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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.

23 **INTRODUCTION**

24 The Type VI secretion system (T6SS) is a multiprotein machine that belongs to the versatile
25 family of contractile injection systems (CISs) (1-4). CISs deliver effectors into target cells
26 using a spring-like mechanism (4-6). Briefly, CISs assemble a needle-like structure, loaded
27 with effectors, wrapped into a sheath built in an extended, metastable, conformation (Fig. 1).
28 Contraction of the sheath propels the needle toward the competitor cell. Genomes of Gram-
29 negative bacteria usually encode one or several T6SSs, with an overrepresentation in
30 Proteobacteria and Bacteroidetes (for a review on the role of T6SS in gut-associated
31 Bacteroidales, see chapter by Coyne and Comstock (7)) (8-10). The broad arsenal of effectors
32 delivered by T6SS includes antibacterial-specific proteins such as peptidoglycan hydrolases,
33 eukaryotic-specific effectors that act on cell cytoskeleton, and toxins that can target all cell
34 types such as DNases, phospholipases, or NAD^+ hydrolases (11-14). As such, the T6SS plays
35 a critical role in reshaping bacterial communities, and directly, or indirectly, in pathogenesis
36 (15-19). Destroying bacterial competitors also provides exogenous DNA that can be acquired
37 in naturally competent bacteria and that serves as reservoir for antibiotic resistance gene
38 spreading (20). This chapter lists the major effector families, and summarizes the current
39 knowledge on the assembly and mode of action of the T6SS.
40

41 **TYPE VI SECRETION SYSTEM EFFECTORS**

42 Several T6SSs have been shown to target eukaryotic cells (21-23). By promoting or
43 preventing cytoskeleton re-arrangements through the action of specific effectors that target
44 actin or tubulin, the T6SSs of *Vibrio cholerae*, *Aeromonas hydrophila*, and *Pseudomonas*
45 *aeruginosa* disable phagocytic cells or stimulate internalization into non-phagocytic cells (21,
46 22, 24-26). Other T6SSs have been demonstrated to manipulate host cells, although the
47 molecular determinants are not yet entirely understood (27-30). However, T6SS gene clusters

48 are widespread in Gram-negative bacterial genomes, and not restricted to pathogens (10).
49 Most of them encode proteins with potent antibacterial activities such as enzymes that cleave
50 essential macromolecules such as DNA, phospholipids or the peptidoglycan mesh, or
51 essential metabolites such as $NAD^+/NADP^+$ (31-36). Additional T6SS antibacterial effectors
52 include ADP-ribosyltransferases that specifically target the Z-ring and hence inhibit cell
53 division (37). Antibacterial effectors are active in the periplasm or cytoplasm of the target
54 cell, and are co-produced with immunity proteins that remain in the producing cell and act as
55 antitoxins to prevent autointoxication during dueling between sister cells (11-13). More
56 recently, T6SS effectors that collect manganese or zinc in the environment to provide metals
57 to the cell have been described (38-40). By deploying antibacterial effectors or scavenging
58 metals, T6SSs play an important role in bacterial communities, and hence T6SS gene clusters
59 are usually highly represented in species present in multispecies microbiota such as the
60 human gut (7, 16-18, 41). In general the regulatory mechanisms and signals underlying
61 expression of T6SS genes, production of T6SS subunits or post-translational activation of the
62 secretion apparatus are tightly linked to environmental cues in the niche in which the T6SS is
63 required destroy competitors (42-45).
64

65 **TYPE VI SECRETION MECHANISM OF ACTION**

66 T6SSs use a contractile mechanism to inject effectors (Fig. 2). This mechanism is shared with
67 all CISs: a sheath, assembled in an extended conformation, wraps a needle. Contraction of the
68 sheath into a stable state propels the needle (1, 3-5). The needle is composed of an inner tube
69 capped by the spike complex that pierces the membrane of the target cell (Fig. 1). The tail
70 tube/sheath complex (TTC) is built on an assembly platform named baseplate (BP) (Fig. 1).
71 TTC and BP are collectively called tail, a structure that is conserved among all CISs. In
72 addition to this common theme to all CISs, T6SSs have evolved (i) a membrane complex

73 (MC), which docks the tail to the cell envelope and serves as channel for the passage of the
74 needle upon sheath contraction, and (ii) a specialized BP component to properly orient the
75 needle toward the cell exterior, by recognizing and binding the MC (2-5, 46-48) (Fig. 1).

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

79

80 **ARCHITECTURE OF THE TYPE VI SECRETION SYSTEM**

81 **The Membrane Complex**

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

94 The electron microscopy structure of the fully assembled 1.7-MDa TssJLM MC from
95 enteroaggregative *Escherichia coli* has been reported (49, 68, 69). The complex has a rocket-
96 like structure: a large base, that contains the cytoplasmic and membrane domains of TssL and
97 TssM, is followed by arches and pillars which correspond to the TssM periplasmic domains

98 and TssJ (68). The TssJLM complex, which has five-fold symmetry *in vivo* and after
99 purification, comprises 15 copies of TssJ, and 10 copies of TssL and of TssM (49, 58). The
100 MC delimits an internal lumen with a diameter insufficient for the passage of the tail tube. In
101 addition, this lumen is partly occluded by a periplasmic constriction gate, suggesting that
102 large conformational changes occur upon BP docking or sheath contraction (49, 58).
103 The MC can be accessorized by additional subunits, such as peptidoglycan-binding
104 proteins (53, 70, 71). MC anchorage to the cell wall likely stabilizes the MC to resist the
105 forces generated during sheath contraction (70). Finally, recent studies have shown that
106 proper assembly of the MC requires the activity of peptidoglycan-degrading enzymes (72,
107 73).
108 Interestingly, while the tail complex is evolutionarily related to contractile injection
109 machines, the evolution history of the MC is less clear. TssL and TssM present significant
110 homologies with two accessory subunits associated with Type IVb secretion systems, DotU
111 and IcmF, respectively (8, 9). No homologue of TssJ is found associated with DotU/IcmF
112 complexes, suggesting that TssJ is from a different ancestry. Indeed, while essential when
113 present, TssJ is lacking in some T6SSs such as that of *Agrobacterium* and *Acinetobacter*.
114 Further studies are required to understand whether other proteins can substitute for the
115 absence of TssJ in these species. The fact that the MC has a distinct history compared to the
116 tail is also exemplified by the observation that no TssJLM complex is present in
117 *Bacteroidales* T6SSs (74, 75). However, putative uncharacterized membrane proteins are
118 encoded within these T6SS gene clusters suggesting that a different transenvelope complex
119 has been domesticated to anchor the tail (74, 75).
120
121 **The Tail**
122 **The baseplate**

123 The baseplate (BP) (Fig. 1) is a large complex of 2.7 MDa comprising > 60 polypeptides of at
124 least six different proteins (50). The role of the baseplate is to initiate the polymerization of
125 the tail tube/sheath complex. While it has not been formally shown yet, the T6SS baseplate is
126 believed to trigger sheath contraction, as demonstrated in other CISs. A specific role of the
127 T6SS baseplate is to anchor the TTC to the MC. The BP is composed of six wedge
128 subcomplexes organized around the central hub, i.e., the N-terminal domain of the VgrG
129 spike (76, 77) (Fig. 1). VgrG hence belongs to two tail sub-complexes: it constitutes the tip of
130 the needle, and the hub for the baseplate. The wedge complex is composed of 4 proteins:
131 TssE, –F, –G, and –K. These four proteins assemble a structure of 1:2:1:6 stoichiometry, the
132 TssG peptide being the central core (77-79). Two TssF subunits wrap TssG to form a
133 triangular shape called trifurcation unit, whereas two extensions of TssG make contacts with
134 two TssK trimers (77). TssE, –F, –G are respectively homologues of phage T4 gp25, gp6 and
135 gp7 and phage Mu Mup46, Mup47 and Mup48 (50, 77, 79, 80), that also constitute the inner
136 part of phage baseplates (79-81). TssK has no homologue in Myoviridae, but shares
137 architectural homologies with receptor-binding proteins (RBP) of Siphoviridae phages (67).
138 The structure of the N-terminal domain of TssK is superimposable with that of Siphoviridae
139 RBP shoulder domains that are anchored into the baseplate (67). Indeed, the TssK N-terminal
140 domain establishes extensive contacts with the $TssF_2G$ complex (67). The TssK C-terminal
141 domain has evolved to bind to the MC, and specifically to the TssL and TssM cytoplasmic
142 domains (57, 64, 66, 67). Similar to the MC, the BP can be accessorized by additional
143 subunits, such as TssA1 in *P. aeruginosa* (82), that may stabilize the complex or provide
144 additional functions.
145
146 **The tail tube/sheath complex**

147 The tail tube/sheath complex comprises the needle and the contractile sheath (Fig. 1). It forms
148 a ~ 1 µm-long tubular structure in the cytoplasm, that is assembled in 30-50 sec (52, 83).

149 The needle is composed of the inner tube topped by the spike complex. The inner tube
150 is made of hexamers of the Hcp protein (84-86). These donut-shaped hexameric Hcp rings
151 (87, 88) stack on each other in a head-to-tail orientation to form a hollow tube (86).
152 Interestingly, despite very low sequence similarities between T6SS Hcps and tube proteins
153 from other CISs, their structure is strictly conserved (5). Hcp tube polymerization starts at the
154 baseplate, through direct recruitment of the first ring to the base of the VgrG hub/spike (89).
155 The spike complex is composed of a trimer of the VgrG protein and, in most instances, of the
156 PAAR-repeat protein (85, 90). VgrG contains several conserved domains (24, 85). The N-
157 terminal domain resembles the phage T4 gp27 protein, and acts as a symmetry adaptor
158 between the six-fold symmetry of the inner tube and the three-fold symmetry of the VgrG
159 central and C-terminal domains, which share homologies with the phage T4 gp5 N-terminal
160 and β-prism domains (89, 91, 92). The VgrG β-prism domain is a triangular β-helix that
161 forms, together with the conical PAAR protein, the penetration device of the T6SS needle
162 (90, 93). The VgrG trimer and the PAAR protein can be extended by additional domains that
163 may act as effectors, or as adaptors for effectors (24, 90).

164 The sheath polymerizes from the baseplate. It is proposed that, similarly to its gp25-
165 like homologues in Myoviridae, the TssE BP subunit constitutes the sheath polymerization
166 initiator (79, 91). By contrast to other CISs, the T6SS sheath is composed of two proteins,
167 TssB and TssC (1, 52, 85, 94, 95), forming a stable dimer that is the repeat unit for sheath
168 polymerization (96-98). Six TssBC dimers form a strand that wraps a Hcp hexameric ring.
169 The TssBC dimer can be divided in three regions: Domains 1 and 2 that resemble CIS sheath
170 proteins, and an additional Domain 3 inserted into Domain 2 (99, 100). Extensive contacts

171 between TssBC dimers from the same strand and from the neighboring -1 and +1 strands
172 stabilize the extended conformation of the sheath polymer (100, 101).
173 In the T6SS, assembly of the inner tube and that of the extended sheath are
174 interdependent (86, 102). The TssA protein coordinates the polymerization of the tail
175 tube/sheath complex (103) (Fig. 2). TssA localizes at the distal extremity of the growing tail
176 tube/sheath (103), at the location in which hexameric tube rings and TssBC strands are
177 incorporated (104). TssA presents a 6-arm starfish-like structure with a central core (103).
178 Protein-protein interaction studies have suggested that the central core of TssA may undergo
179 large conformational changes to insert new Hcp hexamers, whereas the arms may facilitate
180 sheath polymerization (103, 105). Tail tube/sheath polymerization proceeds in the cytoplasm,
181 and is stopped when the distal end hits the membrane on the opposite membrane of the
182 bacterial cell (104, 106). A recent study has identified TagA, a protein that interacts with
183 TssA to stop the assembly of the tail and to maintain the sheath under the extended
184 conformation (106) (Fig. 2). However, the TssA cap protein and the TagA stopper are not
185 conserved in T6SS gene clusters, suggesting that different mechanisms control tail
186 tube/sheath assembly and termination in different T6SS^{+} species (105-107).
187 Contraction of the T6SS sheath, which occurs in less than 2-5 msec, is believed to start
188 at the BP. The cryo-electron microscopy structure of the *Vibrio cholerae* T6SS sheath has
189 been solved in the two states: extended and contracted, allowing a reconstitution of the
190 molecular events leading to contraction (99, 100). Contraction consists to a reorganization of
191 the TssBC strands, and notably an outward rotation of the sheath subunits (100). By doing so,
192 the sheath compacts on the BP, and contacts with the inner tube are abolished, thus,
193 promoting its expulsion (5, 100, 101). The free energy released during contraction is
194 estimated to > 44,000 kcal.mol^{-1} for a 1-µm-long sheath (100).

195 After contraction, the sheath is disassembled by a dedicated AAA$^+$ ATPase, ClpV (94,
196 102) (Fig. 2). ClpV binds to an N-terminal helix of TssC that belongs to sheath Domain 3
197 (108, 109), which is only accessible in the contracted conformation (98, 100). Although this is
198 not clearly established, it is proposed that contracted sheath subunits are recycled rather than
199 conveyed to degradation (102).

200

201 **LOADING AND TRANSPORT OF EFFECTORS**

202 As summarized above, a broad repertoire of anti-bacterial and anti-host activities have been
203 already described for T6SS effectors. In addition, the mode of loading and transfer of these
204 effectors into target cells is also variable. The common theme is that these effectors are
205 associated with needle components, as the needle is the only portion of the T6SS to be
206 propelled into the target cell (12, 13) (Fig. 3). Effectors can be additional domains fused to
207 needle components such as Hcp, VgrG, or PAAR, or independent proteins that directly or
208 indirectly bind to Hcp, VgrG, or PAAR (12, 13). Recruitment of these independent cargo
209 effectors to Hcp, VgrG or PAAR can be mediated by adaptors, which are themselves domains
210 of the needle components, or independent proteins (110) (Fig. 3).

211

212 **Specialized Hcp, VgrG and PAAR**

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

220

221 **Cargo Effectors**

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

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

234 Adaptors can be isolated proteins, or domains fused to the cargo or the vehicle (110).
235 Adaptors from distinct families, such as DUF1795 (EagT6, EagR), DUF2169, DUF2345,
236 DUF4123 (Tap-1 or Tec), transthyretin (TTR) or Recombination hot-spot (Rhs) have been
237 described and studied (35, 90, 116-125). When several copies of VgrG or PAAR proteins are
238 encoded within the genome, these adaptor modules specify the carriers on which the effector
239 should be mounted (112, 118, 122, 123, 126). In addition to loading the effector on the
240 vehicle, some of these adaptors have been shown to act as chaperones to stabilize the effector,
241 or to wrap hydrophobic transmembrane segments to prevent effector aggregation (112, 124,
242 125).

243

244 **CONCLUDING REMARKS**

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

256

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672 **LEGEND TO FIGURES**

673

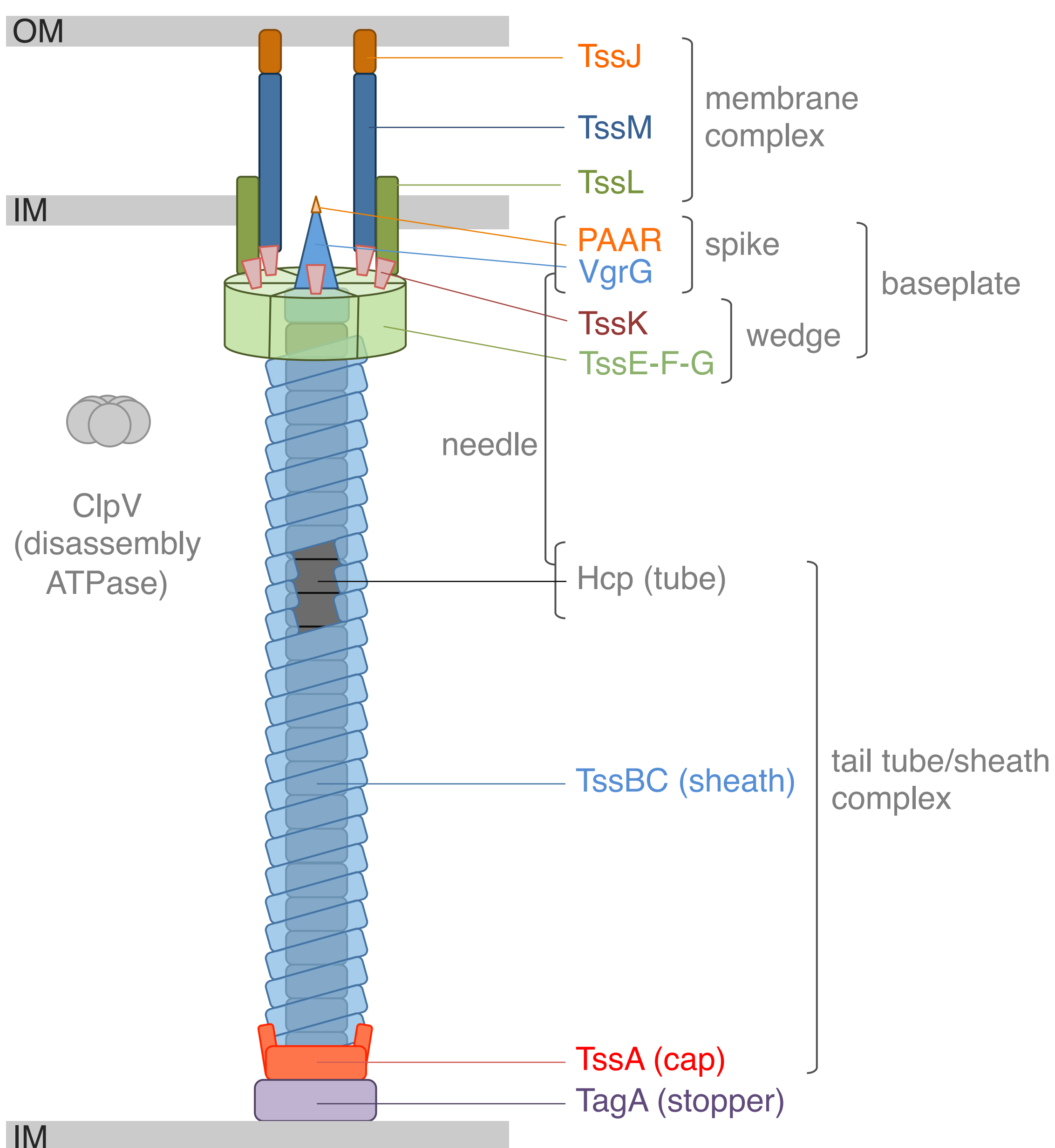
674 **FIGURE 1** Schematic representation of the Type VI secretion system. The different subunits
675 are labeled, as well as the different subcomplexes. IM, inner membrane; OM, outer
676 membrane.

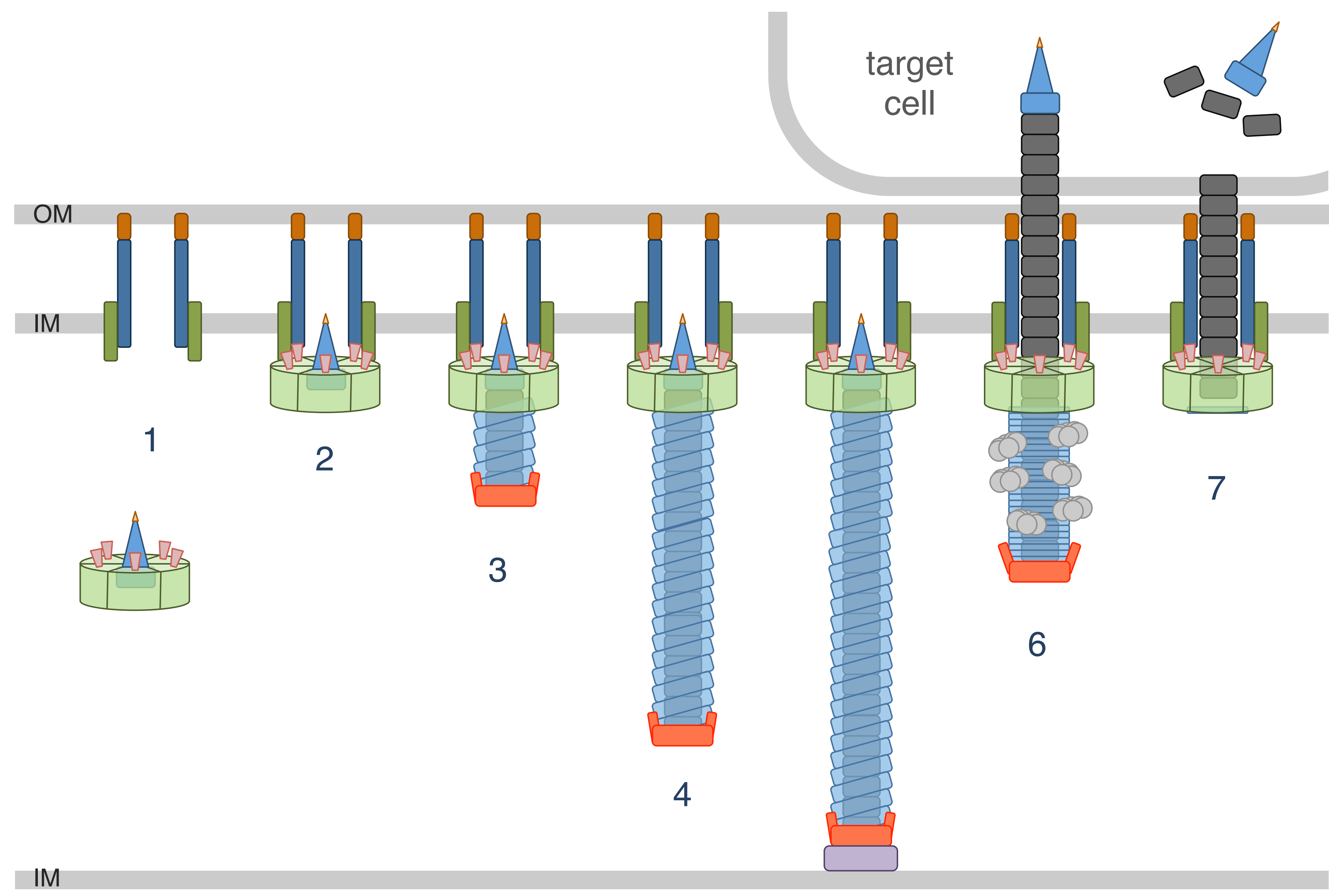
677

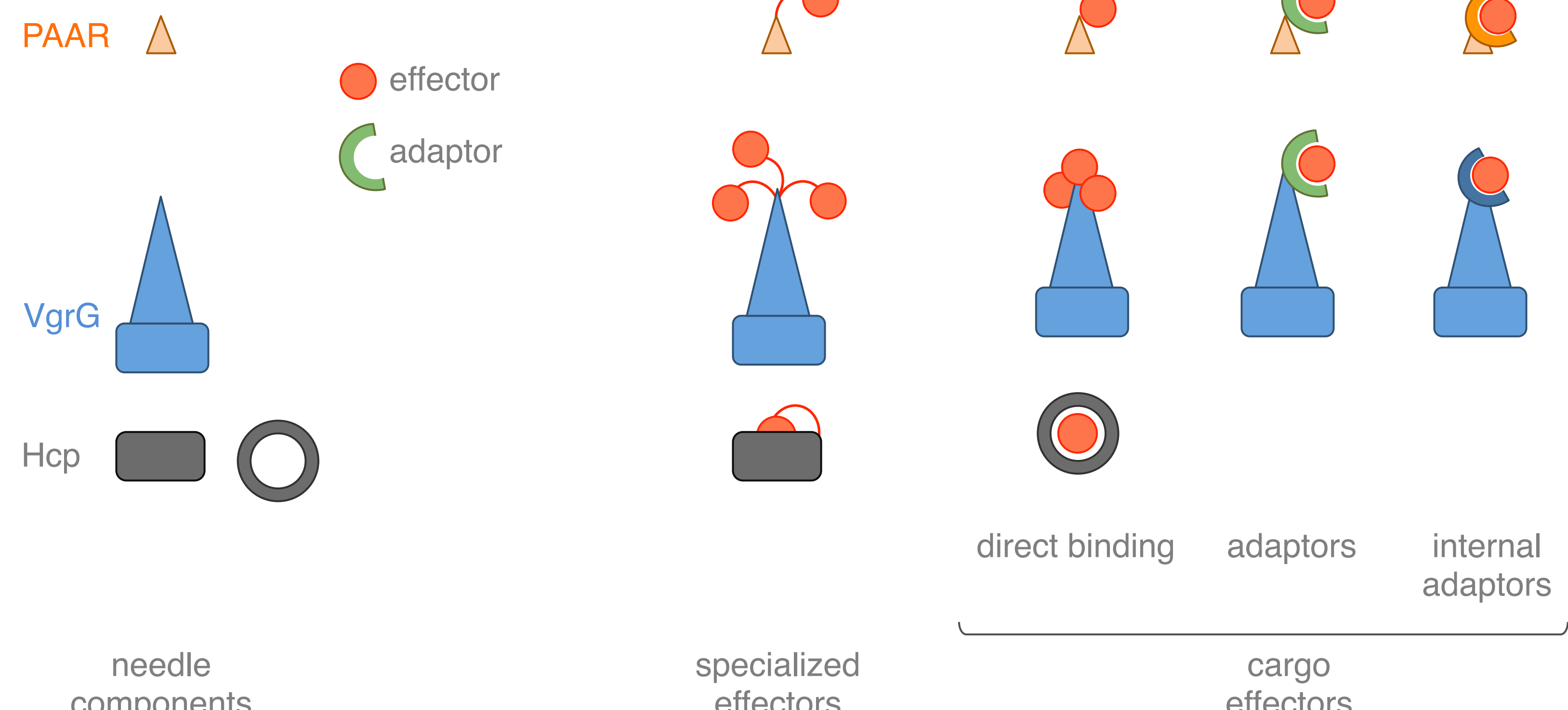
678 **FIGURE 2** Assembly and mechanism of firing of the Type VI secretion system. T6SS
679 biogenesis starts with the positioning and assembly of the membrane complex, and the
680 assembly of the baseplate (1). The recruitment and docking of the baseplate on the membrane
681 complex (2) initiates the TssA-mediated polymerization of the tail tube/sheath tubular
682 structure (3, 4, 5), which is stopped when hitting the opposite membrane by the TagA stopper
683 (5). Sheath contraction propels the tube/spike needle into the target (6). The ClpV ATPase is
684 recruited to the contracted sheath to recycle sheath subunits (6). Needle components, and
685 effectors associated to them, are delivered inside the target (7).

686

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


OM
IM
IM
TssJ
TssM
TssL
membrane
complex
PAAR
VgrG
spike
TssK
TssE-F-G
wedge
baseplate
needle
ClpV
(disassembly
ATPase)
Hcp (tube)
TssBC (sheath)
tail tube/sheath
complex
TssA (cap)
TagA (stopper)


target cell
OM
IM
1
2
3
4
5
6
7
IM


PAAR
effector
adaptor
VgrG
Hcp
direct binding
adaptors
internal
adaptors
needle
components
specialized
effectors
cargo
effectors