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Biochemical and structural characterization of TraE from the plasmid pKM101

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In all organisms, secretion systems mediate the passage of macromolecules across cellular membranes. The bacterial type IV secretion system (T4SS) family can be divided into three functional groups. First, as typified by the Brucella suis system, T4SSs deliver effector macromolecules into eukaryotic cells during the course of infection. Second, in some Gram-negative bacteria, such as in Helicobacter pylori (ComB system), T4SSs mediate DNA uptake from and release into the extracellular environment. Thirdly, as in the IncN plasmid pKM101, T4SSs can mediate the conjugative transfer of plasmid DNA or transposons into a wide range of bacterial species. This conjugation phenomenon contributes to the spread of antibiotic resistance genes among pathogenic bacteria, leading to the emergence of multidrug-resistant pathogenic strains. TraE of the IncN plasmid pKM101 belongs to the VirB8 family of proteins, an essential component of most T4SSs that form functional dimmers in the T4SS core. Here, we present the X-ray crystallographic structure of the periplasmic domain of TraE at 2.4 Å resolution. The structure shows many similarities to the known VirB8-like protein structures from Brucella suis [1] and Agrobacterium tumefaciens [2]. However, the nature and the number of residues implicated in the dimerization interface differ considerably from those in the TraE structure [2]. Similar to other VirB8 homologs we have shown by analytical gel filtration that there is a concentration dependant equilibrium between monomeric and dimeric forms of TraE. Moreover, using a bacterial two-hybrid assay, in vivo dimerization has been demonstrated with full-length TraE and key residues for dimerization were identified by site-directed mutagenesis. Our work adds novel insights into the growing body of knowledge on VirB8-like proteins and it will inform future strategies aimed at developing inhibitors of TraE protein interactions and of plasmid transfer.

[1] *Terradot, L., et al., Structures of two core subunits of the bacterial type IV secretion system, VirB8 from Brucella suis and ComB10 from Helicobacter pylori. Proc Natl Acad Sci U S A, 2005. 102(12): p. 4596-601.,* ***[2]*** *Bailey, S., et al., Agrobacterium tumefaciens VirB8 structure reveals potential protein-protein interaction sites. Proc Natl Acad Sci U S A, 2006. 103(8): p. 2582-7.*

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