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Structure and Activity of the Type VI Secretion System

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12 **ABSTRACT**

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

22

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 $\text{NAD}^+/\text{NADP}^+$ (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 TssF₂G 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 μ m-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- μ m-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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264

265

266 **REFERENCES**

- 267 1. **Bönemann G, Pietrosiuk A, Mogk A.** 2010. Tubules and donuts: a type VI secretion
268 story. *Mol Microbiol* **76**:815–821.
- 269 2. **Zoued A, Brunet YR, Durand E, Aschtgen MS, Logger L, Douzi B, Journet L,**
270 **Cambillau C, Cascales E.** 2014. Architecture and assembly of the type VI secretion
271 system. *Biochim Biophys Acta* **1843**:1664–1673.

- 272 3. **Cascales E.** 2017. Microbiology: and *Amoebophilus* invented the machine gun! *Curr*
273 *Biol* **27**:R1170–R1173.
- 274 4. **Taylor NMI, van Raaij MJ, Leiman PG.** 2018. Contractile injection systems of
275 bacteriophages and related systems. *Mol Microbiol* **108**:6–15.
- 276 5. **Brackmann M, Nazarov S, Wang J, Basler M.** 2017. Using force to punch holes:
277 mechanics of contractile nanomachines. *Trends Cell Biol* **27**:623–632.
- 278 6. **Cianfanelli FR, Monlezun L, Coulthurst SJ.** 2016. Aim, load, fire: the type VI
279 secretion system, a bacterial nanoweapon. *Trends Microbiol.* **24**:51–62. doi:
280 10.1016/j.tim.2015.10.005.
- 281 7. **Coyne MJ, Comstock LE.** 2019. Type VI secretion systems and the gut microbiota.
282 *Microbiol Spectr* **7**:2.
- 283 8. **Bingle LE, Bailey CM, Pallen MJ.** 2008. Type VI secretion: a beginner's guide. *Curr*
284 *Opin Microbiol* **11**:3–8.
- 285 9. **Cascales E.** 2008. The type VI secretion toolkit. *EMBO Rep* **9**:735–741.
- 286 10. **Boyer F, Fichant G, Berthod J, Vandenbrouck Y, Attree I.** 2009. Dissecting the
287 bacterial type VI secretion system by a genome wide in silico analysis: what can be
288 learned from available microbial genomic resources? *BMC Genomics* **10**:104.
- 289 11. **Russell AB, Peterson SB, Mougous JD.** 2014. Type VI secretion system effectors:
290 poisons with a purpose. *Nat Rev Microbiol* **12**:137–148.
- 291 12. **Durand E, Cambillau C, Cascales E, Journet L.** 2014. VgrG, Tae, Tle, and beyond:
292 the versatile arsenal of type VI secretion effectors. *Trends Microbiol* **22**:498–507.
- 293 13. **Alcoforado Diniz J, Liu YC, Coulthurst SJ.** 2015. Molecular weaponry: diverse
294 effectors delivered by the Type VI secretion system. *Cell Microbiol* **17**:1742–1751.
- 295 14. **Hachani A, Wood TE, Filloux A.** 2016. Type VI secretion and anti-host effectors.
296 *Curr Opin Microbiol* **29**:81–93.
- 297 15. **Fu Y, Waldor MK, Mekalanos JJ.** 2013. Tn-Seq analysis of *Vibrio cholerae*
298 intestinal colonization reveals a role for T6SS-mediated antibacterial activity in the
299 host. *Cell Host Microbe.* **14**:652–63. doi: 10.1016/j.chom.2013.11.001.
- 300 16. **Sana TG, Flaugnatti N, Lugo KA, Lam LH, Jacobson A, Baylot V, Durand E,**
301 **Journet L, Cascales E, Monack DM.** 2016. *Salmonella* Typhimurium utilizes a
302 T6SS-mediated antibacterial weapon to establish in the host gut. *Proc Natl Acad Sci*
303 *USA.* **113**:E5044–51. doi: 10.1073/pnas.1608858113.
- 304 17. **Sana TG, Lugo KA, Monack DM.** 2017. T6SS: The bacterial "fight club" in the host
305 gut. *PLoS Pathog.* **13**:e1006325. doi: 10.1371/journal.ppat.1006325.
- 306 18. **Chassaing B, Cascales E.** 2018. Antibacterial weapons: targeted destruction in the
307 microbiota. *Trends Microbiol* **26**:329–338.
- 308 19. **García-Bayona L, Comstock LE.** 2018. Bacterial antagonism in host-associated
309 microbial communities. *Science.* **361** pii: eaat2456. doi: 10.1126/science.aat2456.
- 310 20. **Veening JW, Blokesch M.** 2017. Interbacterial predation as a strategy for DNA
311 acquisition in naturally competent bacteria. *Nat Rev Microbiol.* **15**:621–629. doi:
312 10.1038/nrmicro.2017.66.
- 313 21. **Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC,**
314 **Heidelberg JF, Mekalanos JJ.** 2006. Identification of a conserved bacterial protein
315 secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc*
316 *Natl Acad Sci USA.* **103**:1528–33.
- 317 22. **Sana TG, Baumann C, Merdes A, Soscia C, Rattei T, Hachani A, Jones C,**
318 **Bennett KL, Filloux A, Superti-Furga G, Voulhoux R, Bleves S.** 2015.
319 Internalization of *Pseudomonas aeruginosa* strain PAO1 into epithelial cells is
320 promoted by interaction of a T6SS effector with the microtubule network. *MBio.*
321 **6**:e00712. doi: 10.1128/mBio.00712-15.

- 322 23. **Schwarz S, Singh P, Robertson JD, LeRoux M, Skerrett SJ, Goodlett DR, West**
323 **TE, Mougous JD.** 2014. VgrG-5 is a *Burkholderia* type VI secretion system-exported
324 protein required for multinucleated giant cell formation and virulence. *Infect Immun.*
325 **82:**1445–52. doi: 10.1128/IAI.01368-13.
- 326 24. **Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ.** 2007. Type VI
327 secretion system translocates a phage tail spike-like protein into target cells where it
328 cross-links actin. *Proc Natl Acad Sci USA.* **104:**15508–13.
- 329 25. **Suarez G, Sierra JC, Erova TE, Sha J, Horneman AJ, Chopra AK.** 2010. A type
330 VI secretion system effector protein, VgrG1, from *Aeromonas hydrophila* that induces
331 host cell toxicity by ADP ribosylation of actin. *J Bacteriol.* **192:**155–68. doi:
332 10.1128/JB.01260-09.
- 333 26. **Durand E, Derrez E, Audoly G, Spinelli S, Ortiz-Lombardia M, Raoult D,**
334 **Cascales E, Cambillau C.** 2012. Crystal structure of the VgrG1 actin cross-linking
335 domain of the *Vibrio cholerae* type VI secretion system. *J Biol Chem.* **287:**38190–9.
336 doi: 10.1074/jbc.M112.390153.
- 337 27. **Aubert DF, Xu H, Yang J, Shi X, Gao W, Li L, Bisaro F, Chen S, Valvano MA,**
338 **Shao F.** 2016. A *Burkholderia* Type VI effector deamidates Rho GTPases to activate
339 the pyrin inflammasome and trigger inflammation. *Cell Host Microbe.* **19:**664–74.
340 doi: 10.1016/j.chom.2016.04.004.
- 341 28. **Eshraghi A, Kim J, Walls AC, Ledvina HE, Miller CN, Ramsey KM, Whitney**
342 **JC, Radey MC, Peterson SB, Ruhland BR, Tran BQ, Goo YA, Goodlett DR,**
343 **Dove SL, Celli J, Veesler D, Mougous JD.** 2016. Secreted effectors encoded within
344 and outside of the *Francisella* pathogenicity island promote intramacrophage growth.
345 *Cell Host Microbe.* **20:**573–583. doi: 10.1016/j.chom.2016.10.008.
- 346 29. **Ledvina HE, Kelly KA, Eshraghi A, Plemel RL, Peterson SB, Lee B, Steele S,**
347 **Adler M, Kawula TH, Merz AJ, Skerrett SJ, Celli J, Mougous JD.** 2018. A
348 phosphatidylinositol 3-kinase effector alters phagosomal maturation to promote
349 intracellular growth of *Francisella*. *Cell Host Microbe.* **24:**285–295.e8. doi:
350 10.1016/j.chom.2018.07.003.
- 351 30. **Lennings J, West TE, Schwarz S.** 2019. The *Burkholderia* Type VI secretion system
352 5: composition, regulation and role in virulence. *Front Microbiol.* **9:**3339. doi:
353 10.3389/fmicb.2018.03339.
- 354 31. **Hood RD, Singh P, Hsu F, Güvener T, Carl MA, Trinidad RR, Silverman JM,**
355 **Ohlson BB, Hicks KG, Plemel RL, Li M, Schwarz S, Wang WY, Merz AJ,**
356 **Goodlett DR, Mougous JD.** 2010. A type VI secretion system of *Pseudomonas*
357 *aeruginosa* targets a toxin to bacteria. *Cell Host Microbe.* **7:**25–37. doi:
358 10.1016/j.chom.2009.12.007.
- 359 32. **Russell AB, Hood RD, Bui NK, LeRoux M, Vollmer W, Mougous JD.** 2011. Type
360 VI secretion delivers bacteriolytic effectors to target cells. *Nature.* **75:**343–7. doi:
361 10.1038/nature10244.
- 362 33. **Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai**
363 **SN, Mougous JD.** 2013. Diverse type VI secretion phospholipases are functionally
364 plastic antibacterial effectors. *Nature.* **496:**508–12. doi: 10.1038/nature12074.
- 365 34. **Ma LS, Hachani A, Lin JS, Filloux A, Lai EM.** 2014. *Agrobacterium tumefaciens*
366 deploys a superfamily of type VI secretion DNase effectors as weapons for
367 interbacterial competition in planta. *Cell Host Microbe.* **16:**94–104. doi:
368 10.1016/j.chom.2014.06.002.
- 369 35. **Whitney JC, Quentin D, Sawai S, LeRoux M, Harding BN, Ledvina HE, Tran**
370 **BQ, Robinson H, Goo YA, Goodlett DR, Raunser S, Mougous JD.** 2015. An

- 371 interbacterial NAD(P)(+) glycohydrolase toxin requires elongation factor Tu for
372 delivery to target cells. *Cell*. **163**:607–19. doi: 10.1016/j.cell.2015.09.027.
- 373 36. **Pissaridou P, Allsopp LP, Wettstadt S, Howard SA, Mavridou DAI, Filloux A.**
374 2018. The *Pseudomonas aeruginosa* T6SS-VgrG1b spike is topped by a PAAR
375 protein eliciting DNA damage to bacterial competitors. *Proc Natl Acad Sci USA*.
376 **115**:12519–12524. doi: 10.1073/pnas.1814181115.
- 377 37. **Ting SY, Bosch DE, Mangiameli SM, Radey MC, Huang S, Park YJ, Kelly KA,**
378 **Filip SK, Goo YA, Eng JK, Allaire M, Veesler D, Wiggins PA, Peterson SB,**
379 **Mougous JD.** 2018. Bifunctional immunity proteins protect bacteria against FtsZ-
380 targeting ADP-ribosylating toxins. *Cell*. **175**:1380–1392 doi:
381 10.1016/j.cell.2018.09.037.
- 382 38. **Wang T, Si M, Song Y, Zhu W, Gao F, Wang Y, Zhang L, Zhang W, Wei G, Luo**
383 **ZQ, Shen X.** 2015. Type VI secretion system transports Zn²⁺ to combat multiple
384 stresses and host immunity. *PLoS Pathog*. **11**:e1005020. doi:
385 10.1371/journal.ppat.1005020.
- 386 39. **Si M, Zhao C, Burkinshaw B, Zhang B, Wei D, Wang Y, Dong TG, Shen X.** 2017.
387 Manganese scavenging and oxidative stress response mediated by type VI secretion
388 system in *Burkholderia thailandensis*. *Proc Natl Acad Sci USA*. **114**:E2233–E2242.
389 doi: 10.1073/pnas.1614902114.
- 390 40. **Si M, Wang Y, Zhang B, Zhao C, Kang Y, Bai H, Wei D, Zhu L, Zhang L, Dong**
391 **TG, Shen X.** 2017. The Type VI secretion system engages a redox-regulated dual-
392 functional heme transporter for zinc acquisition. *Cell Rep*. **20**:949–959. doi:
393 10.1016/j.celrep.2017.06.081.
- 394 41. **Verster AJ, Ross BD, Radey MC, Bao Y, Goodman AL, Mougous JD, Borenstein**
395 **E.** 2017. The landscape of type VI secretion across human gut microbiomes reveals Its
396 role in community composition. *Cell Host Microbe*. **22**:411–419 doi:
397 10.1016/j.chom.2017.08.010.
- 398 42. **Bernard CS, Brunet YR, Gueguen E, Cascales E.** 2010. Nooks and crannies in type
399 VI secretion regulation. *J Bacteriol*. **192**:3850–60. doi: 10.1128/JB.00370-10.
- 400 43. **Silverman JM, Brunet YR, Cascales E, Mougous JD.** 2012. Structure and
401 regulation of the type VI secretion system. *Annu Rev Microbiol*. **66**:453–72. doi:
402 10.1146/annurev-micro-121809-151619.
- 403 44. **Miyata ST, Bachmann V, Pukatzki S.** 2013. Type VI secretion system regulation as
404 a consequence of evolutionary pressure. *J Med Microbiol*. **62**:663–76. doi:
405 10.1099/jmm.0.053983-0.
- 406 45. **LeRoux M, Peterson SB, Mougous JD.** 2015. Bacterial danger sensing. *J Mol Biol*.
407 **427**:3744–53. doi: 10.1016/j.jmb.2015.09.018.
- 408 46. **Ho BT, Dong TG, Mekalanos JJ.** 2014. A view to a kill: the bacterial type VI
409 secretion system. *Cell Host Microbe* **15**:9–21.
- 410 47. **Basler M.** 2015. Type VI secretion system: secretion by a contractile nanomachine.
411 *Philos Trans R Soc Lond B Biol Sci* **370**:20150021.
- 412 48. **Coulthurst S.** 2019. The Type VI secretion system: a versatile bacterial weapon.
413 *Microbiology*. doi: 10.1099/mic.0.000789.
- 414 49. **Durand E, Nguyen VS, Zoued A, Logger L, Péhau-Arnaudet G, Aschtgen MS,**
415 **Spinelli S, Desmyter A, Bardiaux B, Dujeancourt A, Roussel A, Cambillau C,**
416 **Cascales E, Fronzes R.** 2015. Biogenesis and structure of a type VI secretion
417 membrane core complex. *Nature* **523**:555–560.
- 418 50. **Brunet YR, Zoued A, Boyer F, Douzi B, Cascales E.** 2015. The type VI secretion
419 TssEFGK-VgrG phage-like baseplate is recruited to the TssJLM membrane complex

- 420 via multiple contacts and serves as assembly platform for tail tube/sheath
421 polymerization. *PLoS Genet* **11**:e1005545.
- 422 51. **Gerc AJ, Diepold A, Trunk K, Porter M, Rickman C, Armitage JP, Stanley-Wall**
423 **NR, Coulthurst SJ.** 2015. Visualization of the *Serratia* type VI secretion system
424 reveals unprovoked attacks and dynamic assembly. *Cell Rep* **12**:2131–2142.
- 425 52. **Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ.** 2012. Type VI
426 secretion requires a dynamic contractile phage tail-like structure. *Nature* **483**:182–
427 186.
- 428 53. **Aschtgen MS, Gavioli M, Dessen A, Llobès R, Cascales E.** 2010. The SciZ protein
429 anchors the enteroaggregative *Escherichia coli* Type VI secretion system to the cell
430 wall. *Mol Microbiol* **75**:886–899.
- 431 54. **Aschtgen MS, Bernard CS, de Bentzmann S, Llobès R, Cascales E.** 2008. SciN is
432 an outer membrane lipoprotein required for type VI secretion in enteroaggregative
433 *Escherichia coli*. *J Bacteriol* **190**:7523–7531.
- 434 55. **Ma LS, Lin JS, Lai EM.** 2009. An IcmF family protein, ImpL_M, is an integral inner
435 membrane protein interacting with ImpK_L, and its walker a motif is required for type
436 VI secretion system-mediated Hcp secretion in *Agrobacterium tumefaciens*. *J*
437 *Bacteriol* **191**:4316–4329.
- 438 56. **Aschtgen MS, Zoued A, Llobès R, Journet L, Cascales E.** 2012. The C-tail
439 anchored TssL subunit, an essential protein of the enteroaggregative *Escherichia coli*
440 Sci-1 type VI secretion system, is inserted by YidC. *Microbiologyopen* **1**:71–82.
- 441 57. **Logger L, Aschtgen MS, Guérin M, Cascales E, Durand E.** 2016. Molecular
442 dissection of the interface between the type VI secretion TssM cytoplasmic domain
443 and the TssG baseplate component. *J Mol Biol* **428**:4424–4437.
- 444 58. **Felisberto-Rodrigues C, Durand E, Aschtgen MS, Blangy S, Ortiz-Lombardia M,**
445 **Douzi B, Cambillau C, Cascales E.** 2011. Towards a structural comprehension of
446 bacterial type VI secretion systems: characterization of the TssJ-TssM complex of an
447 *Escherichia coli* pathovar. *PLoS Pathog* **7**:e1002386.
- 448 59. **Rao VA, Shepherd SM, English G, Coulthurst SJ, Hunter WN.** 2011. The
449 structure of *Serratia marcescens* Lip, a membrane-bound component of the type VI
450 secretion system. *Acta Crystallogr D Biol Crystallogr.* **67**:1065-72. doi:
451 10.1107/S0907444911046300.
- 452 60. **Robb CS, Assmus M, Nano FE, Boraston AB.** 2013. Structure of the T6SS
453 lipoprotein TssJ1 from *Pseudomonas aeruginosa*. *Acta Crystallogr Sect F Struct Biol*
454 *Cryst Commun.* **69**:607–10. doi: 10.1107/S1744309113012220.
- 455 61. **Durand E, Zoued A, Spinelli S, Watson PJ, Aschtgen MS, Journet L, Cambillau**
456 **C, Cascales E.** 2012. Structural characterization and oligomerization of the TssL
457 protein, a component shared by bacterial type VI and type IVb secretion systems. *J*
458 *Biol Chem* **287**:14157–14168.
- 459 62. **Robb CS, Nano FE, Boraston AB.** 2012. The structure of the conserved type six
460 secretion protein TssL (DotU) from *Francisella novicida*. *J Mol Biol.* **419**:277–83.
461 doi: 10.1016/j.jmb.2012.04.003.
- 462 63. **Chang JH, Kim YG.** 2015. Crystal structure of the bacterial type VI secretion system
463 component TssL from *Vibrio cholerae*. *J Microbiol.* **53**:32–7. doi: 10.1007/s12275-
464 015-4539-0.
- 465 64. **Zoued A, Cassaro CJ, Durand E, Douzi B, España AP, Cambillau C, Journet L,**
466 **Cascales E.** 2016. Structure-function analysis of the TssL cytoplasmic domain reveals
467 a new interaction between the type VI secretion baseplate and membrane complexes. *J*
468 *Mol Biol* **428**:4413–4423.

- 469 65. **Zoued A, Duneau JP, Durand E, España AP, Journet L, Guerlesquin F, Cascales**
470 **E.** 2018. Tryptophan-mediated dimerization of the TssL transmembrane anchor is
471 required for type VI secretion system activity. *J Mol Biol* **430**:987–1003.
- 472 66. **Zoued A, Durand E, Bebeacua C, Brunet YR, Douzi B, Cambillau C, Cascales E,**
473 **Journet L.** 2013. TssK is a trimeric cytoplasmic protein interacting with components
474 of both phage-like and membrane anchoring complexes of the type VI secretion
475 system. *J Biol Chem* **288**:27031–27041.
- 476 67. **Nguyen VS, Logger L, Spinelli S, Legrand P, Huyen Pham TT, Nhung Trinh TT,**
477 **Cherrak Y, Zoued A, Desmyter A, Durand E, Roussel A, Kellenberger C,**
478 **Cascales E, Cambillau C.** 2017. Type VI secretion TssK baseplate protein exhibits
479 structural similarity with phage receptor-binding proteins and evolved to bind the
480 membrane complex. *Nat Microbiol* **2**:17103.
- 481 68. **Rapisarda C, Cherrak Y, Kooger R, Schmidt V, Pellarin R, Logger L, Cascales**
482 **E, Pilhofer M, Durand E, Fronzes R.** 2019. *In situ* and high-resolution cryo-EM
483 structure of the type VI secretion membrane complex. *EMBO J in press*
484 doi:10.15252/embj.2018100886.
- 485 69. **Yin M, Yan Z, Li X.** 2019. Architecture of type VI secretion system membrane core
486 complex. *Cell Res* **29**:251–253.
- 487 70. **Aschtgen MS, Thomas MS, Cascales E.** 2010. Anchoring the type VI secretion
488 system to the peptidoglycan: TssL, TagL, TagP... what else? *Virulence* **1**:535–540.
- 489 71. **Santin YG, Camy CE, Zoued A, Doan T, Aschtgen MS, Cascales E.** 2019. Role
490 and recruitment of the TagL peptidoglycan-binding protein during Type VI secretion
491 system biogenesis. *J Bacteriol.* pii: JB.00173-19. doi: 10.1128/JB.00173-19.
- 492 72. **Weber BS, Hennon SW, Wright MS, Scott NE, de Berardinis V, Foster LJ, Ayala**
493 **JA, Adams MD, Feldman MF.** 2016. Genetic dissection of the type VI secretion
494 system in *Acinetobacter* and identification of a novel peptidoglycan hydrolase, TagX,
495 required for its biogenesis. *MBio.* **7**:e01253–16.
- 496 73. **Santin YG, Cascales E.** 2017. Domestication of a housekeeping transglycosylase for
497 assembly of a Type VI secretion system. *EMBO Rep* **18**:138–149.
- 498 74. **Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ, Goo YA, Tran BQ,**
499 **Barry NA, Zheng H, Peterson SB, Chou S, Gonen T, Goodlett DR, Goodman AL,**
500 **Mougous JD.** 2014. A type VI secretion-related pathway in Bacteroidetes mediates
501 interbacterial antagonism. *Cell Host Microbe.* **16**:227–236. doi:
502 10.1016/j.chom.2014.07.007.
- 503 75. **Coyne MJ, Roelofs KG, Comstock LE.** 2016. Type VI secretion systems of human
504 gut *Bacteroidales* segregate into three genetic architectures, two of which are
505 contained on mobile genetic elements. *BMC Genomics.* **17**:58. doi: 10.1186/s12864-
506 016-2377-z.
- 507 76. **Nazarov S, Schneider JP, Brackmann M, Goldie KN, Stahlberg H, Basler M.**
508 2018. Cryo-EM reconstruction of Type VI secretion system baseplate and sheath
509 distal end. *EMBO J* **37**:e97103.
- 510 77. **Cherrak Y, Rapisarda C, Pellarin R, Bouvier G, Bardiaux B, Allain F, Malosse**
511 **C, Rey M, Chamot-Rooke J, Cascales E, Fronzes R, Durand E.** 2018. Biogenesis
512 and structure of a type VI secretion baseplate. *Nat Microbiol* **3**:1404–1416.
- 513 78. **English G, Byron O, Cianfanelli FR, Prescott AR, Coulthurst SJ.** 2014.
514 Biochemical analysis of TssK, a core component of the bacterial type VI secretion
515 system, reveals distinct oligomeric states of TssK and identifies a TssK-TssFG
516 subcomplex. *Biochem J* **461**:291–304.
- 517 79. **Taylor NM, Prokhorov NS, Guerrero-Ferreira RC, Shneider MM, Browning C,**
518 **Goldie KN, Stahlberg H, Leiman PG.** 2016. Structure of the T4 baseplate and its

- 519 function in triggering sheath contraction. *Nature*. **533**:346–52. doi:
520 10.1038/nature17971.
- 521 80. Büttner CR, Wu Y, Maxwell KL, Davidson AR. 2016. Baseplate assembly of phage
522 Mu: Defining the conserved core components of contractile-tailed phages and related
523 bacterial systems. *Proc Natl Acad Sci USA*. **113**:10174–9. doi:
524 10.1073/pnas.1607966113.
- 525 81. Kostyuchenko VA, Leiman PG, Chipman PR, Kanamaru S, van Raaij MJ,
526 Arisaka F, Mesyanzhinov VV, Rossmann MG. 2003. Three-dimensional structure
527 of bacteriophage T4 baseplate. *Nat Struct Biol*. **10**:688–93.
- 528 82. Planamente S, Salih O, Manoli E, Albesa-Jové D, Freemont PS, Filloux A. 2016.
529 TssA forms a gp6-like ring attached to the type VI secretion sheath. *EMBO J*.
530 **35**:1613–27. doi: 10.15252/emj.201694024.
- 531 83. Brunet YR, Espinosa L, Harchouni S, Mignot T, Cascales E. 2013. Imaging type
532 VI secretion-mediated bacterial killing. *Cell Rep*. **3**:36–41. doi:
533 10.1016/j.celrep.2012.11.027.
- 534 84. Ballister ER, Lai AH, Zuckermann RN, Cheng Y, Mougous JD. 2008. *In vitro*
535 self-assembly of tailorable nanotubes from a simple protein building block. *Proc Natl*
536 *Acad Sci USA* **105**:3733–3738.
- 537 85. Leiman PG, Basler M, Ramagopal UA, Bonanno JB, Sauder JM, Pukatzki S,
538 Burley SK, Almo SC, Mekalanos JJ. 2009. Type VI secretion apparatus and phage
539 tail-associated protein complexes share a common evolutionary origin. *Proc Natl*
540 *Acad Sci USA* **106**:4154–4159.
- 541 86. Brunet YR, Hénin J, Celia H, Cascales E. 2014. Type VI secretion and
542 bacteriophage tail tubes share a common assembly pathway. *EMBO Rep* **15**:315–321.
- 543 87. Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL,
544 Joachimiak G, Ordoñez CL, Lory S, Walz T, Joachimiak A, Mekalanos JJ. 2006.
545 A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus.
546 *Science*. **312**:1526–30.
- 547 88. Douzi B, Spinelli S, Blangy S, Roussel A, Durand E, Brunet YR, Cascales E,
548 Cambillau C. 2014. Crystal structure and self-interaction of the type VI secretion tail-
549 tube protein from enteroaggregative *Escherichia coli*. *PLoS One*. **9**:e86918. doi:
550 10.1371/journal.pone.0086918.
- 551 89. Renault MG, Zamarreno Beas J, Douzi B, Chabaliier M, Zoued A, Brunet YR,
552 Cambillau C, Journet L, Cascales E. 2018. The gp27-like hub of VgrG serves as
553 adaptor to promote Hcp tube assembly. *J Mol Biol* **430**:3143–3156.
- 554 90. Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ, Leiman PG. 2013.
555 PAAR-repeat proteins sharpen and diversify the type VI secretion system spike.
556 *Nature* **500**:350–353.
- 557 91. Leiman PG, Shneider MM. 2012. Contractile tail machines of bacteriophages. *Adv*
558 *Exp Med Biol*. **726**:93–114. doi: 10.1007/978-1-4614-0980-9_5.
- 559 92. Spínola-Amilibia M, Davó-Siguero I, Ruiz FM, Santillana E, Medrano FJ,
560 Romero A. 2016. The structure of VgrG1 from *Pseudomonas aeruginosa*, the needle
561 tip of the bacterial type VI secretion system. *Acta Crystallogr D Struct Biol*. **72**:22–
562 33. doi: 10.1107/S2059798315021142.
- 563 93. Uchida K, Leiman PG, Arisaka F, Kanamaru S. 2014. Structure and properties of
564 the C-terminal β -helical domain of VgrG protein from *Escherichia coli* O157. *J*
565 *Biochem*. **155**:173–82. doi: 10.1093/jb/mvt109.
- 566 94. Bönemann G, Pietrosiuk A, Diemand A, Zentgraf H, Mogk A. 2009. Remodelling
567 of VipA/VipB tubules by ClpV-mediated threading is crucial for type VI protein
568 secretion. *EMBO J*. **28**:315–25. doi: 10.1038/emboj.2008.269.

- 569 95. **Lossi NS, Dajani R, Freemont P, Filloux A.** 2011. Structure-function analysis of
570 HsiF, a gp25-like component of the type VI secretion system, in *Pseudomonas*
571 *aeruginosa*. *Microbiology*. **157**:3292–305. doi: 10.1099/mic.0.051987-0.
- 572 96. **Bröms JE, Ishikawa T, Wai SN, Sjöstedt A.** 2013. A functional VipA-VipB
573 interaction is required for the type VI secretion system activity of *Vibrio cholerae* O1
574 strain A1552. *BMC Microbiol*. **13**:96. doi: 10.1186/1471-2180-13-96.
- 575 97. **Zhang XY, Brunet YR, Logger L, Douzi B, Cambillau C, Journet L, Cascales E.**
576 2013. Dissection of the TssB-TssC interface during type VI secretion sheath complex
577 formation. *PLoS One*. **8**:e81074. doi: 10.1371/journal.pone.0081074.
- 578 98. **Kube S, Kapitein N, Zimniak T, Herzog F, Mogk A, Wendler P.** 2014. Structure of
579 the VipA/B type VI secretion complex suggests a contraction-state-specific recycling
580 mechanism. *Cell Rep*. **8**:20–30. doi: 10.1016/j.celrep.2014.05.034.
- 581 99. **Kudryashev M, Wang RY, Brackmann M, Scherer S, Maier T, Baker D, DiMaio**
582 **F, Stahlberg H, Egelman EH, Basler M.** 2015. Structure of the type VI secretion
583 system contractile sheath. *Cell* **160**:952–962.
- 584 100. **Wang J, Brackmann M, Castaño-Díez D, Kudryashev M, Goldie KN, Maier T,**
585 **Stahlberg H, Basler M.** 2017. Cryo-EM structure of the extended type VI secretion
586 system sheath-tube complex. *Nat Microbiol* **2**:1507–1512
- 587 101. **Brackmann M, Wang J, Basler M.** 2018. Type VI secretion system sheath inter-
588 subunit interactions modulate its contraction. *EMBO Rep*. **19**:225–233. doi:
589 10.15252/embr.201744416.
- 590 102. **Kapitein N, Bönemann G, Pietrosiuk A, Seyffer F, Hausser I, Locker JK, Mogk**
591 **A.** 2013. ClpV recycles VipA/VipB tubules and prevents non-productive tubule
592 formation to ensure efficient type VI protein secretion. *Mol Microbiol*. **87**:1013-28.
593 doi: 10.1111/mmi.12147.
- 594 103. **Zoued A, Durand E, Brunet YR, Spinelli S, Douzi B, Guzzo M, Flaugnatti N,**
595 **Legrand P, Journet L, Fronzes R, Mignot T, Cambillau C, Cascales E.** 2016.
596 Priming and polymerization of a bacterial contractile tail structure. *Nature* **531**:59–63.
- 597 104. **Vettiger A, Winter J, Lin L, Basler M.** 2017. The type VI secretion system sheath
598 assembles at the end distal from the membrane anchor. *Nat Commun* **8**:16088.
- 599 105. **Zoued A, Durand E, Santin YG, Journet L, Roussel A, Cambillau C, Cascales**
600 **E.** 2017. TssA: The cap protein of the Type VI secretion system tail. *Bioessays* **39**:10.
- 601 106. **Santin YG, Doan T, Lebrun R, Espinosa L, Journet L, Cascales E.** 2018. *In vivo*
602 TssA proximity labelling during type VI secretion biogenesis reveals TagA as a
603 protein that stops and holds the sheath. *Nat Microbiol* **3**:1304–1313.
- 604 107. **Dix SR, Owen HJ, Sun R, Ahmad A, Shastri S, Spiewak HL, Mosby DJ, Harris**
605 **MJ, Batters SL, Brooker TA, Tzokov SB, Sedelnikova SE, Baker PJ, Bullough**
606 **PA, Rice DW, Thomas MS.** 2018. Structural insights into the function of type VI
607 secretion system TssA subunits. *Nat Commun* **9**:4765.
- 608 108. **Pietrosiuk A, Lenherr ED, Falk S, Bönemann G, Kopp J, Zentgraf H, Sinning**
609 **I, Mogk A.** 2011. Molecular basis for the unique role of the AAA+ chaperone ClpV in
610 type VI protein secretion. *J Biol Chem*. **286**:30010-21. doi:
611 10.1074/jbc.M111.253377.
- 612 109. **Douzi B, Brunet YR, Spinelli S, Lensi V, Legrand P, Blangy S, Kumar A,**
613 **Journet L, Cascales E, Cambillau C.** 2016. Structure and specificity of the Type VI
614 secretion system ClpV-TssC interaction in enteroaggregative *Escherichia coli*. *Sci*
615 *Rep*. **6**:34405. doi: 10.1038/srep34405.
- 616 110. **Unterweger D, Kostiuk B, Pukatzki S.** 2017. Adaptor proteins of type VI secretion
617 system effectors. *Trends Microbiol*. **25**:8–10. doi: 10.1016/j.tim.2016.10.003.

- 618 111. **Ma J, Pan Z, Huang J, Sun M, Lu C, Yao H.** 2017. The Hcp proteins fused with
619 diverse extended-toxin domains represent a novel pattern of antibacterial effectors in
620 type VI secretion systems. *Virulence*. **8**:1189-1202. doi:
621 10.1080/21505594.2017.1279374.
- 622 112. **Brooks TM, Unterweger D, Bachmann V, Kostiuk B, Pukatzki S.** 2013. Lytic
623 activity of the *Vibrio cholerae* type VI secretion toxin VgrG-3 is inhibited by the
624 antitoxin TsaB. *J Biol Chem*. **288**:7618–25. doi: 10.1074/jbc.M112.436725.
- 625 113. **Toesca IJ, French CT, Miller JF.** 2014. The Type VI secretion system spike
626 protein VgrG5 mediates membrane fusion during intercellular spread by *pseudomallei*
627 group *Burkholderia* species. *Infect Immun*. **82**:1436–44. doi: 10.1128/IAI.01367-13.
- 628 114. **Silverman JM, Agnello DM, Zheng H, Andrews BT, Li M, Catalano CE, Gonen
629 T, Mougous JD.** 2013. Haemolysin coregulated protein is an exported receptor and
630 chaperone of type VI secretion substrates. *Mol Cell*. **51**:584-93. doi:
631 10.1016/j.molcel.2013.07.025.
- 632 115. **Whitney JC, Beck CM, Goo YA, Russell AB, Harding BN, De Leon JA,
633 Cunningham DA, Tran BQ, Low DA, Goodlett DR, Hayes CS, Mougous JD.**
634 2014. Genetically distinct pathways guide effector export through the type VI
635 secretion system. *Mol Microbiol*. **92**:529–42. doi: 10.1111/mmi.12571.
- 636 116. **Unterweger D, Miyata ST, Bachmann V, Brooks TM, Mullins T, Kostiuk B,
637 Provenzano D, Pukatzki S.** 2014. The *Vibrio cholerae* type VI secretion system
638 employs diverse effector modules for intraspecific competition. *Nat Commun*. **5**:3549.
639 doi: 10.1038/ncomms4549.
- 640 117. **Liang X, Moore R, Wilton M, Wong MJ, Lam L, Dong TG.** 2015. Identification
641 of divergent type VI secretion effectors using a conserved chaperone domain. *Proc
642 Natl Acad Sci USA*. **112**:9106–11. doi: 10.1073/pnas.1505317112.
- 643 118. **Unterweger D, Kostiuk B, Ötjengerdes R, Wilton A, Diaz-Satizabal L, Pukatzki
644 S.** 2015. Chimeric adaptor proteins translocate diverse type VI secretion system
645 effectors in *Vibrio cholerae*. *EMBO J*. **34**:2198–210. doi: 10.15252/embj.201591163.
- 646 119. **Alcoforado Diniz J, Coulthurst SJ.** 2015. Intraspecies competition in *Serratia
647 marcescens* is mediated by type VI-secreted Rhs effectors and a conserved effector-
648 associated accessory protein. *J Bacteriol*. **197**:2350–60. doi: 10.1128/JB.00199-15.
- 649 120. **Flaugnatti N, Le TT, Canaan S, Aschtgen MS, Nguyen VS, Blangy S,
650 Kellenberger C, Roussel A, Cambillau C, Cascales E, Journet L.** 2016. A
651 phospholipase A1 antibacterial type VI secretion effector interacts directly with the C-
652 terminal domain of the VgrG spike protein for delivery. *Mol Microbiol* **99**:1099–1118.
- 653 121. **Bondage DD, Lin JS, Ma LS, Kuo CH, Lai EM.** 2016. VgrG C terminus confers
654 the type VI effector transport specificity and is required for binding with PAAR and
655 adaptor-effector complex. *Proc Natl Acad Sci USA*. **113**:E3931–40. doi:
656 10.1073/pnas.1600428113.
- 657 122. **Cianfanelli FR, Alcoforado Diniz J, Guo M, De Cesare V, Trost M, Coulthurst
658 SJ.** 2016. VgrG and PAAR proteins define distinct versions of a functional type VI
659 secretion system. *PLoS Pathog*. **12**:e1005735. doi: 10.1371/journal.ppat.1005735.
- 660 123. **Ma J, Sun M, Dong W, Pan Z, Lu C, Yao H.** 2017. PAAR-Rhs proteins harbor
661 various C-terminal toxins to diversify the antibacterial pathways of type VI secretion
662 systems. *Environ Microbiol*. **19**:345–360. doi: 10.1111/1462-2920.13621.
- 663 124. **Quentin D, Ahmad S, Shanthamoorthy P, Mougous JD, Whitney JC, Raunser
664 S.** 2018. Mechanism of loading and translocation of type VI secretion system effector
665 Tse6. *Nat Microbiol*. **3**:1142–1152. doi: 10.1038/s41564-018-0238-z.

- 666 125. **Burkinshaw BJ, Liang X, Wong M, Le ANH, Lam L, Dong TG.** 2018. A type VI
667 secretion system effector delivery mechanism dependent on PAAR and a chaperone-
668 co-chaperone complex. *Nat Microbiol.* **3**:632–640. doi: 10.1038/s41564-018-0144-4.
- 669 126. **Hachani A, Allsopp LP, Oduko Y, Filloux A.** 2014. The VgrG proteins are "à la
670 carte" delivery systems for bacterial type VI effectors. *J Biol Chem.* **289**:17872–84.
671 doi: 10.1074/jbc.M114.563429.

672 **LEGEND TO FIGURES**

673

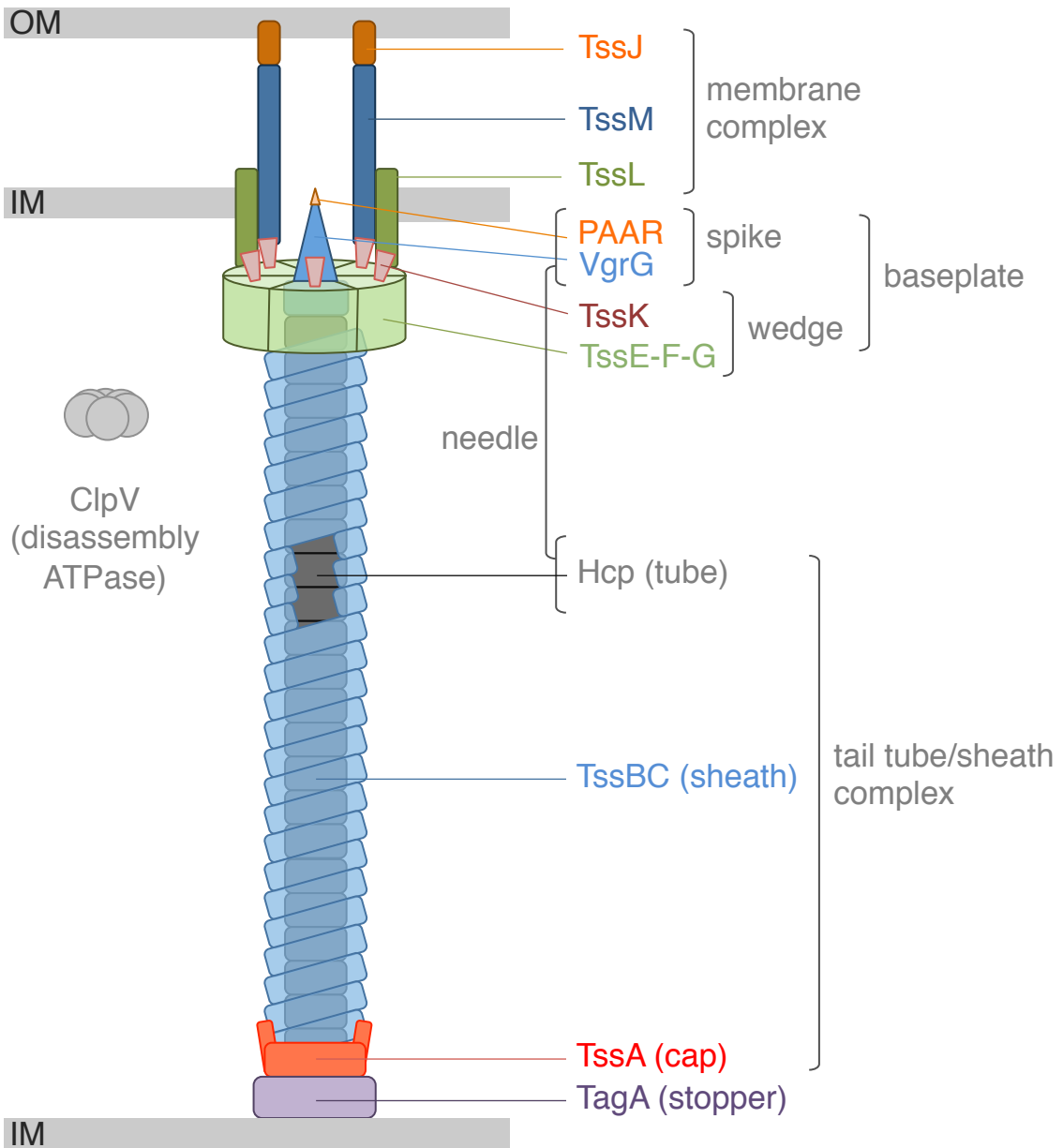
674 **FIGURE 1** Schematic representation of the Type VI secretion system. The different subunits
675 are labeled, as well as the different subcomplexes. IM, inner membrane; OM, outer
676 membrane.

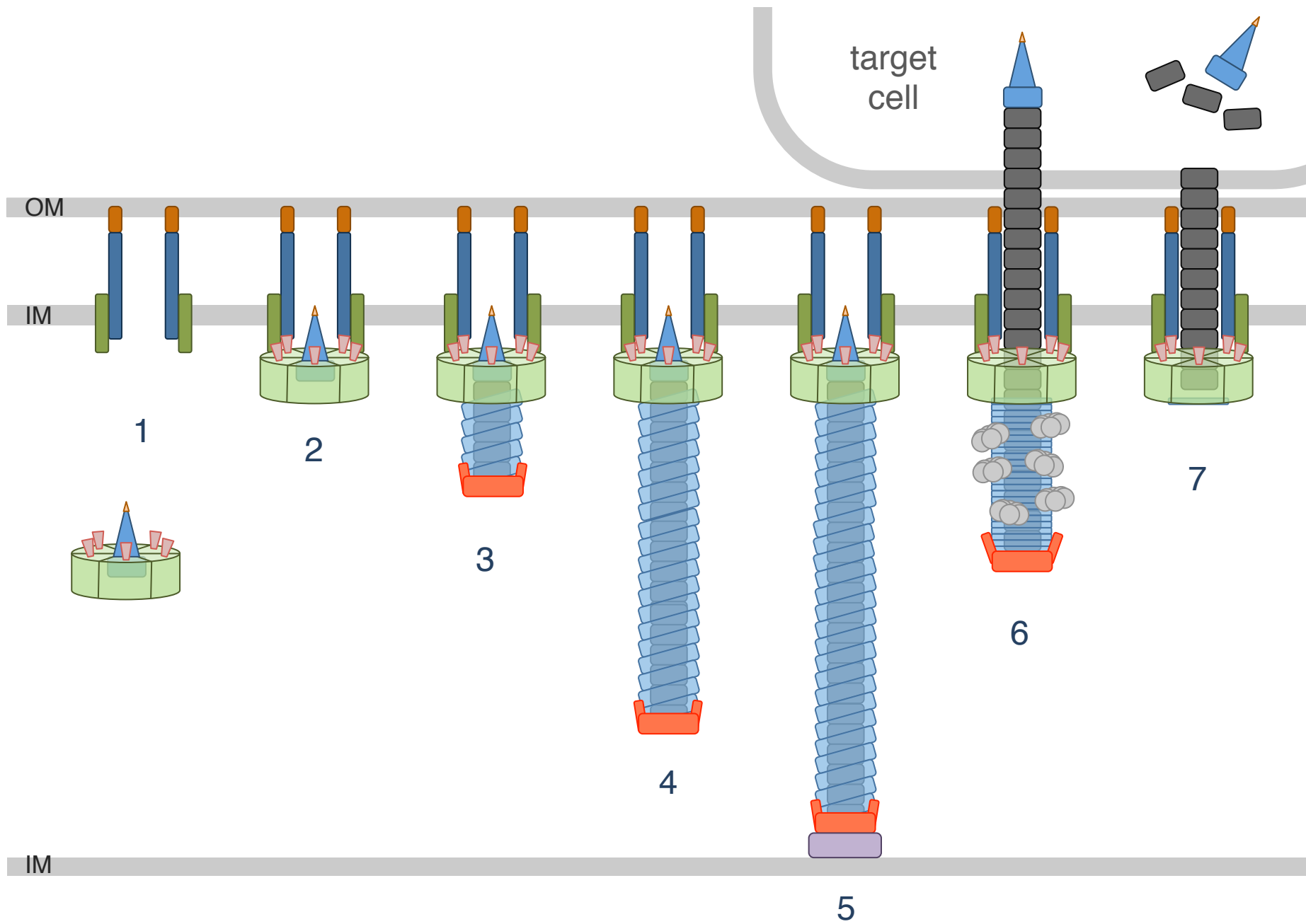
677

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).





PAAR 

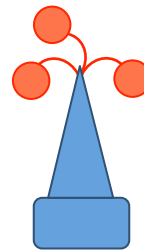
 effector

 adaptor

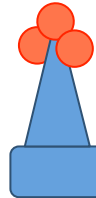
VgrG 

Hcp  

needle
components



specialized
effectors



direct binding



cargo
effectors



internal
adaptors

