Structural Studies of the Conjugative Entry Exclusion Protein from the F and R100 Plasmids, TraG

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Conjugative type IV secretion systems (T4SS) transmit mobile DNA elements in bacteria and are a significant contributor to the evolution of antibiotic resistance. Of the proteins produced from the F and R100 plasmids of Escherichia coli; the representative conjugative plasmids of gram-negative bacteria, TraG is among the largest and consists of a membrane-bound N-terminal domain and a periplasmic C-terminal domain denoted TraG*. Each domain has its own function, the membrane bound N-terminal domain is involved in pilus assembly while TraG* is bifunctional. In the donor cell, it interacts with TraN within the outer membrane to facilitate mating pair stabilisation. However, TraG* is also essential in preventing redundant DNA transfer through its interaction with a cognate TraS in the inner membrane of the recipient cell when the recipient carries the same plasmid. Thermofluor experiments showed N-terminal truncation mutants of TraG* displayed higher stability relative to full-length TraG*, and SEC MALS provides evidence of higher levels of aggregation in the full-length protein relative to the N-terminal truncation mutants. SEC-MALS-SAXS was performed to obtain low-resolution structural models to visualize the conformational changes resulting from the truncation, and crystal trials of TraG* mutants provide evidence of a higher propensity for the crystallisation of N-terminal truncations of TraG*. The 45 N-terminal residues of TraG* are predicted to be highly dynamic, possibly serving as a flexible linker between two independently functioning domains.