

Publication

A translocation motif in relaxase TrwC specifically affects recruitment by its conjugative Type IV Secretion System

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Type IV Secretion System (T4SS) substrates are recruited through a translocation signal that is poorly defined for conjugative relaxases. The relaxase TrwC of plasmid R388 is translocated by its cognate conjugative T4SS, and it can also be translocated by the VirB/D4 T4SS of *Bartonella henselae*, causing DNA transfer to human cells. In this work, we have constructed a series of TrwC variants and assayed them for DNA transfer to bacteria and human cells to compare recruitment requirements by both T4SS. Comparison with other reported relaxase translocation signals allowed us to determine two putative translocation sequence motifs, TS1 and TS2. Mutations affecting TS1 drastically affected conjugation frequencies, while mutations affecting either motif had only a mild effect on DNA transfer rates through the VirB/D4 T4SS of *B. henselae*. These results indicate that a single substrate can be recruited by two different T4SS through different signals. The C-terminus affected DNA transfer rates through both T4SS tested, but no specific sequence requirement was detected. The addition of a *Bartonella* Intracellular Delivery (BID) domain, the translocation signal for the *Bartonella* VirB/D4 T4SS, increased DNA transfer up to 4% of infected human cells, providing an excellent tool for DNA delivery to specific cell types. We show that the R388 coupling protein TrwB is also required for this high-efficiency TrwC-BID translocation. Other elements apart from the coupling protein may also be involved in substrate recognition by T4SS.

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