

S 1-3

**Determining the function of the cytoplasmic membrane protein RipA**

B. Mortensen<sup>1</sup>, J. Fuller<sup>1</sup>, M. Huang<sup>1</sup>, T. Kijek<sup>1</sup>, C. Miller<sup>1</sup>, S. Taft-Benz<sup>1</sup>, T. Kawula<sup>1</sup>

<sup>1</sup>University of North Carolina, Microbiology and Immunology, Chapel Hill, United States

**Aims:** To determine the function of RipA, a cytoplasmic membrane protein that is conserved among *Francisella* and is required for growth within host cells.

**Methods:** We generated alanine substitution mutants at conserved residues within RipA. These mutants were assayed for intracellular replication and IL-1 $\beta$  production. Secondly, pull-down assays were performed using HA-tagged RipA expressed in LVS to identify proteins that physically interact with RipA. We then generated the deletion strain lacking *iclR*, the gene encoding the RipA-interacting protein IclR, and examined the effects on gene expression, intracellular growth, cytokine induction by infected cells and virulence in a mouse model of pulmonary Tularemia.

**Results:** Pull-down assays yielded 3 RipA-interacting proteins, the strongest interaction being with the transcriptional regulator IclR (FTL\_1364). Growth of LVS $\Delta$ *iclR* within alveolar macrophages was indistinguishable from wild-type LVS and the virulence of LVS $\Delta$ *iclR* was equivalent to wild-type in the mouse model. Likewise, IL-1b expression by LVS $\Delta$ *iclR* infected BMMs was not significantly different from wild-type infected cells. Transcriptional profiling of LVS $\Delta$ *iclR* by microarray revealed that IclR regulated the expression of a limited number of genes, including some putative and known virulence factors. Using the RipA point mutants, we identified residues that are involved in intracellular replication and expression of IL-1 $\beta$ .

**Conclusions:** Specific residues in RipA are important for the function of RipA as it applies to intracellular growth and cytokine expression in host cells. In *F. tularensis* subsp. *holarctica* LVS RipA appears to physically interact with the IclR transcriptional regulator. Gene expression and phenotypic analysis of LVS $\Delta$ *iclR* support the conclusion that IclR directly or indirectly suppresses the expression of a several virulence-associated genes. We hypothesize that under specific conditions RipA sequesters IclR, thereby alleviating repression of genes that are necessary for intracellular growth.