Coordinated modulation of multiple processes through phase variation of a c-di-GMP phosphodiesterase in Clostridioides difficile

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Introduction

• *C. difficile* is a gram-positive, spore-forming, obligate anaerobe that causes antibiotic-associated intestinal disease.

• *C. difficile* is one of the leading causes of hospital acquired infections in the United States.1

• Phase variation is a mechanism by which bacteria introduce phenotypic heterogeneity to ensure survival.2 3 One mechanism that drives phase variation is an ON/OFF phenotypic switch regulated by an invertible element upstream of a gene.

• An invertible element has been identified upstream of the phosphodiesterase encoding gene pdcB, and its orientation regulates pdcB expression in an ON/OFF manner.4

• Phosphodiesterases are enzymes that degrade the signaling molecule c-di-GMP. c-di-GMP is known to regulate many phenotypes in bacterial species, including motility and virulence.5

Results

Figure 5: Transcription from TSS1 is affected by the orientation of the downstream invertible element. OFF TSS1+TSS2 shows the promoter activity as low as the promoterless negative control. However, in ON TSS1+TSS2, the promoter activity is 50- to 70-fold higher, suggesting that transcription from TSS1 is dependent on the orientation of the downstream invertible element. ON TSS1 shows an average level of activity similar to ON TSS1+TSS2.

Figure 6: There is an element downstream of TSS1 that affects TSS1 activity. Previous studies suggest that the nutrient dependent transcription factor, CodY, binds downstream of TSS1. To test the potential effect of CodY on TSS1 activity, AP activity was measured from cultures in (A) exponential phase and (B) stationary phase. In both conditions, ON-1 TSS1, which lacks the CodY binding site, showed much lower AP activity.

Methods

1. **ONtrunc1::pdcB**

2. **ONtrunc2::pdcB**

3. **ONtrunc2::pdcB**

4. **ONtrunc2::pdcB**

5. **ONtrunc2::pdcB**

Figure 4: Alkaline phosphatase (AP) assay. Overnight cultures of *C. difficile* were back-diluted and grown to exponential phase. Cultures were collected by centrifugation, supernatant was discarded and cells were lysed to release the phosphatase. Buffers and pNPP were added to start the reaction. Once the sample turned yellow, signaling the pNPP was broken down, the optical density was measured to determine phosphatase activity.

Figure 3: Construction of strains. To study the role of the two identified TSS, DNA fragments consisting of different regions of the invertible element were fused to the phoZ reporter gene. Resulting plasmids were transformed into *C. difficile*. ON-1 TSS1 differs from ON TSS1 in that it lacks the binding site of the transcription factor CodY.

Figure 2: The pdcB switch contains an invertible promoter. (A) An AP assay was used to identify the location of a possible invertible promoter. A promoterless phoZ was used as a control. A significant decrease in activity between Cdi2-ONtrunc1::phoZ and Cdi2-ONtrunc2::phoZ indicated the presence of a promoter near the center of the switch. (B) and (C) A 5'RACE experiment identified a transcriptional start site (TSS) upstream of the invertible element (B) and a second TSS was identified in the center of the invertible element (C). (D) Diagram of the region upstream of pdcB indicating the positions of TSS1 and TSS2 (arrows).

Conclusions and Future Directions

• *PdcB* expression is regulated by an invertible promoter (TSS2) switch.

• An additional promoter (TSS1) upstream of the pdcB switch also controls pdcB expression and seems to be dependent on the orientation of the invertible element.

• An element near the left invertible repeat modulates transcription from TSS1.

Future Directions

• Test if CodY affects expression of TSS1 by transforming these plasmids into a CodY mutant strain and comparing AP activity.

• Determine why the invertible element in the OFF orientation affects expression of TSS1, which is upstream of the invertible element.

References


