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Deadly syringes: type VI secretion system activities in pathogenicity and interbacterial competition

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Among specialized bacterial secretion systems, the most widespread is the type VI secretion system (T6SS). This transports effector molecules into target cells in a single, cell-contact dependent step. T6SSs are structurally related to the cell-puncturing device of tailed bacteriophages and predicted to function as contractile injection machineries that perforate eukaryotic and prokaryotic target membranes for effector delivery. Activities of T6SSs can play important roles in virulence by modifying the eukaryotic host cytoskeleton through actin crosslinking. They are also efficient weaponry in interbacterial warfare and provide a fitness advantage by hydrolyzing cell walls of opponent bacteria. The role of T6SSs in interbacterial competition might enable pathogens to outcompete commensal bacteria and facilitate host colonization.

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Introduction

The secretion of bacterial effector proteins into the extracellular space is a crucial step in pathogenesis and interbacterial virulence. In Gram-negative bacteria various specialized secretion systems mediate the transport of effector proteins in a single step across both the inner and outer membranes. In 2006, Mekalanos and coworkers identified the novel type VI secretion system (T6SS) [1•,2], which has since been predicted in 25% of all sequenced Gram-negative bacteria, making T6SSs the most widespread specialized secretion system [3]. Some bacterial species even harbor multiple, distinct T6SSs. T6SSs are encoded by a gene cluster of 13 conserved core components that are all required for function [4,5]. Together these mediate the translocation of toxic effectors into target cells in a cell-contact dependent manner [1•,6,7,8••]. The export of hemolysin coregulated protein (Hcp) and valine–glycine repeat protein G (VgrG) proteins represents the unifying activity of all T6SSs. Apart from essential core components T6SSs also harbor accessory proteins, which allow for T6S regulation and host adaptation and might have led to evolvement of multiple, distinct T6SSs within several bacterial species.

In this review, we will describe the current view of the mechanism and physiological functions of T6SSs. Because a universally accepted nomenclature for the T6SS components does not yet exist, we refer to the names used in the original studies.

Contractile nanomachines: T6SSs puncture target membranes

Bioinformatic and structural analyses suggest that T6SSs are contractile injection systems and use a mechanism similar to tailed bacteriophages, which infect bacterial cells with high specificity and efficiency [9,10•,11]. These bacteriophages employ a syringe-like macromolecular nanomachine to puncture the membrane of host cells. The infectious device consists of a baseplate, a contractile sheath that harbors an internal noncontractile tube and an associated tail spike complex [12]. Upon contact of the phage with the host cell surface conformational changes within the baseplate trigger contraction of the tail sheath, causing ejection of the internal tube and the tail spike complex and penetration of the host envelope [12] (Figure 1a).

Several T6SS components exhibit sequence or structural similarity to the viral infection machinery. For example, *Vibrio cholerae* VCA0109 shares sequence homology with gp25, a component of the T4 bacteriophage baseplate [10•]. The crystal structure of the T6SS exoprotein Hcp exhibits structural similarity to viral tail tube proteins [10•,11]. Hcp forms hexameric rings with an outer diameter of 90 Å and a central channel with a width of 40 Å [2]. Hcp rings can form tubules *in vitro* if the individual stacks are stabilized by artificially introduced disulfide bonds [13]. The exoprotein VgrG forms a trimer that is highly similar to the viral tail spike complex, sharing a needle-like β-helix at the tip as the structural device to puncture target membranes [10•,14]. The T6SS components VipA and VipB form tubular complexes up to 500 nm long, with an outer diameter of 300 Å and an inner channel diameter of 100 Å width [15]. These tubules are

reminiscent in shape and dimension to viral tail sheath proteins and have been suggested to engulf Hcp tubes and, in analogy to the viral infection mechanism, to eject Hcp and associated VgrG upon contraction [10•,16]. VipA/VipB tubules have been visualized by electron cryotomography in intact *V. cholerae* cells [17••]. The tubules are oriented near-perpendicular to the inner membrane and appear in an extended or contracted conformation. Extended structures appear to contain electron-dense material in the central channel, whereas contracted structures are hollow, providing indirect evidence for Hcp engulfment by VipA/VipB tubules [17••]. VipA/VipB tubules are highly dynamic assemblies, showing cycles of polymerization, contraction, and disassembly in *V. cholerae* cells [17••] (Figure 1b). Together these findings support the model that T6SSs function as contractile injection systems, sharing the mechanism of cell puncturing with tailed bacteriophages (Figure 1). One major difference between these contractile systems is that the injection machinery of bacteriophages is only used once and does not need to be recycled and reassembled for future infection rounds. In contrast, contracted VipA/VipB tubules of T6SS are disassembled by the AAA+ protein ClpV under ATP consumption [15,18]. In *V. cholerae* ClpV exhibits a dynamic localization that is dependent on the presence of VipA/VipB tubules [18]. ClpV specifically binds to the contracted conformation of VipA/VipB, restricting its disassembly activity to a post secretion step and allowing for multiple rounds of secretion and effector protein delivery (Figure 1b) [18]. ClpV dynamics and T6SS activity are reduced in *Pseudomonas aeruginosa* [18]. Here, T6SS activity is under additional posttranslational control, involving opposing activities of a kinase (PpkA) and phosphatase (PppA) pair that is controlling the phosphorylation status of the T6SS component Fha [19]. Kinase activity is suggested to be regulated by environmental cues, allowing to integrate external signals for activation of T6SS on demand [20]. Interestingly, an increase in T6SS activity was noticed in adjacent *P. aeruginosa* cells, suggesting that cell-to-cell signaling can modulate T6SS activity [18].

The syringe-like complex of T6SSs must be positioned and anchored to the bacterial cell envelope, in order to couple the energy from VipA/VipB contraction to transport across the inner and outer membranes. A membrane-spanning T6SS complex has been identified in enteroaggregative *Escherichia coli* (EAEC), consisting of TssL (DotU), TssM (IcmF), TssJ (SciN), and TagL (SciZ) [21•]. TagL contains a peptidoglycan-binding domain, which is fused to TssL in other T6SSs, and anchors the T6SS assembly to the cell wall [21•]. In EAEC TagL interacts with the inner membrane protein TssL, which forms a complex with TssM (IcmF), that also localizes to the inner membrane [21•,22,23]. The periplasmic domain of TssM binds to the lipoprotein TssJ, which is anchored to the outer membrane [24]. This cell-envelope spanning complex is suggested to accommodate the phage-like injection machinery. In support of such a model, interactions have been reported between VipA and Hcp and the periplasmic domain of TssM (IcmF) and TssL, respectively [4,25]. Notably, TssM (IcmF) is an essential ATPase of T6SSs and ATP hydrolysis is required for recruitment of Hcp to the membrane fraction, which potentially triggers Hcp polymerization [22,25].

Two routes of toxin delivery by T6SS

The puncturing of target membranes by T6SS drives a path for toxin delivery. Two different classes of effectors have been characterized. The first class is represented by evolved VgrG proteins, which are present in various T6SSs, including the pathogenic genera *Burkholderia*, *Pseudomonas*, *Yersinia* and *Vibrio* [9]. Evolved VgrGs harbor an additional domain, which is C-terminally fused to the β-helix needle (Figure 2a) [9]. The fusion position ensures exposure of the extra domain upon perforation of target membranes. On the basis of sequence analysis distinct activities of evolved VgrGs have been predicted, including cell adhesion, chitosan degradation and actin filament binding and modification [9,26]. Translocation of the actin crosslinking domain of *V. cholerae* VgrG1 into target cells has been observed in cell culture systems and infant mice [9,27•,28]. *Aeromonas hydrophila* VgrG1 exerts an actin ADP-ribosylation upon T6SS-dependent exposure in host cells [29]. Therefore, VgrG proteins have dual functions, and act as both integral structural components of the T6SS assembly and direct effectors.

The second class of effectors is represented by classical toxins, which do not represent core components of T6SSs and are not required for Hcp and VgrG export [4,8••,30,31]. Toxin encoding genes are often not directly associated with T6SS-encoding gene clusters. These effectors are thought to be delivered into target cells by passing through an Hcp channel. The inner diameter of the assumed Hcp conduit is wide enough (40 Å) to allow for secretion of globular proteins with a size of up to approx. 50 kDa. However, the β-helix of associated VgrG trimers is too narrow to allow for effector passage, suggesting that VgrGs dissociate from Hcp tubes upon cell puncturing, which would free the block in the translocation channel. In agreement with this model, all classical T6SS effectors identified to date are small-sized proteins, except *V. cholerae* VasX [30,32].

Dual role of T6SS in pathogenicity and interbacterial competition

The expression of T6SS encoding gene clusters in pathogenic bacteria is increased during infection [33–35] and is frequently regulated by environmental cues that mimic host conditions (for review see: [36]), which suggests a function in pathogenesis. The contributions of T6SSs to virulence development are diverse. In cell culture systems T6SSs have been reported to play crucial roles in