Solution Characterization of The Dynamic Conjugative Entry Exclusion Protein Trag

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The R100 plasmid is representative of F-like conjugative type IV secretion systems for the transmission of mobile DNA elements in gram-negative bacteria, serving as a major contributor to antibiotic resistance.[1] The TraG protein of F-like systems consists of a membrane-bound N-terminal domain and a periplasmic C-terminal domain, denoted TraG*. TraG* is essential in preventing redundant DNA transfer through a process termed entry exclusion. In the donor cell it interacts with TraN to facilitate mating pair stabilization, however if a mating pore forms between bacteria with identical plasmids, TraG* interacts with its cognate TraS in the inner membrane of the recipient bacterium to prevent redundant donor-donor conjugation. Structural studies of TraG* from the R100 plasmid have revealed the presence of a dynamic region between the N- and C-terminal domains of TraG; thermofluor, circular dichroism, collision induced unfolding mass spectrometry and SEC- MALS-SAXS experiments showed N-terminal truncation mutants displayed higher stability and less disordered content relative to full-length TraG*.[2] The 45 N-terminal residues of TraG* are hypothesized to serve as a flexible linker between the two independently functioning domains. These studies guide further crystallization trials to elucidate a high- resolution structure of TraG* and determine the mechanism of the TraG-TraS interaction.

{1} Bragagnolo, N. et al. Protein dynamics in f-like bacterial conjugation. Biomedicines 8, (2020).
{2} Bragagnolo, N. & Audette, G. F. Solution characterization of the dynamic conjugative entry exclusion protein TraG. Struct. Dyn. 9, (2022).

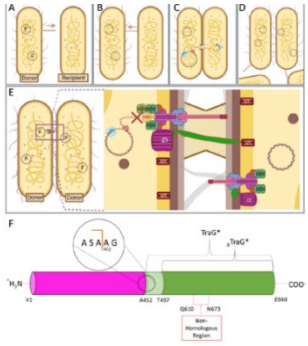

Figure 1: A Model of the F-like type IV secretion systems (T4SS) based on available structural information, including a cryo-electron tomography (cryo-ET) model of the pED208 core complex and a cryo-electron microscopy (cryo-EM) model of the assembled pED208 pilus (PDR ID: 5LEG). Tra proteins are labeled with capital letters and Trb proteins are shown with lower case letters. Proteins are colored based on function; the pilin TraA is white, pilin processing proteins are purple, proteins responsible for pilus assembly/extension are colored in fuchsia, the core complex is colored in dark blue, white pilus retraction proteins are colored in light blue, green colored proteins are responsible for mating pair stabilization,and the red proteins are responsible for exclusion. ATpase Proteins TraC and TraD are complexed in the cytosol based on the vryo-ET image but are present in yhr inner membrane (IM) as well. The outer membrane (OM) complex in blue is composed of TraK and TraV in a complex that created 13-fold symmetry. Figure created with BioRender.com.

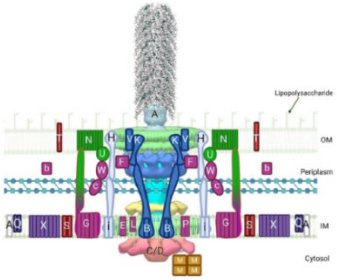

Figure 2: A Simplified depiction of gram-negative bacterial conjugation and entry exclusion by TraG in F-like T4SSs. For more details see Bragagnolo, N. and Audette, G.F.2022.