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## *Structural, Functional and Dynamic Studies of F Plasmid T4SS Proteins*

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The transfer of genetic material within a bacterial population through the process of conjugation distributes novel genetic elements for survival in unique environments. Bacterial conjugation is important to public health as the spread antibiotic resistance genes among bacteria results in multi-drug resistance. Indeed, approximately 70% of bacteria that cause hospital-acquired infections are resistant to at least one antibiotic. Conjugative systems, such as the F plasmid of Escherichia coli, consist of proteins that share similarities to type IV secretion systems (T4SS). T4SS proteins of the F plasmid form a membrane spanning protein complex and surface exposed pilus. The periplasmic T4SS proteins TraF, TraW and TrbC play important roles during the F pilus assembly and DNA transfer. Functional analysis of a series of TraF mutants has shown that modification to TraF abolishes pilus synthesis and in turn F plasmid conjugation. In addition, dynamic analysis of TraF using time-resolved hydrogen-deuterium exchange mass spectrometry has revealed a well structured C-terminal thioredoxin-like domain and a more dynamic N-terminal domain that is predicted to interact with companion T4SS protein TraH. In addition, interaction analysis of the putative pore forming proteins TraW and TrbC indicate that the C-terminal domain of TrbC is not required for interaction with TraW, unlike previous models of F T4SS assembly. Rather the C-terminal domain of TraW preferentially interacts with the N-terminal domain of TrbC. These studies are providing a clearer picture of the structures and interactions that occur within the F T4SS assembly during the conjugative process.

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