Dissecting bacterial virulence regulation: from the bench to the cell

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Regulating *Shigella* virulence through the type three secretion system
Shigella flexneri as a Pathogen

- Non motile Gram negative rod
- Causative agent of shigellosis (bacillary dysentery)
- Invades cells and tissues of the colonic epithelium
- High morbidity in developing world
  - >100 million reported cases annually
  - >100,000 deaths
What provides the energetics required for secretion through the *Shigella* T3SA?
Spa47 is an active *Shigella* T3SS ATPase

**A**

<table>
<thead>
<tr>
<th>kDa</th>
<th>WC</th>
<th>Sup</th>
<th>Chitin</th>
<th>Q</th>
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<tr>
<td>35</td>
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</tbody>
</table>

**B**

- Spa47 Concentration = 1.35 μM
- $k_{\text{cat}}$: Species 1 = 1.18 ± 0.03 sec$^{-1}$
- Species 2 = 0.15 ± 0.01 sec$^{-1}$

J. Burgess et al., 2016, *Protein Science*
Biophysical characterization identifies a novel “activated” Spa47 trimer

J. Burgess et al., 2016, Protein Science
The Spa47 N-terminus is needed for oligomer formation and activation

J. Burgess et al., 2016, Journal of Biological Chemistry
2.4 Å crystal structure of Spa47

J. Burgess et al., 2016, *Journal of Biological Chemistry*

B. Grigorenko et al., 2006, *PNAS*
A Spa47 hexamer model based on F$_1$ ATP synthase provides mechanistic insight

J. Burgess et al., 2016, Journal of Biological Chemistry
Spa47 mutant structures

- Spa47Δ1-79 K165A  
  2.15Å

- Spa47Δ1-79 E188A  
  2.70Å

- Spa47Δ1-79 R350A  
  1.80Å
Effects of ATPase deficient Spa47 on *Shigella* virulence

<table>
<thead>
<tr>
<th>S. flexneri Strain</th>
<th>Complementation</th>
<th>Relative Invasion (% ± SD)</th>
<th>Relative Hemolysis (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2457T (WT)</td>
<td>None</td>
<td>100</td>
<td>100</td>
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<tr>
<td>spa47 null</td>
<td>None</td>
<td>0.8 ± 1.1</td>
<td>0.9 ± 2.0</td>
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<tr>
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<td>spa47/pWPsf4</td>
<td>81.7 ± 10.8</td>
<td>81.4 ± 11.6</td>
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<td>1.1 ± 1.9</td>
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<td>1.2 ± 2.5</td>
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<td>0.2 ± 0.1</td>
<td>0.9 ± 2.2</td>
</tr>
</tbody>
</table>

Congo Red induced secretion

J. Burgess et al., 2016, *Journal of Biological Chemistry*


http://www.cimst.ethz.ch/
Spa47 catalyzed ATP hydrolysis is needed for apparatus formation

J. Burgess et al., 2016, *Journal of Biological Chemistry*
Conclusion 1: Mechanistic insights and a proposed model for Spa47 regulation of *Shigella* virulence

- Spa47 is a *Shigella* T3SS ATPase.
- Spa47 oligomers (trimer) exhibit enhanced activity.
- The N-terminal domain of Spa47 (1-79) is needed for oligomerization and ultimately activation.
- A 2.4 Å crystal structure identified critical active site residues including Arginine 350 which participates in hydrolysis.
- Spa47 ATPase activity is critical for *Shigella* virulence and provides an attractive target for anti-infective agents.
The “Crew”
Acknowledgements

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