nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-018-0260-1

Biogenesis and structure of a type VI secretion baseplate

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To support their growth in a competitive environment and cause pathogenesis, bacteria have evolved a broad repertoire of macromolecular machineries to deliver specific effectors and toxins. Among these multiprotein complexes, the type VI secretion system (T6SS) is a contractile nanomachine that targets both prokaryotic and eukaryotic cells. The T6SS comprises two functional subcomplexes: a bacteriophage-related tail structure anchored to the cell envelope by a membrane complex. As in other contractile injection systems, the tail is composed of an inner tube wrapped by a sheath and built on the baseplate. In the T6SS, the baseplate is not only the tail assembly platform, but also docks the tail to the membrane complex and hence serves as an evolutionary adaptor. Here we define the biogenesis pathway and report the cryo-electron microscopy (cryo-EM) structure of the wedge protein complex of the T6SS from enteroaggregative *Escherichia coli* (EAEC). Using an integrative approach, we unveil the molecular architecture of the whole T6SS baseplate and its interaction with the tail sheath, offering detailed insights into its biogenesis and function. We discuss architectural and mechanistic similarities but also reveal key differences with the T4 phage and Mu phage baseplates.

The bacterial type VI secretion system is one of the key players for microbial competition and an important virulence factor during bacterial infections. This versatile nanomachine delivers a wide arsenal of effector proteins directly into prokaryotic and eukaryotic target cells[1–4]. T6SS antibacterial activities promote privileged access to the niche, to nutrients or to DNA. In most cases, T6SS causes damage within competitor bacterial cells and therefore participates in the reshaping of bacterial communities such as the microbiota[5,6]. In addition, some T6SS confer antihost capabilities, such as phagocytosis inhibition, by remodelling the host cell cytoskeleton[7–10].

The T6SS belongs to the broad family of contractile injection systems (CISs) that includes bacteriophages, high-molecular-weight tailocins such as R-pyocins and specific apparati necessary for the establishment of symbiosis or for the induction of morphological changes[11–16]. All these structures comprise a common core: the tail. CIS tails are composed of an inner tube wrapped by a sheath built under an extended, metastable conformation on an assembly platform, the baseplate. The T6SS tail tube/sheath is a hundred-nanometre-long cytoplasmic structure. It is made of TssB/C subunits that polymerize to form the contractile sheath[17,18], which surrounds the attacking arrow composed of an inner tube of stacked haemolysin coregulated protein (Hcp) hexameric rings[19,20] tipped by the trimeric VgrG puncturing spike[21]. Various signals, such as contact with the target cell, chemical signals released by competitor or kin cells, response to attacking cells or conjugative transfer, induce structural rearrangements of the sheath leading to its contraction and to the propulsion of the Hcp-VgrG arrow into the target cell [22–25]. Assembly of the tail tube/sheath is initiated on the baseplate. In addition to controlling sheath extension, the baseplate also serves to trigger sheath contraction. During T6SS biogenesis, the baseplate docks to a trans-envelope complex[17,26–28] composed of TssJ, TssL and TssM[29,30]. By connecting the tail to the membrane complex and initiating tail tube/sheath polymerization, the baseplate is a central piece of the T6SS machinery. In addition, by binding cargo effectors through VgrG, the T6SS baseplate also serves as an effector-sorting platform[2,3,31].

CIS baseplates comprise a minimal core of five proteins that share homology with the prototypical T4 phage gp6, gp7, gp25, gp53 and gp27 proteins[11]. Gp6, gp7, gp25 and gp53 assemble into a unit called a wedge[32]. Biogenesis of the baseplate occurs by the polymerization of six wedges around the central gp27 hub[32,33]. The T6SS baseplate is composed of five essential subunits: TssE, TssF, TssG, TssK and VgrG[27]. TssE is a structural homologue of gp25[34] and has been recently identified as the sheath initiator[35]; TssF shares a homology with the amino (N)-terminal region of gp6, whereas TssG has been proposed to fulfil the role of gp7 or gp53[27,36]. VgrG is a chimeric protein in which the gp27 hub is fused to the oligonucleotide/oligosaccharide-binding (OB)-fold/β-helix needle of gp5[7,21]. TssF and TssG interact tightly and stabilize each other[27]. TssK interacts with the TssFG complex[27,37]. Taylor *et al.* recently reported the purification of the TssKFG complex bound to TssE[36]. Hence, it is proposed that TssFG, TssKFG and TssKFGE are assembly intermediates of the T6SS baseplate and have structural and functional homologies to the bacteriophage wedges. In agreement with this hypothesis, contacts between the TssFG complex and VgrG have

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