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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 RhyB 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 r*yhB*

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 to determine a mechanism of this system.

B-galactosidase assay:

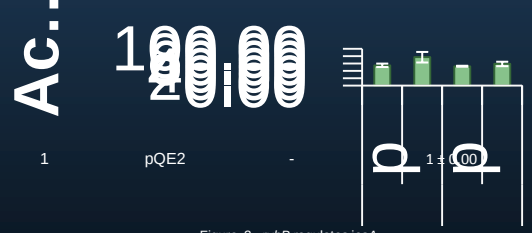

Figure 2. *ryhB* regulates *icsA*

The result of this assay shows that the transcription from the *icsA* promoter is not significantly lower when *ryhB* is induced than when it is not induced. This suggests that over-expression of *ryhB* may not directly effect promoter activity of *icsA*. Additional experiments are needed to test this further.

2	pryhB	-	1.07 ± 0.22

Materials and Methods

1. Transforming a wildtype *Shigella* strain (2457T) with a reporter plasmid and pryhB. 2457T was back diluted for 1 hour at a 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 activity 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 (b) Longer back dilution times

References

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