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The ZnuABC High Affinity Zinc Transporter Plays a Critical Role in *Salmonella* Pathogenicity and is a Promising Target for Novel Antimicrobial Strategies

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Introduction: Adequate zinc supply is critical for all microorganisms as this metal plays essential roles in a very large number of proteins. Under conditions of severe metal shortage, zinc uptake is ensured by ZnuABC, the unique high affinity zinc transporter present in Gram-negative bacteria. Our research aims at understanding the relevance of this transporter in the host-*Salmonella* interaction.

Methods: *Salmonella* mutants were generated by standard methods.

Results: We have shown that: a) *znuABC* expression is repressed in bacteria cultivated in media containing zinc concentrations as low as 1 μM, but is strongly activated in bacteria recovered from the spleens of infected mice or from cultured epithelial or macrophagic cells, b) deletion of either the whole *znuABC* operon or of the single *znuA* gene, encoding for the periplasmic component of the transporter, causes a dramatic reduction of *Salmonella* pathogenicity. These findings prompted us to start investigations on the use of *znuABC* mutant strains as attenuated vaccines and on the possibility to develop ZnuABC inhibitors. We have found that *Salmonella znuABC* mutant strains, when administered to mice by the oral route, induce short lasting infections which lead to a solid and durable immune-based protection against systemic or gastrointestinal challenge with homologous virulent strains. As a starting point for the rational design of ZnuABC inhibitors, the X-ray structure of *Salmonella* Typhimurium ZnuA was solved at 1.7 Å resolution. The structure of partially metallated ZnuA provides novel insights into the mechanism of zinc binding/release operative in this protein.

Discussion: Our results demonstrate that: a) even though the intracellular zinc concentration is nearly millimolar, the free metal quota available for microorganisms during infections is limited, b) ZnuABC-mediated zinc import is critical to ensure bacterial virulence, c) zinc homeostasis is a promising target for antimicrobial strategies.

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