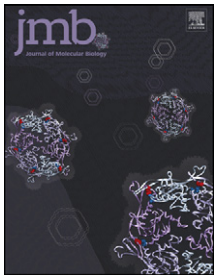

Structure–Function Analysis of the C-Terminal Domain of the Type VI Secretion TssB Tail Sheath Subunit

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Abstract

The type VI secretion system (T6SS) is a specialized macromolecular complex dedicated to the delivery of protein effectors into both eukaryotic and bacterial cells. The general mechanism of action of the T6SS is similar to the injection of DNA by contractile bacteriophages. The cytoplasmic portion of the T6SS is evolutionarily, structurally and functionally related to the phage tail complex. It is composed of an inner tube made of stacked Hcp hexameric rings, engulfed within a sheath and built on a baseplate. This sheath undergoes cycles of extension and contraction, and the current model proposes that the sheath contraction propels the inner tube toward the target cell for effector delivery. The sheath comprises two subunits: TssB and TssC that polymerize under an extended conformation. Here, we show that isolated TssB forms trimers, and we report the crystal structure of a C-terminal fragment of TssB. This fragment comprises a long helix followed by a helical hairpin that presents surface-exposed charged residues. Site-directed mutagenesis coupled to functional assay further showed that these charges are required for proper assembly of the sheath. Positioning of these residues in the extended T6SS sheath structure suggests that they may mediate contacts with the baseplate.
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Introduction

The type VI secretion system (T6SS) is a specialized machine dedicated to the delivery of protein effectors into target cells by a contact-dependent mechanism [1–5]. T6SS gene clusters are widely distributed in Gram-negative proteobacteria with an overrepresentation in the γ phyllum [6–8]. By contrast to other specialized secretion systems, the T6SS is versatile as it can deliver protein effectors in both prokaryotic and eukaryotic cells. In agreement with these functions, T6SS protein effectors that bear broad (nucleases, phospholipases), anti-bacterial-specific (peptidoglycan hydrolases) or anti-host-specific (actin cross-linking) activities have been identified and characterized in the recent years [9–13].

At the molecular level, the T6SS is thought to function as a crossbow. It is composed of two sub-complexes: a cytoplasmic tubular structure anchored to the cell envelope by a membrane complex [3,4,14,15]. The 1.7-MDa membrane complex comprises 10 copies of the TssJ outer-membrane lipoprotein in interaction with 10 copies of the TssM–TssL inner membrane heterodimer that shares homologies with the type IVb secretion system IcmF–IcmH heterodimer [16–23]. By contrast, the cytoplasmic structure is evolutionarily, structurally and functionally homologous to the tail of contractile bacteriophages [2,24,25]. It is composed of a needle formed by the Hcp inner tube tipped by the VgrG/PAAR spike, wrapped by the TssBC contractile sheath and built onto an assembly platform, the baseplate [3,4,15,26–28]. The VgrG protein is structurally similar to the gp27–gp5 phage cell-puncturing device complex and is therefore considered as both the hub for baseplate assembly and the spike for target cell penetration [29,30]. The Hcp protein forms hexameric

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