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Structure and Activity of the Type VI Secretion System

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ABSTRACT The type VI secretion system (T6SS) is a multiprotein machine that uses a spring-like mechanism to inject effectors into target cells. The injection apparatus is composed of a baseplate on which is built a contractile tail tube/sheath complex. The inner tube, topped by the spike complex, is propelled outside of the cell by the contraction of the sheath. The injection system is anchored to the cell envelope and oriented towards the cell exterior by a trans-envelope complex. Effectors delivered by the T6SS are loaded within the inner tube or on the spike complex and can target prokaryotic and/or eukaryotic cells. Here we summarize the structure, assembly, and mechanism of action of the T6SS. We also review the function of effectors and their mode of recruitment and delivery.

INTRODUCTION

The type VI secretion system (T6SS) is a multiprotein machine that belongs to the versatile family of contractile injection systems (CISs) (1–4). CISs deliver effectors into target cells using a spring-like mechanism (4–6). Briefly, CISs assemble a needle-like structure, loaded with effectors, wrapped into a sheath built in an extended, metastable conformation (Fig. 1). Contraction of the sheath propels the needle toward the competitor cell. Genomes of Gram-negative bacteria usually encode one or several T6SSs, with an overrepresentation in *Proteobacteria* and *Bacteroidetes* (8–10; for a review on the role of T6SS in gut-associated *Bacteroidales*, see the chapter by Coyne and Comstock [7]). The broad arsenal of effectors delivered by T6SSs includes antibacterial proteins such as peptidoglycan hydrolases, eukaryotic effectors that act on cell cytoskeleton, and toxins that can target all cell types, such as DNases, phospholipases, and NAD^+ hydrolases (11–14). Consequently, the T6SS plays a critical role in reshaping bacterial communities and, directly or indirectly, in pathogenesis (15–19). Destroying bacterial competitors also provides exogenous DNA that can be acquired in naturally competent bacteria and that serves as a reservoir for antibiotic resistance gene spread (20). This chapter lists the major effector families and summarizes the current knowledge on the assembly and mode of action of the T6SS.

TYPE VI SECRETION SYSTEM EFFECTORS

Several T6SSs have been shown to target eukaryotic cells (21–23). By promoting or preventing cytoskeleton rearrangements through the action of specific effectors that target actin or tubulin, the T6SSs of *Vibrio cholerae*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa*

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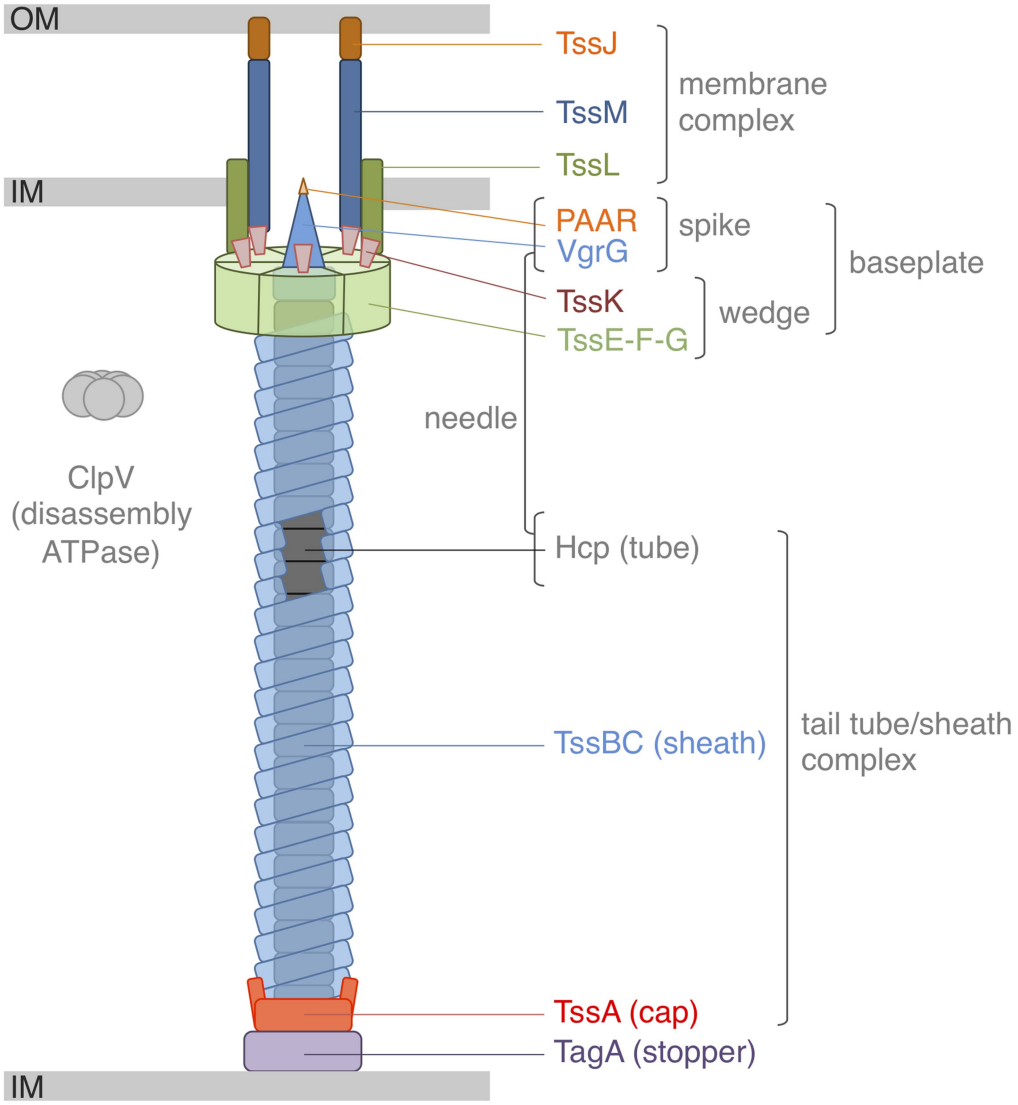


FIGURE 1 Schematic representation of the T6SS. The different subunits are labeled, as are the different subcomplexes. IM, inner membrane; OM, outer membrane.

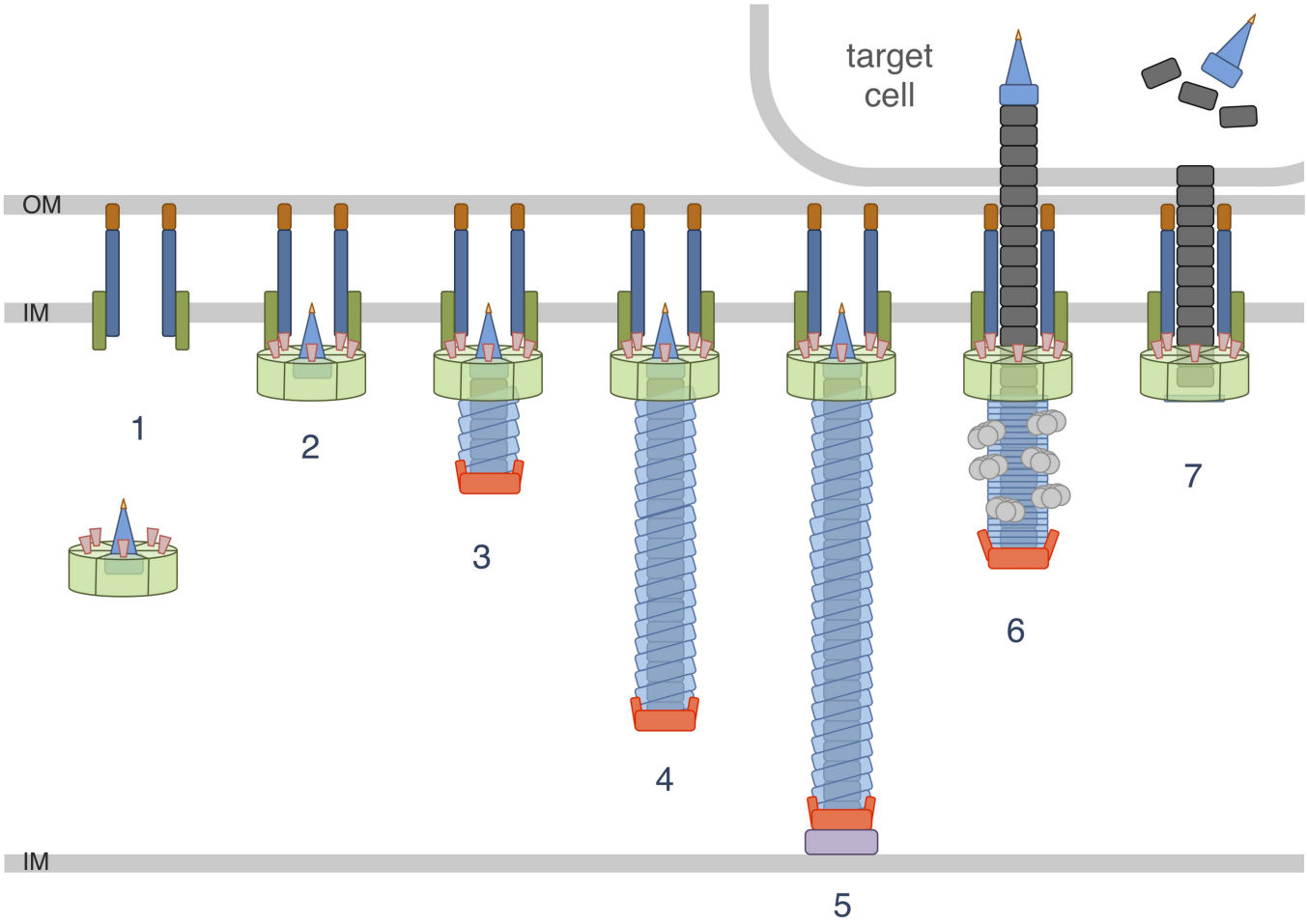
disable phagocytic cells or stimulate internalization into nonphagocytic cells (21, 22, 24–26). Other T6SSs have been demonstrated to manipulate host cells, although the molecular determinants are not yet entirely understood (27–30). However, T6SS gene clusters are widespread in Gram-negative bacterial genomes and not restricted to pathogens (10). Most of them encode proteins with potent antibacterial activities, such as enzymes that cleave essential macromolecules such as DNA, phospholipids, or the peptidoglycan mesh or essential metabolites such as $NAD^+/NADP^+$ (31–36). Additional T6SS antibacterial effectors include ADP-ribosyltransferases that specifically target the Z ring and hence inhibit cell division (37). Antibacterial effectors are active in the periplasm or cytoplasm of the target cell and are coproduced with immunity proteins that remain in the producing cell and act as antitoxins to prevent autointoxication during dueling between sister cells (11–13). More recently, T6SS effectors that collect manganese or zinc in the environment to provide metals to the cell have been described (38–40). By deploying antibacterial effectors or scavenging metals, T6SSs play an important role in bacterial communities, and hence T6SS gene clusters are usually highly represented in species present in multispecies microbiota such as the human gut (7, 16–18, 41). In general, the regulatory mechanisms

and signals underlying expression of T6SS genes, production of T6SS subunits, or posttranslational activation of the secretion apparatus are tightly linked to environmental cues in the niche in which the T6SS is required to destroy competitors (42–45).

TYPE VI SECRETION MECHANISM OF ACTION

T6SSs use a contractile mechanism to inject effectors (Fig. 2). This mechanism is shared with all CISs: a sheath, assembled in an extended conformation, wraps a needle. Contraction of the sheath into a stable state propels the needle (1, 3–5). The needle is composed of an inner tube capped by the spike complex that pierces the membrane of the target cell (Fig. 1). The tail tube/sheath complex (TTC) is built on an assembly platform named the baseplate (BP) (Fig. 1). The TTC and BP are collectively called the tail, a structure that is conserved among all CISs. In addition to this theme common to all CISs, T6SSs have evolved (i) a membrane complex (MC), which docks the tail to the cell envelope and serves as a channel for the passage of the needle upon sheath contraction, and (ii) a specialized BP component

FIGURE 2 Assembly and mechanism of firing of the T6SS. T6SS biogenesis starts with the positioning and assembly of the membrane complex and the assembly of the BP (1). The recruitment and docking of the BP on the membrane complex (2) initiate the TssA-mediated polymerization of the tail tube/sheath tubular structure (3 to 5), which is stopped when hitting the opposite membrane by the TagA stopper (5). Sheath contraction propels the tube/spike needle into the target (6). The ClpV ATPase is recruited to the contracted sheath to recycle sheath subunits (6). Needle components, and effectors associated with them, are delivered inside the target (7).



to properly orient the needle toward the cell exterior, by recognizing and binding the MC (2–5, 46–48) (Fig. 1).

T6SS biogenesis starts with the assembly of the MC in the cell envelope and that of the BP in the cytoplasm (49–51) (Fig. 2). Once the BP is docked to the MC, the inner tube and sheath are coordinately assembled (49–52) (Fig. 2).

ARCHITECTURE OF THE TYPE VI SECRETION SYSTEM

The Membrane Complex

The vast majority of T6SS gene clusters of proteobacterial species encode three membrane proteins: TssJ, TssL, and TssM (8–10, 53) (Fig. 1). TssJ is an outer membrane-associated lipoprotein that protrudes in the periplasm (54). TssL and TssM are anchored in the inner membrane (55–57). The structures of several TssJ homologues have been reported: they all share a classical transthyretin fold with an additional loop, of variable length and composition, located between β-strands 1 and 2 (58–60). TssL bears a single C-terminal membrane-spanning segment (56) and a cytoplasmic domain that comprises two bundles of α-helices (61–63). TssM possesses three transmembrane helices followed by a large periplasmic region (55, 57). The periplasmic region of TssM comprises three domains, including the C-terminal domain that engages in interaction with the TssJ extra loop (49, 58). TssL and TssM interact through their transmembrane segments (55, 64, 65). The cytoplasmic domains of TssL and TssM mediate contacts with the BP (50, 57, 64, 66, 67).

The electron microscopy structure of the fully assembled 1.7-MDa TssJLM MC from enteroaggregative *Escherichia coli* has been reported (49, 68, 69). The complex has a rocket-like structure: a large base, which contains the cytoplasmic and membrane domains of TssL and TssM, is followed by arches and pillars which correspond to the TssM periplasmic domains and TssJ (68). The TssJLM complex, which has 5-fold symmetry *in vivo* and after purification, comprises 15 copies of TssJ and 10 copies of TssL and of TssM (49, 58). The MC delimits an internal lumen with a diameter insufficient for the passage of the tail tube. In addition, this lumen is partly occluded by a periplasmic constriction gate, suggesting that large conformational changes occur upon BP docking or sheath contraction (49, 58).

The MC can be accessorized by additional subunits, such as peptidoglycan-binding proteins (53, 70, 71). MC anchorage to the cell wall likely stabilizes the MC to resist the forces generated during sheath contraction (70). Finally, recent studies have shown that proper assembly of the MC requires the activity of peptidoglycan-degrading enzymes (72, 73).

Interestingly, while the tail complex is evolutionarily related to contractile injection machines, the evolution history of the MC is less clear. TssL and TssM present significant homologies with two accessory subunits associated with type IVb secretion systems, DotU and IcmF, respectively (8, 9). No homologue of TssJ is associated with DotU/IcmF complexes, suggesting that TssJ is from a different ancestry. Indeed, while essential when present, TssJ is lacking in some T6SSs, such as those of *Agrobacterium* and *Acinetobacter*. Further studies are required to understand whether other proteins can compensate for the absence of TssJ in these species. The fact that the MC has a distinct history compared to the tail is also exemplified by the observation that no TssJLM complex is present in *Bacteroidales* T6SSs (74, 75). However, putative uncharacterized membrane proteins are encoded within these T6SS gene clusters, suggesting that a different transenvelope complex has been domesticated to anchor the tail (74, 75).

The Tail

The baseplate

The BP (Fig. 1) is a large complex, 2.7 MDa, comprising >60 polypeptides of at least six different proteins (50). The role of the BP is to initiate the polymerization of the TTC. While it has not been formally shown yet, the T6SS BP is believed to trigger sheath contraction, as demonstrated in other CISs. A specific role of the T6SS BP is to anchor the TTC to the MC. The BP is composed of six wedge subcomplexes organized around the central hub, i.e., the N-terminal domain of the VgrG spike (76, 77) (Fig. 1). VgrG hence belongs to two tail subcomplexes: it constitutes the tip of the needle and the hub for the BP. The wedge complex is composed of four proteins: TssE, -F, -G, and -K. These four proteins assemble a structure of 1:2:1:6 stoichiometry, the TssG peptide being the central core (77–79). Two TssF subunits wrap TssG to form a triangular shape called the trifurcation unit, whereas two extensions of TssG make contacts with two TssK trimers (77). TssE, -F, -G are, respectively, homologues of phage T4 gp25, gp6, and gp7 and phage Mu Mup46, Mup47, and Mup48 (50, 77, 79, 80), which also constitute the inner part of phage BPs (79–81). TssK has no homologue in *Myoviridae* but shares architectural homologies with receptor-binding proteins (RBP) of *Siphoviridae* phages (67). The structure of the N-terminal domain of TssK is superimposable with that of *Siphoviridae* RBP shoulder domains that are anchored

into the BP (67). Indeed, the TssK N-terminal domain establishes extensive contacts with the $TssF_2G$ complex (67). The TssK C-terminal domain has evolved to bind to the MC and specifically to the TssL and TssM cytoplasmic domains (57, 64, 66, 67). Similar to the MC, the BP can be accessorized by additional subunits, such as TssA1 in *P. aeruginosa* (82), that may stabilize the complex or provide additional functions.

The tail tube/sheath complex

The TTC comprises the needle and the contractile sheath (Fig. 1). It forms an ~1-μm-long tubular structure in the cytoplasm that is assembled in 30 to 50 s (52, 83).

The needle is composed of the inner tube topped by the spike complex. The inner tube is made of hexamers of the Hcp protein (84–86). These donut-shaped hexameric Hcp rings (87, 88) stack on each other in a head-to-tail orientation to form a hollow tube (86). Interestingly, despite very low sequence similarities between T6SS Hcps and tube proteins from other CISs, their structure is strictly conserved (5). Hcp tube polymerization starts at the BP, through direct recruitment of the first ring to the base of the VgrG hub/spike (89). The spike complex is composed of a trimer of the VgrG protein and, in most instances, of the PAAR repeat protein (85, 90). VgrG contains several conserved domains (24, 85). The N-terminal domain resembles the phage T4 gp27 protein and acts as a symmetry adaptor between the 6-fold symmetry of the inner tube and the 3-fold symmetry of the VgrG central and C-terminal domains, which share homologies with the phage T4 gp5 N-terminal and β-prism domains (89, 91, 92). The VgrG β-prism domain is a triangular β-helix that forms, together with the conical PAAR protein, the penetration device of the T6SS needle (90, 93). The VgrG trimer and the PAAR protein can be extended by additional domains that may act as effectors or as adaptors for effectors (24, 90).

The sheath polymerizes from the BP. It is proposed that, similarly to its gp25-like homologues in *Myoviridae*, the TssE BP subunit constitutes the sheath polymerization initiator (79, 91). In contrast to other CISs, the T6SS sheath is composed of two proteins, TssB and TssC (1, 52, 85, 94, 95), forming a stable dimer that is the repeat unit for sheath polymerization (96–98). Six TssBC dimers form a strand that wraps an Hcp hexameric ring. The TssBC dimer can be divided into three regions: domains 1 and 2 resemble CIS sheath proteins, and domain 3 is inserted into domain 2 (99, 100). Extensive contacts between TssBC dimers from the same strand and from the neighboring −1 and +1 strands stabilize the extended conformation of the sheath polymer (100, 101).

In the T6SS, assembly of the inner tube and that of the extended sheath are interdependent (86, 102). The TssA protein coordinates the polymerization of the TTC (103) (Fig. 2). TssA localizes at the distal extremity of the growing tail tube/sheath (103), at the location in which hexameric tube rings and TssBC strands are incorporated (104). TssA presents a 6-arm starfish-like structure with a central core (103). Protein-protein interaction studies have suggested that the central core of TssA may undergo large conformational changes to insert new Hcp hexamers, whereas the arms may facilitate sheath polymerization (103, 105). Tail tube/sheath polymerization proceeds in the cytoplasm and is stopped when the distal end hits the membrane on the opposite membrane of the bacterial cell (104, 106). A recent study has identified TagA, a protein that interacts with TssA to stop the assembly of the tail and to maintain the sheath under the extended conformation (106) (Fig. 2). However, the TssA cap protein and the TagA stopper are not conserved in T6SS gene clusters, suggesting that different mechanisms control tail tube/sheath assembly and termination in different $T6SS^+$ species (105–107).

Contraction of the T6SS sheath, which occurs in less than 2–5 ms, is believed to start at the BP. The cryo-electron microscopy structure of the *Vibrio cholerae* T6SS sheath has been solved in the two states, extended and contracted, allowing a reconstitution of the molecular events leading to contraction (99, 100). Contraction consists of a reorganization of the TssBC strands and, notably, an outward rotation of the sheath subunits (100). By doing so, the sheath compacts on the BP and contacts with the inner tube are abolished, thus promoting its expulsion (5, 100, 101). The free energy released during contraction is estimated to >44,000 $kcal \cdot mol^{-1}$ for a 1-μm-long sheath (100).

After contraction, the sheath is disassembled by a dedicated AAA^+ ATPase, ClpV (94, 102) (Fig. 2). ClpV binds to an N-terminal helix of TssC that belongs to sheath domain 3 (108, 109), which is accessible only in the contracted conformation (98, 100). Although this is not clearly established, it is proposed that contracted sheath subunits are recycled rather than conveyed to degradation (102).

LOADING AND TRANSPORT OF EFFECTORS

As summarized above, a broad repertoire of antibacterial and antihost activities has been described already for T6SS effectors. In addition, the mode of loading and transfer of these effectors into target cells is also variable. The common theme is that these effectors are associated

with needle components, as the needle is the only portion of the T6SS to be propelled into the target cell (12, 13) (Fig. 3). Effectors can be additional domains fused to needle components such as Hcp, VgrG, or PAAR or independent proteins that directly or indirectly bind to Hcp, VgrG, or PAAR (12, 13). Recruitment of these independent cargo effectors to Hcp, VgrG, or PAAR can be mediated by adaptors, which are themselves domains of the needle components, or independent proteins (110) (Fig. 3).

Specialized Hcp, VgrG, and PAAR

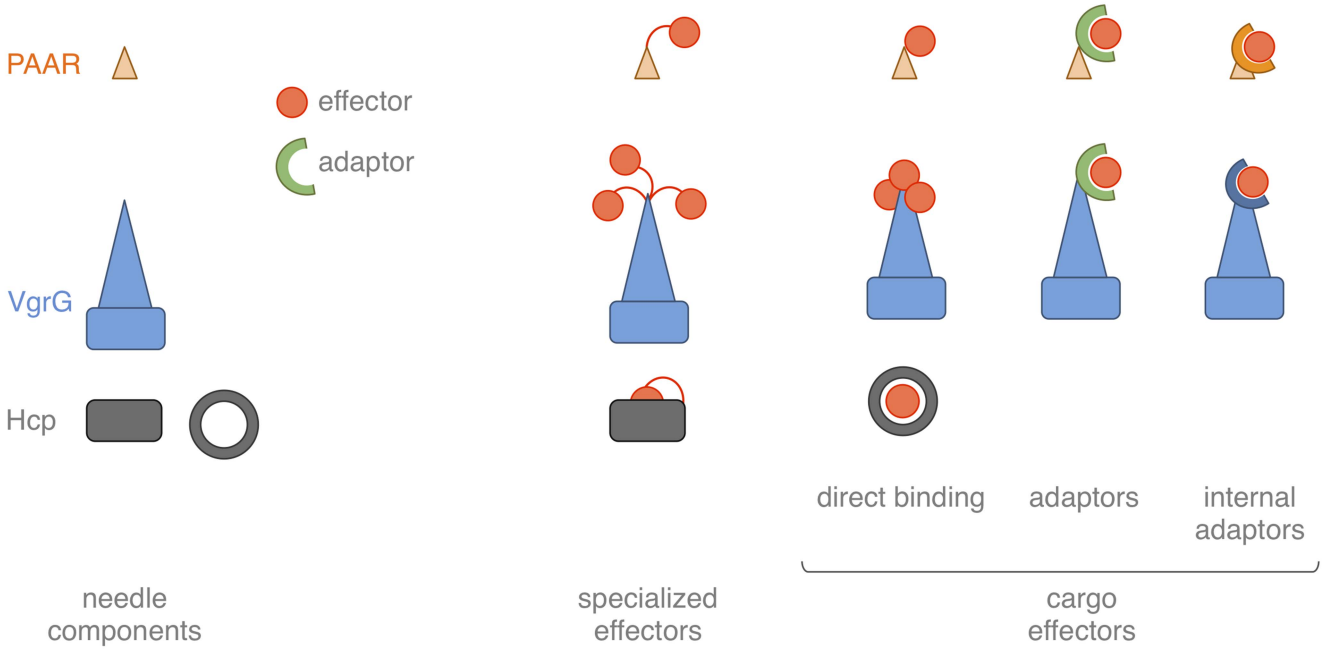
When the effector module is on the same polypeptide as the needle component, the T6SS subunit is called "specialized" or "evolved." Although effectors fused to Hcp or PAAR have been described (36, 90, 111), the best-characterized examples are C-terminal extensions of specialized VgrGs such as *V. cholerae* VgrG1, which cross-links actin, and VgrG3, which has peptidoglycan glycoside hydrolase activity; *A. hydrophila* VgrG1, which ADP-ribosylates actin; *P. aeruginosa* VgrG2b, which interacts with the tubule cap complex; and *Burkholderia pseudomallei* VgrG5, which induces host cell membrane fusion (21–26, 112, 113).

Cargo Effectors

Cargo effectors are independent proteins that need to recognize their Hcp, VgrG, or PAAR carrier for transfer. This recognition could be direct, such as the case of effectors that bind Hcp, or may require an additional adaptor module that binds VgrG or PAAR (12, 13, 110) (Fig. 3). Usually the effector genes are genetically linked to genes encoding their vehicle, their adaptors (if any), and, in the case of antibacterial toxins, their immunity proteins. These genetic elements could be found within T6SS gene clusters or as Hcp-VgrG islands scattered on the genome (9, 10).

When associated with Hcp, the effector is embedded in the lumen of the hexameric ring and is thus likely found inside the channel of the inner tube during T6SS assembly (16, 114). Consequently, it is protected and stabilized (114, 115). However, the available space in the Hcp ring lumen limits the size of the effector to be transported, which is estimated to be <25 kDa (114).

FIGURE 3 Schematic representation of the mechanisms of effector loading. Effectors are depicted as red circles. Specialized effectors are chimeric needle proteins with extensions encoding the effector. Cargo effectors are independent proteins that associate with needle components (Hcp, VgrG, and PAAR). Binding of cargo effectors to needle components could be direct or mediated by adaptor modules that are independent proteins (adaptors) or extensions of VgrG and PAAR (internal adaptors).



Adaptors can be isolated proteins or domains fused to the cargo or the vehicle (110). Adaptors from distinct families, such as DUF1795 (EagT6 and EagR), DUF2169, DUF4123 (Tap-1 and Tec), transthyretin, and recombination hot spot (Rhs), have been described and studied (35, 90, 116–125). When several copies of VgrG or PAAR proteins are encoded within the genome, these adaptor modules specify the carriers on which the effector should be mounted (112, 118, 122, 123, 126). In addition to loading the effector on the vehicle, some of these adaptors have been shown to act as chaperones to stabilize the effector or to wrap hydrophobic transmembrane segments to prevent effector aggregation (112, 124, 125).

CONCLUDING REMARKS

Although the T6SS is one of the most recently identified secretion apparatuses, we now have a detailed view on how the system is assembled, how it is structurally arranged, and how effectors are loaded and transported. The broad repertoire of effectors has only recently started to emerge, and it is likely that many effectors with interesting activities will be identified and characterized in the next years. The discovery of the T6SS 13 years ago and its role as an antibacterial weapon have altered our view of bacterial communities. It is now broadly admitted that not only do bacteria cohabitate peacefully but also complex interactions are established to maintain stable ecosystems, such as the human gut microbiota. Further fundamental and translational works are required to better understand how T6SS activation or inhibition may impact microbial communities and may perturb complex ecosystems.

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