Molecular Dissection of the Interface between the Type VI Secretion TssM Cytoplasmic Domain and the TssG Baseplate Component

Laureen Logger, Marie-Stéphanie Aschtgen, Marie Guérin, Eric Cascales and Eric Durand

Laboratoire d'Ingénierie des Systèmes Macromoléculaires, *Institut de Microbiologie de la Méditerranée, Aix-Marseille Université, CNRS – UMR 7255, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France*

Correspondence to Eric Cascales and Eric Durand: *cascales@imm.cnrs.fr; edurand@imm.cnrs.fr*
http://dx.doi.org/10.1016/j.jmb.2016.08.032
Edited by Bert Poolman

Abstract

The type VI secretion system (T6SS) is a multiprotein complex that catalyses toxin secretion through the bacterial cell envelope of various Gram-negative bacteria including important human pathogens. This machine uses a bacteriophage-like contractile tail to puncture the prey cell and inject harmful toxins. The T6SS tail comprises an inner tube capped by the cell-puncturing spike and wrapped by the contractile sheath. This structure is built on an assembly platform, the baseplate, which is anchored to the bacterial cell envelope by the TssJLM membrane complex (MC). This MC serves as both a tail docking station and a channel for the passage of the inner tube. The TssM transmembrane protein is a key component of the MC as it connects the inner and outer membranes. In this study, we define the TssM topology, highlighting a large but poorly studied 35-kDa cytoplasmic domain, $TssM_{Cyto}$, located between two transmembrane segments. Protein–protein interaction assays further show that $TssM_{Cyto}$ oligomerises and makes contacts with several baseplate components. Using computer predictions, we delineate two subdomains in $TssM_{Cyto}$, including a nucleotide triphosphatase (NTPase) domain, followed by a 110-aa extension. Finally, site-directed mutagenesis coupled to functional assays reveals the contribution of these subdomains and conserved motifs to the interaction with T6SS partners and to the function of the secretion apparatus.

Introduction

The type VI secretion system (T6SS) is a versatile multiprotein secretory machine that is implicated in both interbacterial competition and anti-eukaryotic host activities. The T6SS delivers a broad arsenal of toxins with peptidoglycan, phospholipid, or DNA hydrolysis activities or induces cytoskeleton rearrangements directly into the target cell [1–4].

For toxin delivery, the T6SS uses a contractile mechanism that is comparable to that of *Myoviridae* phages or R-pyocins [5–10]. This machine is composed of 13 core subunits, categorised in three subcomplexes [8,10–12]: a cytoplasmic tubular structure built on an assembly platform—or baseplate (BP)—that is evolutionarily, structurally, and functionally related to bacteriophage contractile tails [5,13–15] and is anchored to the cell envelope by a membrane complex (MC) [16].

The T6SS tail is composed of an inner tube made of stacked Hcp hexameric rings and is wrapped into a sheath-like structure, formed by the polymerisation of TssB–TssC heterodimeric complexes and that is assembled in an extended conformation [14,17–19]. Indeed, the assembly of the tail can be followed by time-lapse microscopy: fluorescent-labelled sheath components assemble a ~600-nm-long tubular structure in tens of seconds, which then contracts in a few milliseconds [14,20]. The contraction of the sheath coincides with bacterial prey lysis, suggesting that similar to phages, sheath contraction propels the inner tube towards the target cell, allowing the delivery of toxin effectors [8,11,20,21]. The assembly of the tube and the sheath is coordinated by TssA, a protein that controls the elongation of the tail at the distal end and that maintains the sheath under the extended conformation [22]. The inner tube is tipped by a spike constituted of a trimer of the VgrG

protein, which is proposed to puncture the target cell membrane [13,23]. The VgrG trimer is also part of the BP that is used as an assembly platform for the tail. Recently, the T6SS BP composition has been revealed. In addition to VgrG, it is composed of the TssE, -F, and -G subunits, the homologues of the phage T4 gp25, gp6, and gp7 proteins, respectively, and of TssK, a protein of unknown function with limited homologies to phage T4 gp8 or gp10 proteins that has been proposed to be a connector to the MC [15,24–27]. This MC is composed of the two TssL and TssM inner membrane (IM) proteins and of the TssJ outer membrane (OM) lipoprotein [28–32]. TssL and TssM interact in the IM, whereas the C-terminal periplasmic domain of TssM contacts the TssJ lipoprotein close to the OM [16,29,30,33–35]. The MC serves as a docking station for the BP and the tail but has also been proposed to serve as a channel for the passage of the inner tube during sheath contraction [16]. In the recent years, the assembly pathway of the T6SS has been well defined. T6SS biogenesis progresses from the OM to the cytoplasm. It starts with the positioning of the TssJ lipoprotein and the successive recruitments of TssM and TssL [16]. Recruitment of TssA then positions the BP complex onto the MC and primes the polymerisation of the tail tube/sheath [15,22,36]. This ordered assembly pathway requires tight contacts between the different subunits. Indeed, docking of the BP onto the MC requires multiple contacts including the interactions of TssE and TssK with TssL and of TssG and TssK with TssM [15,24]. TssM is therefore a key component as it mediates contact with the OM TssJ lipoprotein and with cytoplasmic BC subunits.

Here, we show that the enteroaggregative *Escherichia coli* (EAEC) TssM protein is a polytopic membrane protein, inserted into the IM by three transmembrane helices (TMH). The C-terminal portion of TssM is in the periplasm and interacts with TssJ [34]. TMH2 and TMH3 delimitates a ~35-kDa cytoplasmic domain, $TssM_{Cyto}$, which is conserved among TssM homologues. Computer analyses show that $TssM_{Cyto}$ is constituted of two subdomains: a subdomain with a nucleotide triphosphatase (NTPase)-like domain, followed by an extension. Indeed, TssM has been previously shown to bind and hydrolyse nucleotide triphosphates (NTPs) [37]. However, the role of the NTP-binding motif and its functional implication during T6SS activity are still a matter of debate [29,33]. The extension comprises a eukaryotic Dumpy-30 (DPY-30)-like dimerisation motif. We show that the NTPase-like domain mediates the interaction with TssK, whereas the extension is necessary and sufficient for $TssM_{Cyto}$ oligomerisation and interaction with TssG. Site-directed mutagenesis of conserved motifs within the extension revealed their contribution for $TssM_{Cyto}$ oligomerisation and $TssM_{Cyto}$–TssG interaction and for proper assembly of the T6SS. Our results thus provide details on the molecular interface between the T6SS membrane and the BP complexes.

Results

TssM is a polytopic IM protein

The TssM protein encoded within the EAEC *sci-1* gene cluster [EC042_4539; Genbank accession (GI): 284924260] is a large protein of 1129 aa. Based on hydrophobicity plots, the most widely used computer tools predict TssM as an IM protein with three TMH (Fig. 1a). Indeed, fractionation experiments showed that TssM co-fractionates with membrane proteins (data not shown). To experimentally define the TssM topology and determine the TMH boundaries, we performed a cysteine accessibility assay using the substituted cysteine accessibility method [38]. This assay relies on the ability of 3-(*N*-maleimidylpropionyl) biocytin (MPB), a sulfhydryl reagent, to cross the OM but not the IM of Gram-negative bacteria including EAEC [30,31]. TssM possesses nine native cysteine residues, with one (C727) predicted to locate in the periplasm. Hence, the wild-type (WT) TssM protein is labelled by MPB *in vivo* (Fig. 1b). In agreement with the computer predictions, a TssM protein, in which the cysteine at position 727 is substituted to serine (C727S), was not labelled with MPB (Fig. 1b). These data suggest that C727 is located in the periplasm, whereas all other eight cysteine residues are located in the cytoplasm or buried into the structure of the protein and are then inaccessible to MPB. We then introduced cysteine substitutions in the C727S TssM variant at various positions along the protein (at positions 37, 67, 352, and 386; Fig. 1a). All these mutated proteins were produced at similar levels (Fig. 1b) and were able to complement the effect of the *tssM* mutant in an Hcp secretion assay (data not shown). The A37C and S386C variants were biotinylated with MPB, suggesting that the A37 and S386 residues are located in the periplasm (Fig. 1b). By contrast, the V67C and S352C variants were not labelled, indicating that the V67 and S352 residues are located in the cytoplasm (Fig. 1b). Altogether, the data of the cysteine accessibility defined the topology of TssM; TssM is constituted of three TMH, with the N terminus in the cytoplasm and the C terminus in the periplasm. TssM spans the IM through two TMH-oriented in-to-out (TMH1, residues 13–29; TMH3, residues 360–382) and one TMH-oriented out-to-in (TMH2, residues 44–62; Fig. 1c). TMH2 and TMH3 thus delimitate a ~35-kDa domain located in the cytoplasm, called $TssM_{Cyto}$ hereafter.

The cytoplasmic domain of TssM oligomerises and interacts with the components of the T6SS membrane and BP complexes

The topology of TssM indicates the existence of two soluble domains, one in the periplasm ($TssM_{Peri}$,