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Structural and Functional Characterization of the Shigella flexneri Type Three Secretion System (T3SS) ATPase Spa47

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Unraveling the Driving Forces Behind Bacterial Infection

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Introduction

Shigella flexneri is a water-borne Gram-negative bacterial pathogen which causes shigellosis, a severe form of dysentery that is hallmarked by massive fluid loss and hemorrhaging of the intestines. *Shigella* is responsible for a least 90 million infections and more than 100,000 deaths per year. The recent emergence of multidrug-resistant *Shigella* strains underscores the need for alternative treatment options which can only be achieved with a better understanding of the means by which Shigella infects human cells.

Shigella relies on a Type Three Secretion System (T3SS) to inject proteins into host cells and ultimately cause infection. We recently identified the T3SS protein Spa47 as an enzyme that provides the energy for protein secretion. Here we solved the crystal structure of Spa47, generated and activated oligomeric model, and identified key amino acid residues necessary for T3SS function and *Shigella* infection.

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Methods

- A series of Spa47 mutants were cloned, expressed, and purified.
- The activity of each of these mutants were measured using a radioactive ATP hydrolysis activity assay
- The Spa47$^{\Delta 1\text{-}79}$ mutant was crystalized and the structure was determined using X-ray diffraction.
- A complete series of phenotypic studies were performed to determine the role of Spa47 activation in *Shigella* infection.

Results

- The first crystal structure of Spa47 was solved and used to generate an activated Spa47 model (see Figure 1).
- Each of the mutations resulted in an elimination of ATP hydrolysis activity.
- *Shigella* harboring the inactive Spa47 mutations could not infect host cells.
- The loss of infection capabilities resulted from the inability of *Shigella* to assemble a proper T3SS apparatus.

Figure 1 – Cartoon depiction of the *Shigella* T3SA, the solved structure of Spa47, and our model of activated Spa47

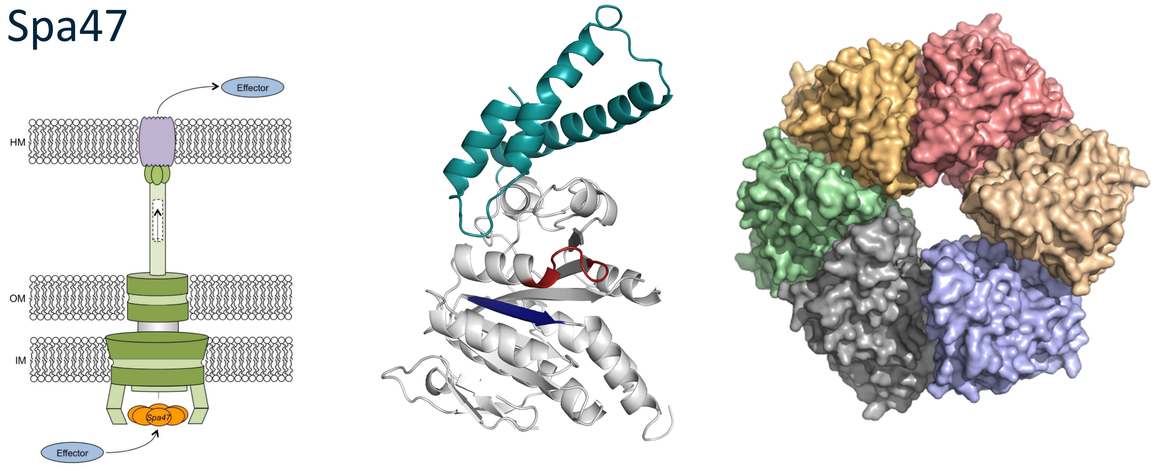
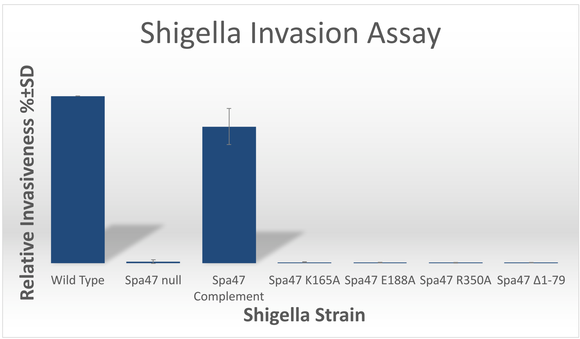


Figure 2 – Effects of engineered Spa47 mutations on *Shigella* infection



Conclusions

ATPases play a critical role in the energetics and regulation of T3SS infection-mediated bacterial infections. This first structure-function characterization of Spa47 provides critical insight into the regulation of *Shigella* infection which clearly requires Spa47 oligomerization to activate the enzyme. It is our hope that these and future studies will support the development of much needed non-antibiotic based treatments for shigellosis and other related diseases.

Burgess, J.L., Burgess, R.A., Morales, Y., Bouvang, J.M., Johnson, S.J., Dickenson N.E. (2016) Structural and Biochemical Characterization of Spa47 Provides Mechanistic Insight into Type III Secretion System ATPase Activation and Shigella Virulence Regulation J.Biol. Chem. ***291****.*

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