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Domestication of a housekeeping transglycosylase for assembly of a Type VI secretion system

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Abstract

The type VI secretion system (T6SS) is an anti-bacterial weapon comprising a contractile tail anchored to the cell envelope by a membrane complex. The TssJ, TssL, and TssM proteins assemble a 1.7-MDa channel complex that spans the cell envelope, including the peptidoglycan layer. The electron microscopy structure of the TssJLM complex revealed that it has a diameter of ~18 nm in the periplasm, which is larger than the size of peptidoglycan pores (~2 nm), hence questioning how the T6SS membrane complex crosses the peptidoglycan layer. Here, we report that the MltE housekeeping lytic transglycosylase (LTG) is required for T6SS assembly in enteroaggregative *Escherichia coli*. Protein–protein interaction studies further demonstrated that MltE is recruited to the periplasmic domain of TssM. In addition, we show that TssM significantly stimulates MltE activity *in vitro* and that MltE is required for the late stages of T6SS membrane complex assembly. Collectively, our data provide the first example of domestication and activation of a LTG encoded within the core genome for the assembly of a secretion system.

Keywords multiprotein assembly; peptidoglycan; protein complex; protein transport; secretion system

Subject Categories Microbiology, Virology & Host Pathogen Interaction; Structural Biology

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Introduction

The cell envelope of Gram-negative bacteria is crossed by multiprotein complexes that participate to the assembly of surface appendages (e.g., the flagellum) or serve as channels for the passage of large molecules such as pili, DNA, or protein effectors (e.g., piliation, conjugation, or secretion systems) [1]. These complexes are usually large and are anchored to both the inner and outer membranes [1]. However, the peptidoglycan layer represents a physical barrier for the assembly of these structures, as they are usually larger than peptidoglycan pores, estimated to have a diameter of ~2 nm [2]. Most of these systems have therefore evolved enzymes, called lytic transglycosylases (LTGs), that locally rearrange the cell wall [3–5]. LTGs cleave the glycan strands but have no action on peptide cross-links, therefore creating lateral separation of the peptidoglycan [6,7]. Endogeneous LTGs are involved in peptidoglycan synthesis, turnover, recycling, and daughter cell separation [7–9]. By contrast, the LTGs dedicated to specific cell-envelope spanning complexes are called specialized LTGs [3–5,8]. The activity of these enzymes needs to be tightly controlled to avoid peptidoglycan breaches and cell lysis [8,10]. In addition, the LTG activity should be spatially controlled to create sufficient space at the site of assembly. The spatial activation of specialized LTGs is secured by their recruitment to the site of assembly through interactions with one or several components of the apparatus. The recruitment of specialized LTGs to their cognate apparatus has been exemplified in the case of several cell-spanning machineries: The *Rhodobacter sphaeroides* SltF LTG is recruited to the flagellar FlgJ subunit [11,12], the PleA protein localizes at the cell pole in *Caulobacter crescentus* and is required for the assembly of the polar pilus and polar flagellum [13], the VirB1-like LTG is recruited to the VirB8-like protein in type IV secretion systems [4,14–19], and the EtgA LTG associates with the type III secretion system EscI rod component [4,20–22]. Interestingly, in a few cases, machine subunits comprise an additional domain with LTG activity, such as the flagellar rod FlgJ protein [23–27] or the *Bordetella pertussis* T4SS PtlE subunit [28]. For several of these enzymes, it has been recently demonstrated that the transglycosylase activity is weak *in vitro* but stimulated in the presence of its partner, suggesting that binding to the cell-envelope spanning structure specifically activates the enzymatic activity and hence controls localized peptidoglycan hydrolysis. The activity of the T3SS EtgA LTG is enhanced by co-incubation with the EscI rod subunit [22]. In the case of the *R. sphaeroides* flagellum, the activity of SltF is modulated by both FlgB and FlgF [29].

Recently, we determined the structure of the 1.7-MDa type VI secretion system (T6SS) membrane complex from enteroaggregative *Escherichia coli* (EAEC) using negative stain electron microscopy [30]. This complex spans the cell envelope, and its diameter was estimated to ~18 nm in the periplasm, suggesting that its proper insertion requires localized peptidoglycan rearrangement or degradation. However, no gene encoding LTG is encoded within T6SS gene clusters [31,32]. The T6SS is a sophisticated multiprotein

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