

Structural Studies of TraG, the Conjugative Entry Exclusion Protein from the F-plasmid

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The F-plasmid of *Escherichia coli* is representative of conjugative type IV secretion systems for the transmission of mobile DNA elements in bacteria, a significant contributor to the evolution of antibiotic resistance. One of the largest proteins of this system, TraG 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 F-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, circular dichroism and HDX-MS experiments showed N-terminal truncation mutants of TraG* displayed higher stability and less disordered content relative to full-length TraG*. The 45 N-terminal residues of TraG* were predicted to be highly dynamic, possibly serving as a flexible linker between two independently functioning domains. Further truncation mutants of TraG* were designed to enable protein crystallisation.