Colicin A binds to a novel binding site of TolA in the *E.coli* periplasm.

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We report the structure of the C-terminal domain of TolA (TolA\(^{III}\)) bound to the translocation domain of colicin A (TA\(^{53-107}\)). The interface region of the TA\(^{53-107}\)-TolA\(^{III}\) complex consists of polar contacts linking residues R92 to R96 of ColA with residues L375-P380 of TolA which constitutes a b-strand addition commonly seen in more promiscuous protein-protein contacts. The interface region also includes three cation-π interactions (Y58-K368, Y90-K379, F94-K396) which have not been observed in any other colicin-Tol protein complex. Mutagenesis of the interface residues of ColA or TolA revealed that the effect on the interaction was cumulative; single mutations of either partner had no effect on ColA activity, whereas mutations of three or more residues significantly reduced ColA activity. TA\(^{53-107}\) binds on the opposite side of TolA\(^{III}\) to that used by g3p, ColN or TolB, illustrating the flexible nature of TolA as a periplasmic hub protein.