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A phospholipase A_1 antibacterial Type VI secretion effector interacts directly with the C-terminal domain of the VgrG spike protein for delivery

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Summary

The Type VI secretion system (T6SS) is a multiprotein machine that delivers protein effectors in both prokaryotic and eukaryotic cells, allowing interbacterial competition and virulence. The mechanism of action of the T6SS requires the contraction of a sheath-like structure that propels a needle towards target cells, allowing the delivery of protein effectors. Here, we provide evidence that the entero-aggregative *Escherichia coli* Sci-1 T6SS is required to eliminate competitor bacteria. We further identify Tle1, a toxin effector encoded by this cluster and showed that Tle1 possesses phospholipase A_1 and A_2 activities required for the interbacterial competition. Self-protection of the attacker cell is secured by an outer membrane lipoprotein, Tli1, which binds Tle1 in a 1:1 stoichiometric ratio with nanomolar affinity, and inhibits its phospholipase activity. Tle1 is delivered into the periplasm of the prey cells using the VgrG1 needle spike protein as carrier. Further analyses demonstrate that the C-terminal extension domain of VgrG1, including a transthyretin-like domain, is responsible for the interaction with Tle1 and its subsequent delivery into target cells. Based on these results, we propose an additional mechanism of transport of T6SS effectors in which cognate effectors are selected by specific motifs located at the C-terminus of VgrG proteins.

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Introduction

The T6SS is built by the assembly of at least 13 proteins encoded by usually clustered genes. A transmembrane complex anchors to the cell envelope a phage-like tail complex that extends from the membrane in the cytoplasm (Coulthurst, 2013; Ho *et al.*, 2014; Zoued *et al.*, 2014; Basler, 2015). The membrane complex serves as docking station for assembly of the tail complex (Durand *et al.*, 2015), a dynamic tubular structure functionally and structurally homologous to the contractile tail of bacteriophages (Bönemann *et al.*, 2009; Leiman *et al.*, 2009; Bönemann *et al.*, 2010; Basler *et al.*, 2012). It is constituted of an inner tube made of stacked hexameric rings of the Hcp protein, whose three-dimensional structure is very similar to that of the bacteriophage tail tube gpV (Mougous *et al.*, 2006; Pell *et al.*, 2009; Ballister *et al.*, 2008; Brunet *et al.*, 2014; Douzi *et al.*, 2014). This Hcp edifice resembles a channel-like tubular structure with a 40-Å internal diameter and is surrounded by a contractile sheath made of the TssB and TssC proteins (Kudryashev *et al.*, 2015). The inner tube/sheath structure is built on an assembly platform – the baseplate – that contacts the membrane