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Insights into bacterial iron acquisition from transferrin through study of the pathway in Avibacterium

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Background

The transferrin binding proteins (TbpA & TbpB) were first discovered in *Neisseria meningitidis* and have since been identified in many important Gram-negative bacterial pathogens as a critical iron acquisition system for sequestering iron from host transferrin (Tf). These proteins are highly specific for the Tf from their mammalian hosts and evidence indicates this strict host specificity is due to selective pressures of driving changes to the surface residues on Tf.

Aim/Methods

This study was initiated to gain mechanistic insights into the Tbp iron acquisition pathway through study of homologous system in *Avibacterium*. Using whole genome sequencing, bioinformatic approaches and structural studies, we compared orthologues of the proteins involved in the iron transport pathway. Deletion mutants were made to probe the function of the transport proteins identified.

Results

To adapt to the changes in the *Avibacterium* taxonomy, we sequenced the genomes of several strains recovered after 30 years of storage that were capable binding ovotransferrin and using it for growth. We identified orthologues of transferrin binding protein A and B (TbpA & TbpB) and ferric binding protein A (FbpA) and confirmed their role in iron acquisition by deletion mutagenesis. Structural modeling of the *Avibacterium* TbpA revealed a smaller loop 3 without a predicted alpha-helical region and the conserved NEVTGLGCK and GAINEYEIE motifs found in the plug domain of the orthologues in mammalian pathogens are replaced with the atypical sequences QNVSGLGEV and SSKNDVEYE instead. Mutations of these key residues resulted in lost in ability to bind Tf. Structural models of TbpB and FbpA were validated by mutations of critical binding residues. We were only able to purify and crystallize the C-lobe of *A. avium* TbpB which resolved a 2.1 angstrom structure x-ray crystallography, revealing a canonical TbpB fold with a discrete handle and barrel domain.

Conclusions

The study of transport proteins involved in iron acquisition from Tf in birds and mammals revealed functional orthologues that retain the core structural features despite evolving separately over 320 million years and providing additional insights into the transport process.