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Structure and mechanistic insights into F/R1 plasmid conjugative relaxase

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Conjugation, a process mediating transport of plasmid DNAs from one bacterium to another, is an important means by which antibiotic resistance genes propagate among bacteria. This process requires a type IV secretion system (T4SS), a pilus, and a DNA processing complex called the "relaxosome"1. The main protein in the relaxosome is the "relaxase", a protein that binds to a plasmid sequence termed "origin of transfer or OriT", nicks it at a site within OriT termed "nic", and covalently attaches to the released 5'-OH of the strand to be transferred1. It is this covalently bound protein-DNA complex that is translocated to the recipient cell through the T4SS.
The relaxase TraI of the F/R1 plasmid systems has two binding sites within OriT on either side of the nic site2. Proteolysis of TraI bound to these ssDNA sites revealed two states, "open" or "closed", depending on whether TraI is bound to the sequence of OriT 5' or 3' to the nic site, respectively. SEC titration experiment shows two molecules can load on the ssDNA forming an ssDNA mediated dimer. Using modified ssDNA we showed that the two TraI molecules are indeed bound to the respective two binding sites. A cryo EM structure of TraI bound to its helicase site, solved to near-atomic resolution reveals a unique organization of domains within the protein and shed light into its helicase mode at OriT.
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