Scientific Report

TRANSPARENT PROCESS

EMBO reports

Type VI secretion and bacteriophage tail tubes share a common assembly pathway

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Abstract

The Type VI secretion system (T6SS) is a widespread macromolecular structure that delivers protein effectors to both eukaryotic and prokaryotic recipient cells. The current model describes the T6SS as an inverted phage tail composed of a sheath-like structure wrapped around a tube assembled by stacked Hcp hexamers. Although recent progress has been made to understand T6SS sheath assembly and dynamics, there is no evidence that Hcp forms tubes *in vivo*. Here we show that Hcp interacts with TssB, a component of the T6SS sheath. Using a cysteine substitution approach, we demonstrate that Hcp hexamers assemble tubes in an ordered manner with a head-to-tail stacking that are used as a scaffold for polymerization of the TssB/C sheath-like structure. Finally, we show that VgrG but not TssB/C controls the proper assembly of the Hcp tubular structure. These results highlight the conservation in the assembly mechanisms between the T6SS and the bacteriophage tail tube/sheath.

Keywords bacteriophage; Hcp; sheath; tail tube; Type VI secretion
Subject Categories Membrane & Intracellular Transport; Microbiology, Virology & Host Pathogen Interaction
DOI 10.1002/embr.201337936 | Received 31 August 2013 | Revised 9 December 2013 | Accepted 12 December 2013 | Published online 31 January 2014
EMBO Reports (2014) 15, 315–321

Introduction

In Gram-negative bacteria, the secretion of toxin proteins is achieved by dedicated, specialized machineries called secretion systems. The Type VI secretion system (T6SS) is highly versatile as it delivers protein effectors in either eukaryotic or prokaryotic target cells [1,2]. The T6SS is composed of 13 Tss (Type six subunits) core components that assemble a macromolecular complex spanning the cell envelope [2,3]. The TssL, TssM and TssJ membrane proteins interact to form the trans-envelope apparatus [4,5]. A number of subunits are evolutionarily, structurally and mechanistically related to proteins that form the tail of contractile bacteriophages. VgrG is a composite protein, structurally related to the trimeric gp27/gp5 hub complex forming the cell-puncturing device of bacteriophage T4 [6]. The membrane of the target cell is supposed to be disrupted by the tip of the VgrG protein that acts as a syringe. Recently, a PAAR-like protein has been shown to associate with the tip of VgrG, and by recruiting effector proteins, has been proposed to translocate these proteins into the target cell [7]. The Hcp protein is structurally related to gpV, the major tail tube protein of phage λ. Hcp is an abundant protein of the T6SS that forms a ring-shaped hexamer [8–11]. While Hcp tubes have been visualized *in vitro* [12], the existence of such tubes *in vivo* has not been evidenced. In contractile bacteriophages, prior to contraction, the tail tube is surrounded by the sheath in an extended conformation [13]. Upon infection, the phage sheath undergoes an extensive structural transition leading to its contraction and propelling the tail tube towards the target cell interior [13]. Interestingly, the TssB (VipA) and TssC (VipB) proteins assemble large tubular structures extending into the cytoplasm [14,15]. The TssB/C sub-complex exhibits cogwheel-like cross-sections resembling the bacteriophage sheath [16]. Time-lapse fluorescence microscopy further showed that these structures are highly dynamic, oscillating between extended and contracted conformations [14,15,17]. Based on homology with the bacteriophage it is thought that contraction of the TssB/C tubule propels the Hcp tube towards the exterior and the target cell. In agreement with this model, a recent study has shown that contraction of the T6SS sheath is correlated with prey cell lysis [17]. However, the existence of Hcp tubes has not been demonstrated *in vivo*. The Hcp crystal structure packing suggested that Hcp hexamers may stack either in a head-to-tail [8], head-to-head [10] or tail-to-tail [11] conformation. However, while the different modes of inter-rings interactions observed for the different Hcp proteins may reflect differences during assembly, it is formally possible that these packing are crystallographic artifacts and are not physiologically relevant. Similarly, the mode of assembly of the tube protein of contractile tailed phages remains unsolved. Cryo-electron microscopy images showed that the phage tail tube is a cylinder lacking discernable surface structures [9,13]. However, the tail tubes of non-contractile phages are composed of stacked hexamers

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