** IcsA production is influenced by ryhB

- **Figure 1b.** Densitometry of figure 1a.

The result of this analysis shows that IcsA protein production is less when ryhB is induced than when ryhB is not induced. Additional experiments will allow for a more comprehensive understanding of this system.

### Materials and Methods

1. Transforming a wildtype *Shigella* strain (2457T) with a reporter plasmid and pryhB. 2457T was back diluted for 1 hour at 1:50 mL dilution at 37°C.
2. Growing and transforming *Shigella* strains in tryptic soy broth with and without IPTG. 1 mM IPTG concentration serves to induce the expression of ryhB. The strains were induced for 3 hours at 37°C. Non-induced strain were grown at 37°C for 3 hours.
3. Performing a β-galactosidase assay to indirectly measure the expression of the icsA promoter.
4. Performing a Western blot analysis to visualize the IcsA protein made in both IPTG-induced and non-induced conditions.

### Conclusion and Future Directions

RyhB appears to negatively affect IcsA protein production, but to have no affect on promoter activity. Future experiments are needed to fully understand this system. These experiments include:

- Performing Western blot analyses and β-galactosidase assays with (a) lower inducer concentrations for longer back dilution times

### References


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**Figure 1a.** IcsA production is influenced by ryhB

**Figure 1b.** Densitometry of figure 1a.