The Role of RdoA in flagellar phase variation in Salmonella enterica

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Introduction

Salmonella enterica serovar Typhimurium alternates expression of two antigenically distinct flagellins that form the flagellar filament.

Flagellar phase variation was first discovered in 1922 (1), but the biological significance and signals controlling flagellin switching are poorly understood.

Although Hin levels control the rate of flagellin switching, there is no obvious reason for the inversion reaction to favour one orientation over the other.

However, FliC is the predominant flagellin in wild-type cells.

We have previously shown that RdoA expression influences the bias towards FliC expression (3).

RdoA is a eukaryotic-like kinase (4) that is upregulated via the Cpx two-component signal transduction pathway (5).

Cpx signaling pathway

Our hypothesis is that RdoA activity helps to maintain the bias toward FliC expression.

To explore this hypothesis, the following questions were asked:

Does RdoA affect Hin expression?

Does RdoA affect the orientation of the invertible segment involved in flagellar phase variation?

Does RdoA affect Hin expression?

Does RdoA affect flagellar phase variation or flagellar function?

Results

Do RdoA or pH affect FljB expression?

We wanted to see how RdoA and Cpx activation (pH 8.5) affect the proportion of cells expressing FljB.

- Measured FljB promoter activity in a chromosomal fljB::lacZ transcriptional reporter fusion
- Therefore, assessing phase variation-dependent FljB expression at a PER-CELL level

- Twice as many cells express FljB in the absence of RdoA
- Therefore, RdoA normally causes more cells to be in the FIC-expression phase
- pH 8.5 data is not significantly different from pH 7 data (not shown)
- Suggests Cpx activation has little or no influence on flagellin expression, compared to RdoA

- In wild-type, Hin expression is higher than in rdoA at both pHs
- pH 8.5 also decreases Hin expression
- Results suggest that Hin levels in all growth conditions are sufficient to promote phase variation
- The effect on Hin due to RdoA, or due to Cpx activation?

Does RdoA affect Hin expression?

We next tried to determine if the target of RdoA’s actions with regard to phase variation are due to effects on Hin

- Used a hin::lacZ transcriptional reporter fusion in wild-type and rdoA cells grown at pH 7.0 or 8.5 to measure Hin expression using a β-galactosidase assay

- In wild-type, Hin expression is higher than in rdoA at both pHs
- pH 8.5 also decreases Hin expression
- Results suggest that Hin levels in all growth conditions are sufficient to promote phase variation
- The effect of Hin on Hin due to RdoA, or due to Cpx activation?

Does RdoA affect phase variation through DNA inversion?

We wanted to see if RdoA affects the orientation of the invertible segment responsible for flagellar phase variation on a POPULATION basis

- Tested using PCR reactions with primers that produce different sized amplicons depending on the orientation of the segment:
  - FliC-expressing orientation: 200 bp amplicon
  - Cpx-expressing orientation: 220 bp amplicon
- Therefore, the effect on DNA inversion occurs predominantly through RdoA

- Twice as many cells express FljB in the absence of RdoA
- Therefore, RdoA normally causes more cells to be in the FIC-expression phase
- pH 8.5 data is not significantly different from pH 7 data (not shown)
- Suggests Cpx activation has little or no influence on flagellin expression, compared to RdoA

Discussion

RdoA normally maintains cells in a FIC-expressing phase.

- RdoA maintains the invertible segment in a FIC-ON orientation

- The absence of RdoA, and alkaline pH, activate the Cpx pathway.

Both of these conditions also cause a decrease in Hin expression.

- Decrease in Hin expression is likely due to Cpx activation, not absence of RdoA
- Therefore, RdoA does not elicit changes in flagellin expression bias by changing Hin levels

RdoA maintains a lower level of flagellation but does not affect function of flagella.

- More abundant flagella in the absence of RdoA
- No change in swimming motility in the absence of RdoA

- Perhaps increased FljB in absence of RdoA is an increase in overall number of flagella by increasing FliC-containing flagella more than FIC-containing flagella.

Taken together, these results strongly support our hypothesis that RdoA maintains the bias towards FIC expression. Our results also suggest that this effect occurs through alteration of the orientation of the invertible segment involved in phase variation and changes in overall flagellin expression.

The effect of RdoA is not through its actions on Hin expression.

Why is RdoA regulated by the Cpx pathway?

- To assist in regulation of surface appendage expression

- To optimize this expression when signals of environmental change are detected, as in infection

- To coordinately regulate flagellar expression with other Cpx-regulated proteins

Future Directions

- To determine how RdoA affects the bias toward FIC expression (i.e. through another protein, or directly?)
- To determine the targets of RdoA’s kinase activity
- To determine if Cpx and RdoA effects occur in conjunction with one another, or through separate routes

Although the mechanism underlying flagellar phase variation in S. typhimurium is well understood, there is still much to be learned about why phase variation occurs and how it is stimulated.

Determining the role of RdoA in flagellar phase variation will help to link this process with cell physiology and environment.

Strains Used in This Study

All strains of S. typhimurium are derived from SL1344.

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<th>Strain Name</th>
<th>Genotype</th>
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<td>NLM2211</td>
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References