Investigating the structure and function of a novel bacterial anti-virulence factor

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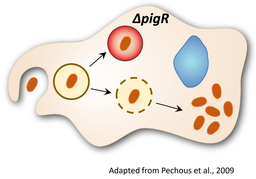
Abstract

Francisella tularensis is a highly infectious intracellular human pathogen. How *F. tularensis* regulates expression of genes important for replication in macrophage, a key host niche, is still incompletely understood. One transcription factor critical for virulence is the response regulator PmrA. This transcription factor is necessary for virulence primarily because it functions to repress expression of PriM (PmrA-repressed-inhibitor of intramacrophage growth). Although how PriM functions to inhibit virulence is still unknown, we have defined the crystal structure of the PriM protein and identified several structural elements that may contribute to its function. We have generated cells that produce PriM with distinct mutations to purposefully disrupt these structural elements. To determine how the structure of PriM contributes to its function, we are assessing the ability of these cells to survive within macrophage. Our goal is to understand how the anti-virulence factor PriM functions at the molecular level; bacterially-encoded anti-virulence pathways may be targets for future therapeutics.

Introduction



Francisella tularensis

Gram-negative, facultative, intracellular pathogen

Highly infectious potential bioweapon

Survival and replication in macrophage necessary for infection

Response regulator PmrA critical for intramacrophage replication

The transcription factor PmrA is required for intramacrophage replication

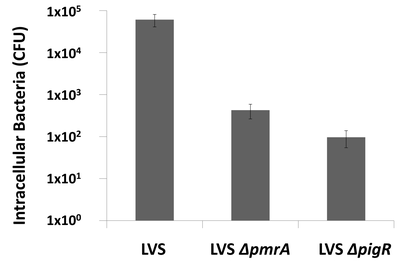


Figure 1. Survival of wild-type or indicated *F. tularensis* mutant cells after infection of J774A cells for 24 hours.

priM is the gene most tightly controlled by PmrA

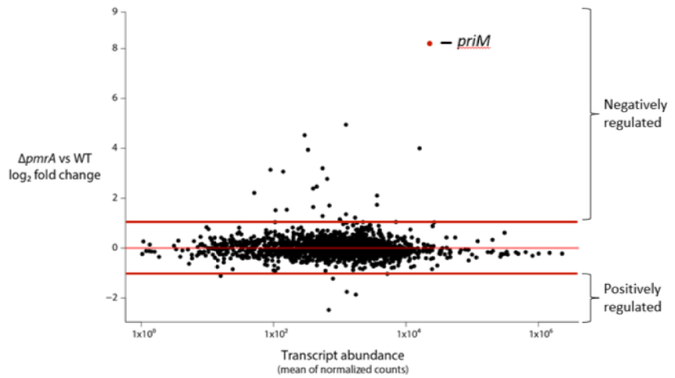


Figure 2. Transcriptomic analysis comparing wild-type cells and cells lacking *pmrA* using RNA-Seq. Points represent individual genes (Ramsey and Dove, 2016).

PmrA directly represses expression of *priM*

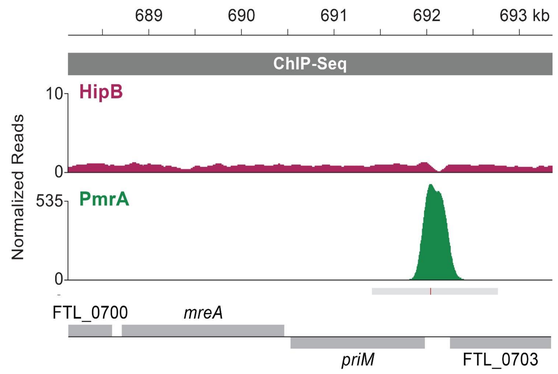


Figure 3. Representative illustration of normalized sequencing reads after ChIP-Seq of HipB and PmrA (Ramsey and Dove 2016).

The primary role of PmrA in intramacrophage replication is repression of *priM*

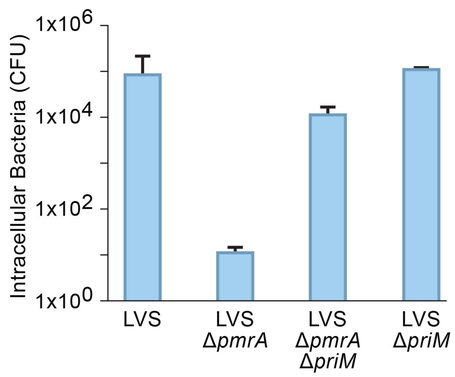
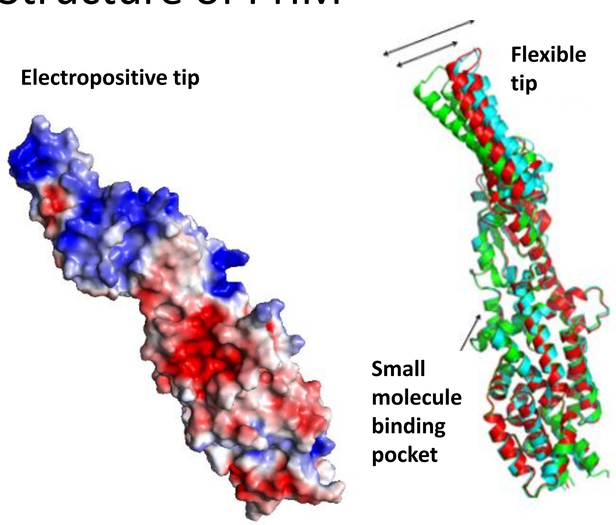


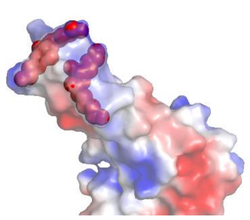
Figure 4. Survival of wild-type or indicated *F. tularensis* mutant cells after infection of J774A cells for 24 hours (Ramsey and Dove, 2016).

Structure of PriM



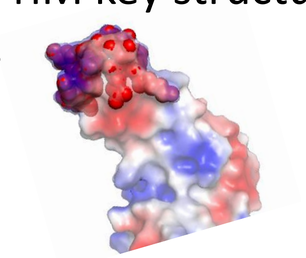
Targeted mutation of PriM key structural features

1.



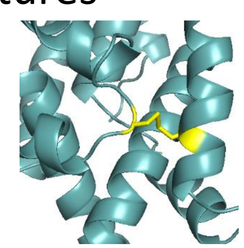
Mutations in the tip region: two lysine doublets to glutamic acid doublets (colored as red spheres)

2.



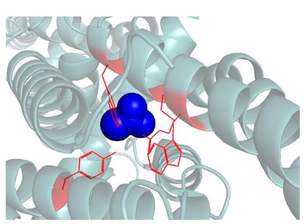
Mutations in the tip region: entire tip region to polyglycine (colored as red spheres)

3.



Mutations changing the cysteines (colored in yellow) to alanines targeting disulfide bond formation.

4.



Mutations in the small molecule binding pocket region: arginine, two tryptophans and one tyrosine to alanine (acetate molecule colored in blue)

Future Directions

Structure-function analyses: Testing strains producing PriM with targeted mutations for ability to survive in macrophage.

Identifying interaction partners: Unbiased genetic selection for mutants able to grow in macrophage in the presence of PriM; loss of key interaction partners should prevent PriM function and allow bacterial cells to survive within macrophage

Additional Information

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Reference

Ramsey KM, Dove SL. A response regulator promotes *Francisella tularensis* intramacrophage growth by repressing an anti-virulence factor. Mol Microbiol. 2016; 101(4):688-700.