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Abstract

"Mechanisms of Surface Lipoprotein Secretion in *Borrelia* Spirochetes"

Bacterial protein secretion is a fundamental physiological process that generates the cell envelope and maintains its integrity throughout the bacterial life cycle. In bacterial pathogens, a variety of protein secretion systems have been shown to deploy important virulence factors to the bacterial surface, into the milieu, or even directly into eukaryotic cells or other bacteria. *Borrelia* spirochetes, the causative agents of tick-borne Lyme disease and relapsing fever, have a unique double-membrane envelope with periplasmic flagella. The *Borrelia* surface lacks lipopolysaccharide and is instead covered by abundant, immunodominant and serotype-defining surface lipoproteins that serve as linchpins for transmission and pathogenesis. Our recent study has shown that two thirds of the about 130 lipoproteins expressed by the Lyme disease bacterium *Borrelia burgdorferi* localize to the surface. Therefore, *B. burgdorferi* is a perfect model organism for investigations into the secretion of bacterial surface lipoproteins. Several seminal studies have demonstrated that (i) *Borrelia* surface lipoprotein secretion determinants commonly localize to N-terminal disordered tether regions of the mature lipoproteins, (ii) translocation through the outer membrane can initiate at a lipoprotein's C terminus and requires an at least partially unfolded conformation, and (iii) *Borrelia* surface lipoproteins are ultimately anchored in the surface leaflet of the outer membrane bilayer. These data support the hypothesis that the *Borrelia* surface lipoprotein secretion pathway includes a periplasmic mechanism that

prevents premature folding of surface lipoprotein and an outer membrane translocon complex that allows for the flipping of lipoproteins from the periplasm to the surface. We are currently defining periplasmic events, taking a high-resolution structure-guided approach to detail the function of the *B. burgdorferi* LolA homolog via dominant negative screens, conditional knockouts, quantitative proteomics and X-ray crystallography. Using a novel *B. burgdorferi*-adapted tunable CRISPR interference (Bb-tCRISPRi) knockdown system, we have begun to deplete OM flippase candidate proteins to study their role in surface lipoprotein localization; preliminary results are indicating an intriguing functional adoption of an orphan outer membrane translocase by *Borrelia*. Together, these experiments will further elucidate how emerging pathogens of global importance generate their interface with the host. This will ultimately yield better tools for diagnostics and improved strategies for prevention and treatment.

Bio-Summary

Dr. Zückert obtained his M.S. and Ph.D. degrees from the University of Basel in Switzerland, working on the complex genome structure of the Lyme disease spirochete *Borrelia burgdorferi*. After a first postdoctoral fellowship investigating the structure-function relationships of the *Listeria monocytogenes* phospholipases at the Univ. of Pennsylvania (with Howard Goldfine), he returned to *Borrelia* for his second postdoctoral fellowship at the Univ. of California, Irvine. Work on structure-function of the unusually abundant surface lipoproteins in *Borrelia* peaked his interest in lipoprotein secretion mechanisms. He joined the faculty at the Univ. of Kansas Medical Center in 2001 and has since trained 5 graduate students and 2 postdoctoral fellows. The laboratory continues to use novel approaches to investigate the biogenesis and structure-function of the *Borrelia* pathogen-host interface, with a particular focus on surface lipoprotein secretion pathway components in the periplasm and outer membrane.