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The Type VI secretion system

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1 # The Type VI Secretion system: a versatile bacterial weapon

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7 **Abstract**

8 The Type VI secretion system (T6SS) is protein nanomachine which is widespread in Gram-negative

9 bacteria and used to translocate effector proteins directly into neighbouring cells. It represents a

10 versatile bacterial weapon which can deliver effectors into distinct classes of target cells, playing key

11 roles in inter-bacterial competition and bacterial interactions with eukaryotic cells. This versatility is

12 underpinned by the ability of the T6SS to deliver a vast array of effector proteins, with many distinct

13 activities and modes of interaction with the secretion machinery. Recent work has highlighted the

14 importance and diversity of interactions mediated by T6SSs within polymicrobial communities and

15 offered new molecular insights into effector delivery and action in target cells.

16

17 **Key Words**

18 Bacterial protein secretion, Type VI secretion system, bacterial effector proteins, polymicrobial

19 communities

20 **Introduction**

21 The Type VI secretion system (T6SS) is a protein nanomachine deployed by many Gram-negative

22 bacteria in order to translocate effector proteins directly into target cells. Following its recognition

23 as the sixth major protein secretion system in Gram-negative bacteria (Mougous *et al.*, 2006;

24 Pukatzki *et al.*, 2006), the T6SS was initially believed to function as a classical virulence factor,

25 namely to deliver effector proteins which destroy or manipulate the cells of eukaryotic host

26 organisms ('anti-eukaryotic T6SS'). However it has subsequently become clear that the primary

27 function of the T6SS is as a device for inter-bacterial competion. In other words, bacteria use the

28 T6SS to deliver toxic anti-bacterial effectors into rival bacterial cells ('anti-bacterial T6SS'). More

29 recently, the range of known uses of the T6SS has expanded further, including action against

30 microbial fungi and scavenging of scarce metal ions. In parallel, recent work has revealed the

31 importance of interactions mediated by anti-bacterial T6SSs in varied polymicrobial communities

32 and offered new molecular insights into effector delivery and action in target cells.

33 It has been estimated that > 25% of proteobacteria encode at least one T6SS in their genome (Bingle

34 *et al.*, 2008). These T6SSs, whilst sharing the same 13 or 14 core components (TssA-M, PAAR), show

35 considerable variation in terms of gene content and function and can be split into six sub-families (1,

36 2, 3, 4a, 4b, 5) (Barret *et al.*, 2011; Boyer *et al.*, 2009). Moreover, two further, evolutionarily-

37 divergent, T6SS-like systems have been reported. One is found on the *Francisella* pathogenicity

38 island and designated T6SS[ii], the other is found in the phylum *Bacteroidetes* and designated T6SS[iii],

39 whilst the widespread and well-characterised proteobacterial T6SS can be termed T6SS[i]. T6SS[ii] and

40 T6SS[iii] have distinct components but a common overall mode of action when compared with the

41 canonical T6SS[i] (Clemens *et al.*, 2015; Russell *et al.*, 2014). A given bacterial species can contain

42 between one and six different T6SSs, with the complement of T6SSs present frequently varying

43 between individual strains. In some bacteria, one T6SS is used for two distinct roles, e.g. the T6SS in

44 *Vibrio cholerae* is both anti-bacterial and anti-eukaryotic (MacIntyre *et al.*, 2010), whilst in other

45 cases different T6SSs fulfil distinct roles, e.g. T6SS-5 and T6SS-1 in *Burkholderia thailandensis* have

46 exclusively anti-host and anti-bacterial activity, respectively (Schwarz *et al.*, 2010). In general,

47 different bacterial species and strains use and tailor their T6SS(s) for specific roles according to the

48 niche and strategy of the organism. In addition to variation in number and type of T6SS itself, there

49 is also considerable diversity in effector portfolio. Furthermore, regulation of T6SS gene expression is

50 highly organism-specific and matched with the biological role the system is required to fulfil. For

51 example, the anti-host T6SS of *B. mallei* is regulated by the global virulence regulator VirAG, whilst

52 the 'defensive' anti-bacterial H1-T6SS of *Pseudomonas aeruginosa* is post-transcriptionally activated

53 by cell damage-derived signals via the RetS/Gac/Rsm pathway (LeRoux *et al.*, 2015; Schell *et al.*,

54   2007). The aim of this review is to showcase the diversity and breadth of functions mediated by the

55   T6SS and highlight the widespread importance of this system in many contexts.

56   **Mechanism of effector delivery by the T6SS**

57   The T6SS is a large and dynamic 'nanomachine' that uses a contraction mechanism to propel an

58   extracellular puncturing structure out of the secreting cell. The force of this propulsion can further

59   drive the puncturing structure, which is decorated with effector proteins, into an immediately-

60   adjacent cell, thus achieving contact-dependent translocation of effector proteins into target cells

61   (summarised in **Figure 1**).

62   *Assembly and firing of the T6SS machinery*

63   As reviewed in detail elsewhere (Brackmann *et al.*, 2017; Cianfanelli *et al.*, 2016b; Clemens *et al.*,

64   2018; Nguyen *et al.*, 2018), the T6SS is an envelope-spanning apparatus assembled from 14 core

65   components (TssA-M, PAAR) and comprising several distinct sub-assemblies: membrane complex,

66   cytoplasmic baseplate, cytoplasmic contractile sheath and expelled puncturing structure. The

67   puncturing structure consists of a tube of stacked hexameric rings of the Hcp (TssD) protein, topped

68   by a spike made of a VgrG (TssI) trimer with a PAAR domain-containing protein tip. Assembly begins

69   with the formation of the bell-shaped membrane complex, comprising ten or twelve copies each of

70   the outer membrane protein TssJ and the inner membrane proteins TssLM (Durand *et al.*, 2015;

71   Nguyen *et al.*, 2018). The cytoplasmic face of the membrane complex then docks the baseplate,

72   which consists of six $TssEF_2G(K_3)_2$ 'wedges' around a central $VgrG_3PAAR$ unit. TssK acts as a connector

73   linking the baseplate to the membrane complex, by interacting with TssFG and the cytoplasmic

74   domains of TssLM (Cherrak *et al.*, 2018; Nazarov *et al.*, 2018; Nguyen *et al.*, 2018). The Hcp tube can

75   then assemble onto the base of VgrG, extending into the cytoplasm. Simultaneously, a helical sheath

76   structure made of TssBC subunits polymerises around the Hcp tube in an extended, high-energy

77   'primed' conformation (Kudryashev *et al.*, 2015; Renault *et al.*, 2018; Wang *et al.*, 2017). Rapid (< 2

78   ms) contraction of the TssBC sheath drives the Hcp-VgrG-PAAR structure through the baseplate and

79   membrane complex, out of the secreting cell. Contraction of the TssBC sheath, which can span the

80   width of the producing cell, provides sufficient power and reach for the expelled Hcp-VgrG-PAAR

81   structure to also breach an appropriately located recipient cell (Basler, 2015; Vettiger *et al.*, 2017;

82   Wang *et al.*, 2017). Following contraction, the contracted sheath is specifically depolymerised by the

83   ATPase TssH, whilst the effectors are somehow released inside the target cell. A role for the final

84   core T6SS component, TssA, in capping the distal end of the Hcp-TssBC structure and co-ordinating

85   assembly of the inner tube and the sheath has recently been described (Dix *et al.*, 2018; Zoued *et al.*,

86   2016).

87   The contraction-based mechanism of the T6SS is related to the injection mechanism of contractile

88   bacteriophages, with close structural similarities between the Hcp-VgrG-PAAR structure and the

89   phage tail tube and tail spike, between the TssBC sheath and the tail sheath, and between the

90   TssEFG and gp25gp6$_2$gp7 wedge units (Brackmann *et al.*, 2017; Cherrak *et al.*, 2018). However in

91   contrast with the phage system, the T6SS can be reused for multiple firing events by the same cell.

92   Indeed dynamic cycles of T6SS assembly-contraction-disassembly have been observed using

93   fluorescence microscopy imaging of individual T6SSs in a number of organisms, including *V. cholerae*,

94   enteroaggregative *E. coli* (EAEC), *P. aeruginosa* and *Serratia marcescens* (Basler *et al.*, 2012; Basler

95   *et al.*, 2013; Durand *et al.*, 2015; Ostrowski *et al.*, 2018).

96   *Accessory components*

97   In addition to the core components above, T6SSs are diversified and further tailored for their

98   function by the possession of accessory structural or regulatory components which are present in

99   some, but not all, T6SSs. In some systems an additional membrane complex component, SciZ/TagL,

100   provides a peptidoglycan binding functionality normally present in TssL, whilst, conversely, T6SSs

101   may also co-opt peptidoglycan hydrolase enzymes to assist in the formation of the membrane

102   complex through the cell wall (Aschtgen *et al.*, 2010; Santin & Cascales, 2017; Weber *et al.*, 2016). In

103   EAEC, a membrane-associated TssA-family protein, TagA, interacts with TssA in order to 'catch', stop

104   and stabilise the extending TssBC sheath when it reaches the opposite side of the cell (Santin *et al.*,

105   2018). Although some other T6SSs possess a second TssA-family protein which is likely to function

106   similarly, many do not, leaving open the question of how else this function might be achieved.

107   Another protein, TagJ, may help to recruit TssH to the contracted sheath in some systems (Forster *et*

108   *al.*, 2014).

109   Many T6SSs also contain conserved post-translational regulatory components. In *P. aeruginosa* and

110   *S. marcescens*, a membrane-bound protein threonine kinase, PpkA, phosphorylates the T6SS-

111   associated Fha protein, overcoming inhibition mediated by a negative regulator,TagF, and thereby

112   allowing assembly of an active T6SS. An antagonistic phosphatase, PppA, promotes spatial relocation

113   of the T6SS machinery between firing events. Interestingly, the two systems are used to increase the

114   efficiency of T6SS anti-bacterial activity in distinct ways. In *P. aeruginosa*, the TagQRST complex

115   detects incoming T6SS-mediated attacks from neighbouring cells, activating PpkA and thus causing

116   the H1-T6SS to assemble and fire back towards the attacker, making it a 'defensive' system. In *S.*

117   *marcescens*, the upstream regulator of PpkA is a distinct protein, RtkS, and the signal for activation is

118   independent of incoming attack or cell-cell contact, resulting in an 'offensive' system which can

119   attack passive or aggressive neighbours (Cianfanelli *et al.*, 2016b; Ostrowski *et al.*, 2018; and

120 references therein). Other variations also exist, including PpkA-mediated phosphorylation of TssL in

121 *A. tumefaciens* (Lin *et al.*, 2014).

122 *Modes of effector delivery*

123 An important aspect of the versatility of the T6SS is its ability to deliver a large variety of different

124 types of effector proteins. To achieve this, effectors can associate with the expelled Hcp-VgrG-PAAR

125 structure through multiple distinct mechanisms in order to be translocated between cells (**Figure 1**).

126 'Cargo' effectors non-covalently interact with specific Hcp, VgrG or PAAR proteins, whilst

127 'specialised' effectors comprise modular proteins in which additional effector domains are

128 covalently fused to the C-terminus of Hcp, VgrG or PAAR proteins (Cianfanelli *et al.*, 2016b; Durand

129 *et al.*, 2014). Hcp-dependent cargo effectors are relatively small, bind within the lumen of the Hcp

130 hexamer and are recognised and stabilised by this interaction, as first described for the anti-bacterial

131 effectors Tse2, Tse1 (Tae1^{PA}) and Tse3 (Tge1 ^{PA}) of *P. aeruginosa* (Silverman *et al.*, 2013). VgrG-

132 dependent cargo effectors interact with specific VgrG proteins to sit on the outside of the spike and

133 include many phospholipase effectors, such as Tle1^{EC} from EAEC (Flaugnatti *et al.*, 2016). Examples of

134 PAAR-interacting cargo effectors have also recently been reported, including TseT in *P. aeruginosa*

135 (Burkinshaw *et al.*, 2018). Many different examples of specialised VgrG effectors (also termed

136 'evolved VgrGs') have been described, including VgrG-1 of *V. cholerae*, which possesses a C-terminal

137 actin crosslinking domain (ACD) (Pukatzki *et al.*, 2007). PAAR domain-containing specialised effectors

138 are also widespread and diverse, including many nuclease toxins. They can be based simply on a

139 PAAR domain followed by an effector domain, but also include a group of large polymorphic toxins

140 known as Rhs proteins. In the latter, a conserved central Rhs repeat domain is predicted to form a

141 shell-like structure around a C-terminal effector domain, with different Rhs proteins possessing a

142 multitude of distinct C-terminal domains and associated immunity proteins (Alcoforado Diniz &

143 Coulthurst, 2015; Hachani *et al.*, 2014; Whitney *et al.*, 2014; Zhang *et al.*, 2012). It also appears that

144 effector domains may on occasion be fused at the N-terminal end of PAAR proteins, further

145 emphasizing their modularity (Shneider *et al.*, 2013). A family of specialised Hcp effectors with a

146 number of distinct C-terminal effector domains, present in members of the *Enterobacteriaceae*, has

147 also recently been described (Ma *et al.*, 2017a). Thus all three components of the expelled

148 puncturing structure can be used for both cargo and specialised effector delivery modes. A given

149 T6SS is typically associated with multiple VgrG, PAAR and/or Hcp homologues, with or without

150 specialised effector domains, allowing for delivery of many effectors. Specific combinations of these

151 homologues define functional tube-spike units and determine the effectors translocated by that

152 firing event (Cianfanelli *et al.*, 2016a). However the frequency and significance of the formation of

153 VgrG heterotrimers, or of 'mixed' Hcp tubes, is currently unclear.

154   In some cases, effector recruitment and therefore delivery by the T6SS requires a further

155   'chaperone' or 'adaptor' protein. Several unrelated but widespread families of such chaperones have

156   been described to date. EagR/EagT (DUF1795) proteins bind the N-terminal PAAR-containing

157   domains of Rhs and Tse6-like effectors, stabilising transmembrane regions which may ultimately

158   permit the effectors to cross the recipient cell inner membrane and allowing the effector to be

159   loaded onto the cognate VgrG (Cianfanelli *et al.*, 2016a; Quentin *et al.*, 2018). Tap-1/TecL (DUF4213)

160   family proteins allow the interaction and loading of VgrG-dependent cargo effectors, including TseL

161   and Tde1, onto the cognate VgrG. They are modular adaptors, where the C-terminal half of the

162   protein varies with the associated effector, providing a mechanism for new effectors to be

163   horizontally acquired and interact with existing VgrG homologues via recombination within the Tap-

164   1 gene (Ma *et al.*, 2014; Unterweger *et al.*, 2015). A chaperone (TecT) facilitating interaction of a

165   PAAR-dependent cargo effector with the cognate PAAR protein has also been reported, with PAAR

166   competing with a co-chaperone for access to the chaperone (Burkinshaw *et al.*, 2018). None of these

167   families of chaperones appear to be secreted with their effectors, rather their role is to protect and

168   load specific effectors onto the machinery prior to secretion. Consistent with the different routes of

169   effector secretion, there is no universal secretion signal for substrates of the T6SS. No such signal is

170   required for specialised effectors, whilst cargo effectors require specific 3D interactions with their

171   cognate VgrG/PAAR/Hcp protein, assisted in many cases by specific chaperones. One secretion motif

172   ('MIX') has been identified in a subset of effectors widespread in *Vibrionaceae* (Salomon *et al.*,

173   2014); this motif is likely to define an adaptor domain interacting with one of the core components.

174   **Anti-bacterial effectors**

175   T6SSs represent widespread and potent weapons for killing or inhibiting rival bacterial cells, both

176   within and between species. This is achieved by the delivery of broad-spectrum anti-bacterial

177   effectors (**Figure 2**), with a given system typically able to deliver one or more representatives of

178   several different effector families.

179   *Classes and modes of action of T6SS-delivered anti-bacterial effectors*

180   A large number of T6SS effectors which target the peptidoglycan cell wall of recipient bacteria have

181   been identified. These peptidoglycan hydrolases can be divided into at least five families of

182   peptidoglycan amidases (Tae1-4, TaeX), which cleave specific bonds within the peptide cross-bridges

183   of the cell wall, and four families of peptidoglycan glycoside hydrolases (Tge1-3, VgrG-3), which

184   cleave the backbone glycan chains (Brooks *et al.*, 2013; Ma *et al.*, 2018; Russell *et al.*, 2012; Whitney

185   *et al.*, 2013). The inner membrane is also a common target of T6SS effectors. Five families of

186   lipase/phospholipase effector (Tle1-4, includes effectors with phospholipase A_1 or A_2 activity, and

187   Tle5, phospholipase D) have been described (Flaugnatti *et al.*, 2016; Russell *et al.*, 2013). Additionally

188   two effectors, VasX and Tse4, have been reported to form pores or channels in the membrane

189   (LaCourse *et al.*, 2018; Miyata *et al.*, 2013). All these effectors targeting the cell wall or membrane

190   act in/from the periplasm, being generally non-toxic if expressed in the cytoplasm and in some cases

191   activated by periplasmic insertion of disulphide bonds (Mariano *et al.*, 2018). This suggests that the

192   major destination for incoming effectors is likely to be the periplasm of target cells, which may also

193   inform on why the T6SS does not appear to act against Gram-positive cells which lack this

194   compartment. However T6SSs also deliver effectors which act in the bacterial cytoplasm. A number

195   of T6SS-dependent nuclease effectors have also been described (e.g. DNases Tde1 and RhsAB) with

196   many more, particularly PAAR-containing specialised effectors, predicted to possess DNase, RNase

197   or deaminase activity according to bioinformatic analyses (Koskiniemi *et al.*, 2013; Ma *et al.*, 2017b;

198   Ma *et al.*, 2014; Zhang *et al.*, 2012). T6SS effectors can also act by degrading an essential cytoplasmic

199   cofactor, as revealed by the identification of two families of NAD(P)$^+$ hydrolase effectors (Tse6/Tne1

200   and Tne2) (Tang *et al.*, 2018; Whitney *et al.*, 2015). Recently a T6SS-delivered ADP-ribosyltransferase

201   toxin, Tre1, which modifies FtsZ by the addition of ADP-ribose and thus inhibits cell division, has

202   been described. Such toxins are often used by bacteria against eukaryotic cells but this work

203   suggests they may also be used by several inter-bacterial toxin delivery systems including the T6SS

204   (Ting *et al.*, 2018). Cytoplasmic acting effectors may reach the cytoplasm in several ways: by a

205   minority of T6SS delivery events reaching the cytoplasm directly, by effectors incorporating

206   transmembrane domains to allow their own traversal of the inner membrane, and/or by target cell

207   protein-mediated import similar to Cdi toxins; to date, some evidence has been presented for each

208   possibility (Quentin *et al.*, 2018; Vettiger & Basler, 2016; Whitney *et al.*, 2015; Willett *et al.*, 2015).

209   Whilst numerous and varied T6SS-dependent antibacterial effectors have already been reported, it

210   seems clear that the portfolio of effectors and of effector modes of action will continue to grow,

211   given that many effectors identified to date still have no known or readily-predictable function and

212   that new effectors will be revealed by experimental and bioinformatic analysis of increasing

213   numbers of bacterial strains and species.

214   *Self-protection by specific immunity proteins*

215   Any bacterial cell possessing an anti-bacterial T6SS must possess a means to prevent self-intoxication

216   by its own effectors (cytoplasmic-acting effectors, prior to secretion) and intoxication by effectors

217   delivered into it by its neighbouring sibling cells (all effectors, incoming). This is achieved through

218   specific immunity proteins, which are encoded adjacent to the gene for the cognate effector protein.

219   Immunity proteins reside in the cellular compartment of action of the effector and normally bind

220   tightly to the effector to physically prevent toxicity (**Figure 2**) (Alcoforado Diniz & Coulthurst, 2015).

221   For example, immunity proteins against peptidoglycan hydrolase effectors are soluble or lipid-

222   anchored periplasmic proteins which specifically bind to their cognate effectors and block the active

223   site (Russell *et al.*, 2011; Srikannathasan *et al.*, 2013; Whitney *et al.*, 2013). Interestingly, the Tri1

224   immunity protein, which protects against the Tre1 ADP-ribosyltransferase effector, has a novel dual

225   function. In addition to a typical, specific active site occlusion mechanism, it also has an enzymatic

226   ADP-ribosylhydrolase activity which removes the modification added by the effector and confers

227   broad resistance to related toxins (Ting *et al.*, 2018).

228   *Evolution and acquisition of effector-immunity pairs*

229   T6SS-mediated inter-bacterial competition occurs between and within bacterial species, mediated

230   by considerable variation in effector-immunity portfolio, even between strains of the same species.

231   In addition to diversity in the number and type of effector, there is also variation within effector

232   families, resulting in related but specific and mutually-incompatible effector-immunity pairs. In

233   general, effector-immunity pairs appear to be horizontally acquired in an inter-bacterial 'arms race'.

234   In the case of specialised effectors, there is some evidence, particularly for Rhs proteins, that

235   homologous recombination events allow the facile exchange of one C-terminal effector domain plus

236   cognate downstream immunity gene for another pair, resulting in highly variable loci which can

237   mediate competition between strains (Koskiniemi *et al.*, 2014). For cargo effectors, whilst simple

238   effector-immunity pairs can be acquired, it appears that they are often acquired together with linked

239   chaperone or VgrG proteins which are predicted to allow their delivery in the recipient background

240   (Barret *et al.*, 2011; Fitzsimons *et al.*, 2018; Unterweger *et al.*, 2015). So-called 'orphan' immunity

241   proteins, which do not confer resistance to effectors currently possessed by the organism, are

242   frequently encoded downstream of a related 'active' effector-immunity pair. They may be retained

243   from formerly-active effector-immunity pairs where the effector has been lost, or represent newly-

244   acquired genes, in both cases likely able to confer protection against effectors delivered by other

245   strains (Alcoforado Diniz *et al.*, 2015; Kirchberger *et al.*, 2017). Many T6SS effectors appear to be

246   modular toxin domains which can be used for inter-bacterial competition in several contexts, such as

247   as cargo or specialised T6SS effectors, effectors delivered by contact-dependent inhibition (Cdi) or

248   Type VII (ESAT/Esx) secretion systems, or toxin domains of colicins. For example, Tne2 domains are

249   found in putative T6SS cargo effectors, in Rhs and smaller PAAR specialised T6SS effectors, and in

250   proteins containing LXG and WXG motifs associated with T7SSs (Tang *et al.*, 2018), whilst CdiA toxin

251   domains are frequently shared with T6SS-associated Rhs proteins and can also be found in putative

252   T6SS cargo effectors (Batot *et al.*, 2017; Poole *et al.*, 2011).

253 One reason why anti-bacterial T6SSs are so effective is likely to be because multiple distinct effectors

254 can be delivered at the same time. One one hand, this means that a rival cell cannot simply protect

255 itself by becoming spontaneously resistant to one effector or acquiring one immunity protein.

256 Perhaps more importantly, simultaneous delivery of effectors targeting the cell wall, cell membrane

257 and cellular DNA, for example, or attacking one target with several different enzymatic activities, is

258 likely to lead to more efficient killing than any effector alone. Indeed synergy between several

259 different combinations of effectors has been demonstrated in *P. aeruginosa* (LaCourse *et al.*, 2018).

260 This study also suggested that multiple effectors may protect against variations in environmental

261 conditions which might reduce the efficacy of an individual toxin.

262 **Roles of anti-bacterial T6SSs in bacterial communities**

263 The killing activity conferred by anti-bacterial T6SSs can be extremely potent during *in vitro* co-

264 culture experiments, with T6SS-wielding cells often able to virtually eliminate similar numbers of

265 susceptible competitor cells within a few hours. The obvious next question, then, is how that activity

266 is used and relevant in 'real-life' microbial communities and niches. Once T6SS-dependent anti-

267 bacterial activity had been identified (Hood *et al.*, 2010), it became clear that reports of virulence

268 defects in T6SS mutants should be carefully evaluated, since decreased fitness of a T6SS mutant

269 could be due to loss of ability to compete against the resident microbiota or co-infecting pathogens,

270 rather than the T6SS having a direct effect on host cells. Some years on, there is now evidence that

271 anti-bacterial T6SSs can markedly influence the composition of host-associated communities and

272 affect the outcome for the host. Anti-bacterial T6SSs can also play roles in the social behaviour of

273 bacteria, for example recognition of self, and facilitate horizontal gene transfer by releasing DNA

274 from prey cells.

275 *Polybacterial host-associated communities*

276 A number of studies have demonstrated a role for anti-bacterial T6SSs in overcoming colonisation

277 resistance of the gut microbiota towards pathogens. The T6SS of *Salmonella typhimurium* was found

278 to be required for successful establishment of infection in the gut if the resident microbial

279 community was intact, whilst the T6SS of *Shigella sonnei* increased its ability to outcompete

280 commensal *E. coli* and persist in a mouse model (Anderson *et al.*, 2017; Sana *et al.*, 2016). In *V.*

281 *cholerae*, T6SS-mediated anti-bacterial activity against the host commensal microbiota was shown to

282 increase intestinal colonisation and activate host innate immunity genes in an infant mouse model,

283 and to contribute to colonisation of the middle small intestine in the infant rabbit model (Fu *et al.*,

284 2018; Zhao *et al.*, 2018). Interestingly, in a *Drosophila* infection model, T6SS-mediated killing of a

285 subpopulation of commensal bacteria was somehow actively required to trigger host destruction by

286 *V. cholerae* (Fast *et al.*, 2018). In contrast, in a zebrafish model, removal of a pre-colonised symbiotic

287 species from the gut in a manner dependent on the action of the *V. cholerae* T6SS was not due to

288 anti-bacterial activity, but rather a direct impact of the VgrG-1 ACD on host intestinal movements,

289 emphasizing the potential complexities of T6SS-dependent *in vivo* interactions (Logan *et al.*, 2018).

290 Members of the *Bacteroidales* are major constituents of the human gut microbiota and commonly

291 possess up to three distinct architectures of T6SS[iii], with T6SS loci frequently transferred among co-

292 resident species in the gut (Coyne *et al.*, 2016). T6SS-mediated competition has been observed

293 between strains of *B. fragilis in vivo* and appears to play a key role in establishing a stable

294 community of compatible *Bacteriodes* strains in a given individual (Chatzidaki-Livanis *et al.*, 2016;

295 Verster *et al.*, 2017; Wexler *et al.*, 2016). Anti-bacterial T6SS activity has also been implicated in

296 generating colonisation resistance. A symbiotic *B. fragilis* strain was shown to utilise its T6SS and the

297 effector Bte2 to exclude a pathogen, a toxigenic strain of *B. fragilis*, *in vivo*, protecting the host from

298 colitis (Hecht *et al.*, 2016). It will be interesting to see whether *Bacteroides* also utilise the T6SS

299 against invading proteobacterial gut pathogens *in vivo*. In the bee gut, competition mediated by anti-

300 bacterial T6SSs, and in particular highly diverse and readily exchangeable Rhs effector domains,

301 appears to be an important driver of fitness and evolution within the microbiota (Steele *et al.*, 2017).

302 Complex polymicrobial communities are also found associated with plant hosts, in particular within

303 the rhizosphere. Anti-bacterial T6SSs are frequently found in both pathogenic and symbiotic or

304 beneficial plant-associated bacteria (Bernal *et al.*, 2018). This suggests that anti-bacterial T6SSs

305 should be involved in establishing and protecting beneficial plant-associated communities and in

306 invasion of these communities by pathogens. In support of this concept, T6SS-dependent anti-

307 bacterial activity in the plant-protecting rhizosphere bacterium *P. putida* was shown to reduce

308 colonisation and necrosis caused by the phytopathogen *Xanthomonas campestris* when co-

309 infiltrated into *Nicotiana* leaves (Bernal *et al.*, 2017). From the other side, the anti-bacterial T6SS of

310 the plant pathogen *A. tumefaciens* is effective against *P. aeruginosa* within a plant but not on lab

311 media (Ma *et al.*, 2014).

312 A very specific symbiotic relationship is that between the *Euprymna scolopes* squid and *V. fischeri*, in

313 which the bacteria colonise individual crypts in the squid light organ and ultimately bioluminesce. It

314 turns out that an anti-bacterial T6SS plays a key role in selecting and spatially separating strains of *V.*

315 *fischeri* colonising the light organ. Individual crypts cannot be colonised by two or more incompatible

316 strains, where incompatibility is conferred by a difference in T6SS and effector-immunity

317 complement (Speare *et al.*, 2018). In general, host-associated bacterial communities typically exist in

318 a biofilm state, where aggregates of cells are adhered to a surface within a polymeric extracellular

319 matrix. This scenario is likely to be conducive to T6SS-dependent interactions, and indeed T6SS

320   genes are frequently co-regulated with biofilm genes and the T6SS has been shown to allow

321   persistence of *Burkholderia* in a mixed biofilm (Schwarz *et al.*, 2010). On the other hand, it has been

322   suggested that extracellular polysaccharide may, in some cases, provide a physical barrier which

323   reduces the effectiveness of T6SS attacks (Toska *et al.*, 2018).

324   *Social behaviour and acquisition of genetic material*

325   Anti-bacterial T6SSs can represent a means by which one individual strain or genotype can

326   distinguish self from non-self (i.e. from closely related strains). In some cases, this manifests as the

327   formation of a boundary between two populations. In *Proteus mirabilis*, the formation of Dienes

328   lines, macroscopic boundaries between swarms of two non-identical strains, is dependent on T6SS-

329   mediated killing of non-siblings via strain-specific effector-immunity pairs (Alteri *et al.*, 2013;

330   Wenren *et al.*, 2013). Similarly, in *Myxococcus xanthus*, delivery of a T6SS nuclease toxin was shown

331   to be required for the inability of colonies of different strains to merge (Gong *et al.*, 2018). The T6SS

332   has also been proposed to be a means of policing quorum sensing (QS) 'cheats', spontaneous QS

333   mutants which benefit from the production of common goods (e.g. extracellular enzymes) by a

334   population, whilst no longer producing the goods themselves. In *B. thailandensis*, QS activation of

335   effector-immunity gene expression was shown to result in QS mutant cheats being eliminated by QS-

336   proficient cells intoxicating them with effectors they are no longer immune to (Majerczyk *et al.*,

337   2016). Even within a genetically uniform population, anti-bacterial T6SSs may be able to promote

338   phenotypic homogeneity by allowing fitter cells to eliminate starving or otherwise less-fit cells from

339   the population. Somewhat similar to elimination of QS cheats, starving cells of *M. xanthus* have

340   reduced levels of T6SS and TsxI immunity proteins, allowing them to be killed by delivery of the TsxE

341   effector by healthy neighbouring cells (Troselj *et al.*, 2018). Mathematical modelling has provided

342   support for the idea that T6SS-mediated inter-bacterial competition can lead to spatial separation of

343   non-identical bacterial populations and suggested that this separation can favour the evolution of

344   co-operation within the segregated population (McNally *et al.*, 2017).

345   A distinct ecological role for anti-bacterial T6SSs is in facilitating DNA uptake, and thus horizontal

346   gene transfer, in naturally competent bacteria. T6SS-mediated lysis of non-self cells, whether closely

347   or more distantly related, results in release of DNA from the targeted cell which can then be taken

348   up by the T6SS-wielding attacker. This role was first identified in *V. cholerae*, where T6SS gene

349   expression is co-regulated with that of the competence machinery (Borgeaud *et al.*, 2015). Further

350   studies in this organism indicated that T6SS effector-immunity genes themselves can be transferred

351   in this way, thus an attacker can acquire new weapons from its prey (Thomas *et al.,* 2017). Similarly,

352   T6SS-mediated horizontal gene acquisition has been demonstrated in *Acinetobacter* (Cooper *et al.,*

353   2017; Ringel *et al.*, 2017). Transfer of genetic material from prey *E. coli* to *Acinetobacter* was

354   frequent enough to allow observation of functional transformation in real time, which may help to

355   explain why *Acinetobacter baumannii* is able to acquire antibiotic resistance so rapidly in the clinic

356   (Cooper *et al.*, 2017).

357   **Anti-host T6SS effectors**

358   In addition to widespread and versatile utilisation of anti-bacterial T6SSs, bacteria can also use T6SSs

359   to directly target eukaryotic cells, including those of host organisms. There have been many reports

360   of virulence, host response and host cell interaction phenotypes dependent on a functional T6SS, in

361   a range of bacterial pathogens (Hachani *et al.*, 2016). However given the caveat that such

362   phenotypes may be an indirect result of the action of an anti-bacterial T6SS, for example against the

363   host microbiota, it is pertinent to consider only those cases where T6SS effectors responsible for

364   direct action against host cells have been identified. During the first decade of T6SS research, these

365   were few in number: VgrG proteins with C-terminal actin crosslinking, actin ADP ribosylase and host

366   membrane fusion domains; another VgrG protein with a tubulin binding domain which modulates

367   microtubule-mediated bacterial internalisation; and two cargo phospholipase D effectors which also

368   facilitate internalisation by binding Akt and activating the PI3K pathway; all reviewed by Hachani and

369   coworkers (Hachani *et al.*, 2016). These phospholipase effectors, PldA and PldB of *P. aeruginosa*, are

370   also anti-bacterial effectors (Tle5 family) which exert toxicity from the periplasm of target cells

371   lacking the cognate immunity proteins. This highlights another aspect of the versatility of the T6SS:

372   not only is the same system sometimes able to deliver dedicated anti-eukaryotic and anti-bacterial

373   effectors (e.g. the ACD-containing VgrG-1 and peptidoglycan hydrolase-containing VgrG-3 proteins

374   delivered by the *V. cholerae* T6SS), but some effectors are able to act trans-kingdom, against both

375   eukaryotic and prokaryotic cells (Brooks *et al.*, 2013; Jiang *et al.*, 2014).

376   More recently, a number of other anti-host effectors have been reported. The T6SS of *Francisella*

377   *tularensis* is required for virulence and intracellular proliferation, via phagosomal escape into the

378   cytoplasm. Four effectors, PdpCD and OpiAB, delivered by this system and contributing to

379   intramacrophage growth have been identified. PdpC is the major determinant of phagosomal

380   escape, whilst OpiA can assist in this process through its PI(3)-kinase activity (Brodmann *et al.*, 2017;

381   Eshraghi *et al.*, 2016; Ledvina *et al.*, 2018). One of the first T6SS effectors identified, EvpP of

382   *Edwardsiella tarda*, was recently shown to prevent activation of the NLRP3 inflammasome by

383   inhibiting the Ca^{2+}-dependent MAPK-Jnk pathway, whilst TecA of *B. cenocepacia* was shown to cause

384   activation of the Pyrin inflammasome via its Rho GTPase deaminase activity and the resulting

385   cytoskeleton disruption (**Figure 3**) (Aubert *et al.*, 2016; Chen *et al.*, 2017). Actin rearrangement is

386 also induced by a CNF1-like toxin delivered by *V. parahaemolyticus*, whilst a Tle4-family

387 phospholipase of *P. aeruginosa* causes disruption of the endoplasmic reticulum (Jiang *et al.*, 2016;

388 Ray *et al.*, 2017). In addition, a catalase effector from EHEC, KatN, was proposed to act against

389 reactive oxygen species within host cells (Wan *et al.*, 2017).

390 **The T6SS as a weapon against eukaryotic microbial competitors**

391 It is clear from the studies described in the previous section that the bacterial T6SS is able to deploy

392 effector proteins against eukaryotic cells, and it is also well-appreciated that many polymicrobial

393 communities, including those relevant clinically, contain both bacteria and fungi (Peleg *et al.*, 2010).

394 Therefore it is perhaps not surprising that the versatility of the T6SS extends to its use against fungal

395 cells. Early indications that this could be the case came from observations of T6SS-dependent

396 inhibition of the yeast *Cryptococcus carnescens* by the phytopathogen *P. syringae* and an increase in

397 T6SS gene expression in a biocontrol strain of *P. fluorescens* when colonising plant roots in the

398 presence of the fungal phytopathogen *Gaeumannomyces graminis* (Haapalainen *et al.*, 2012; Marchi

399 *et al.*, 2013). Recently, the T6SS of *S. marcescens*, previously believed to be exclusively an anti-

400 bacterial T6SS, was shown to possess anti-fungal activity against *Sacchromyces cerevisiae* and

401 *Candida* spp., and it was by studying this system that the first T6SS-delivered anti-fungal effector

402 proteins were identified. These effectors, Tfe1 and Tfe2, have distinct actions against target fungal

403 cells, ultimately leading to fungal cell death. Tfe1-mediated intoxication leads to plasma membrane

404 depolarisation without the formation of aspecific pores, whilst Tfe2 intoxication disrupts nutrient

405 uptake and amino acid metabolism and leads to the induction of autophagy, probably as a starvation

406 reponse (**Figure 3**) (Trunk *et al.*, 2018). Tfe1 and Tfe2 represent new classes of effector proteins,

407 with no obvious similarity to other effectors or proteins of known function, and their precise mode

408 of action remains to be elucidated. It is likely that T6SS-dependent anti-fungal activity is widespread.

409 Not only can homologues of Tfe1 and Tfe2 be detected in other T6SS-wielding bacteria, but typical

410 T6SS effector identification criteria are likely to miss anti-fungal effectors. These criteria include (1)

411 association with T6SS genes, (2) anti-bacterial activity and adjacently-encoded immunity protein,

412 and/or (3) the presence of known toxin or effector domains, all of which would have excluded Tfe1

413 and Tfe2. This highlights the importance of unbiased approaches such as secretomics for the

414 identification of new effectors. The discovery of T6SS-dependent anti-fungal activity suggests that

415 the contribution of the T6SS to shaping polymicrobial communities is broader and more important

416 than previously appreciated. Extending this idea further, there is also evidence that the T6SS can

417 also act against other single-celled eukaryotes, namely amoebae. Indeed the T6SS was first identified

418 through the requirement for an intact T6SS for *V. cholerae* to resist predation by *Dictyostelium*

13

419   *discoideum* (Pukatzki et al., 2006). Further work revealed that the pore-forming effector VasX and

420   the actin crosslinking effector VgrG-1 are required for virulence against *Dictyostelium*, in addition to

421   having anti-bacterial activity or anti-host activity, respectively (Miyata *et al.*, 2011; Zheng *et al.*,

422   2011). Thus the *V. cholerae* T6SS can be utilised against bacterial competitors in the host or

423   environment, against amoebal predators in the environment, and against the host directly,

424   representing a truly versatile and multi-purpose weapon.

425   **A contact-independent role for T6SS in metal uptake**

426   A further role for the T6SS, this time not requiring effector delivery into target cells, has also been

427   described. In this case, the T6SS is responsible for secretion of effectors to the extracellular milieu

428   which allow the bacteria to take up particular metal ions. In *Yersinia pseudotuberculosis*, T6SS-4

429   secretes a Zn^{2+}-binding protein, YezP, under conditions of oxidative stress. Secreted YezP is proposed

430   to scavenge Zn^{2+} ions and thus contribute to survival in the presence of reactive oxygen species

431   produced by the host (Wang *et al.*, 2015). Similarly, *B. thailandensis* T6SS-4 secretes a related Zn^{2+}-

432   binding effector, TseZ, which allows Zn^{2+} uptake under conditions of oxidative stress via interaction

433   of extracellular TseZ with the TonB-dependent outer membrane haem receptor HmuR (Si *et al.*,

434   2017a). The *B. thailandensis* T6SS-4 is also responsible for the secretion of another metallophore

435   effector, TseM. TseM is required for manganese uptake under conditions of oxidative stress and

436   interacts with MnoT, another TonB-dependent outer membrane receptor, to achieve transport of

437   Mn^{2+} into the cell (**Figure 3**) (Si *et al.*, 2017b). Importantly, it was demonstrated that possession of

438   TseZ/HmuR or TseM/MnoT, and T6SS-4, by *B. thailandensis* provides a competitive advantage

439   against other bacteria during co-culture in conditions where the respective metal ion is limiting but

440   cells are not in contact. Thus T6SS-dependent secretion of metallophores to the media allows the

441   producing organism to effectively scavenge scarce metal ions from the environment and thus

442   outcompete bacterial competitors without directly harming them. Both metal uptake systems were

443   also required for full virulence in a *Galleria* model, indicating an important role in metal acquisition

444   within a host environment (Si *et al.*, 2017a; Si *et al.*, 2017b). It is not yet clear whether T6SS-4 in *B.*

445   *thailandensis* and *Y. pseudotuberculosis*, and related systems in other organisms, are also used to

446   deliver toxic effector proteins into target cells, or whether they are used exclusively for

447   metallophore secretion; certainly their transcriptional regulation, responsive to oxidative stress and

448   metal limitation, appears to be tailored for a metal scavenging function.

449   A distinct role for a T6SS effector in metal uptake has been reported for the *P. aeruginosa* H3-T6SS-

450   dependent effector TseF. Extracellular TseF binds $PQS-Fe^{2+}$ complexes incorporated within outer

451   membrane vesicles (OMVs). (PQS, Pseudomonas Quinolone Signal, is a quorum sensing signalling

452 molecule with iron chelating properties.) TseF also interacts with the outer membrane siderophore

453 receptor FptA and porin OprF and is proposed to mediate Fe2+ uptake by delivering OMV-associated

454 PQS-Fe^{2+} to these receptors for transport into the cell (Lin *et al.*, 2017). It is currently unclear why

455 TseF or the metallophores above would be secreted by the T6SS, a system normally considered to be

456 designed for effector translocation into cells. Nevertheless, these effectors once again highlight the

457 breadth and versatility of T6SS function, as well as raising the possibility that other proteins that

458 function extracellularly may utilise the T6SS for their secretion.

459 **Concluding Remarks**

460 It is now clear that the bacterial T6SS is used for an impressively broad range of functions, all linked

461 by the purpose of increasing the competitive fitness of the secreting cell. While anti-host T6SSs allow

462 the bacterium to compete with host defence mechanisms, anti-bacterial and anti-fungal T6SSs allow

463 the bacterium to compete with rival microbes, both closely and distantly related. Furthermore,

464 those T6SSs and effectors used for contact-independent metal scavenging allow the secreting

465 bacterium to compete with both host defences and co-resident microbes. This functional versatility

466 is permitted by an ever-growing repertoire of diverse effector proteins, and the ability to hook them

467 up to the delivery device in many different ways. It is likely that the portfolio of known effector

468 proteins will continue to increase rapidly, particularly given the evolutionary pressures of inter-

469 bacterial competition and the recent realisation that effectors may also have non-toxic extracellular

470 roles. The extent of T6SS-related contributions to myriad bacterial interactions has further expanded

471 by the validated inclusion of T6SS[ii] (*Francisella*) and T6SS[iii] (*Bacteriodetes*) alongside the canonical

472 T6SS[i] systems, with an even more distantly-related T6SS-like system ('T6SS[iv]') recently described

473 (Bock *et al.*, 2017). Importantly, recent work has not only revealed new molecular and atomic-level

474 details of the mechanism of this intriguing machinery, but it has also begun to demonstrate the

475 importance of T6SS-mediated cell-cell interactions in a variety of 'real-life', or at least relevant

476 model, communities. It is likely that the future will reveal many more examples of communities

477 whose composition, dynamics and proprties will be shaped by T6SSs of several flavours. Finally, it is

478 exciting to note that T6SS could provide several potential opportunities for the development of new

479 antimicrobial strategies. It might be possible to inhibit the T6SS itself to reduce the fitness of a T6SS-

480 wielding pathogen, although the broad distribution of the system in other bacteria including

481 commensals would require consideration of specificity. Another avenue, although with similar issues

482 when considering complex communities, could be generating engineered 'biocontrol' strains to

483 target pathogens of concern. Finally, by studying T6SS effectors and the impact that they have on

484 target cells, we may learn more about the basic physiology of host, bacterial or fungal cells and how

485   to effectively inhibit them. From both a fundamental and translational point of view, exciting times

486   lie ahead regarding this versatile, widespread and effective bacterial nanoweapon.

487   **Author statement**

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491   **References**

492   **Alcoforado Diniz, J. & Coulthurst, S. J. (2015).** Intraspecies Competition in *Serratia marcescens* Is
493   Mediated by Type VI-Secreted Rhs Effectors and a Conserved Effector-Associated Accessory Protein.
494   *J Bacteriol* **197**, 2350-2360.
495
496   **Alcoforado Diniz, J., Liu, Y. C. & Coulthurst, S. J. (2015).** Molecular weaponry: diverse effectors
497   delivered by the Type VI secretion system. *Cell Microbiol* **17**, 1742-1751.
498
499   **Alteri, C. J., Himpsl, S. D., Pickens, S. R., Lindner, J. R., Zora, J. S., Miller, J. E., Arno, P. D., Straight,**
500   **S. W. & Mobley, H. L. (2013).** Multicellular Bacteria Deploy the Type VI Secretion System to
501   Preemptively Strike Neighboring Cells. *PLoS Pathogens* **9**, e1003608.
502
503   **Anderson, M. C., Vonaesch, P., Saffarian, A., Marteyn, B. S. & Sansonetti, P. J. (2017).** *Shigella*
504   *sonnei* Encodes a Functional T6SS Used for Interbacterial Competition and Niche Occupancy. *Cell*
505   *Host Microbe* **21**, 769-776 e763.
506
507   **Aschtgen, M. S., Gavioli, M., Dessen, A., Lloubes, R. & Cascales, E. (2010).** The SciZ protein anchors
508   the enteroaggregative *Escherichia coli* Type VI secretion system to the cell wall. *Mol Microbiol* **75**,
509   886-899.
510
511   **Aubert, D. F., Xu, H., Yang, J. & other authors (2016).** A *Burkholderia* Type VI Effector Deamidates
512   Rho GTPases to Activate the Pyrin Inflammasome and Trigger Inflammation. *Cell Host Microbe* **19**,
513   664-674.
514
515   **Barret, M., Egan, F., Fargier, E., Morrissey, J. P. & O'Gara, F. (2011).** Genomic analysis of the type VI
516   secretion systems in *Pseudomonas* spp.: novel clusters and putative effectors uncovered.
517   *Microbiology* **157**, 1726-1739.
518
519   **Basler, M., Pilhofer, M., Henderson, G. P., Jensen, G. J. & Mekalanos, J. J. (2012).** Type VI secretion
520   requires a dynamic contractile phage tail-like structure. *Nature* **483**, 182-186.
521
522   **Basler, M., Ho, B. T. & Mekalanos, J. J. (2013).** Tit-for-tat: type VI secretion system counterattack
523   during bacterial cell-cell interactions. *Cell* **152**, 884-894.
524
525   **Basler, M. (2015).** Type VI secretion system: secretion by a contractile nanomachine. *Philos Trans R*
526   *Soc Lond B Biol Sci* **370**, 1679.
527
528   **Batot, G., Michalska, K., Ekberg, G. & other authors (2017).** The CDI toxin of *Yersinia kristensenii* is a
529   novel bacterial member of the RNase A superfamily. *Nucleic Acids Res* **45**, 5013-5025.
530
531   **Bernal, P., Allsopp, L. P., Filloux, A. & Llamas, M. A. (2017).** The *Pseudomonas putida* T6SS is a plant
532   warden against phytopathogens. *The ISME Journal* **11**, 972-987.
533
534   **Bernal, P., Llamas, M. A. & Filloux, A. (2018).** Type VI secretion systems in plant-associated bacteria.
535   *Environ Microbiol* **20**, 1-15.
536
537   **Bingle, L. E., Bailey, C. M. & Pallen, M. J. (2008).** Type VI secretion: a beginner's guide. *Curr Opin*
538   *Microbiol* **11**, 3-8.
539

540   **Bock, D., Medeiros, J. M., Tsao, H. F., Penz, T., Weiss, G. L., Aistleitner, K., Horn, M. & Pilhofer, M.**
541   **(2017).** In situ architecture, function, and evolution of a contractile injection system. *Science* **357**,
542   713-717.
543
544   **Borgeaud, S., Metzger, L. C., Scrignari, T. & Blokesch, M. (2015).** Bacterial evolution. The type VI
545   secretion system of *Vibrio cholerae* fosters horizontal gene transfer. *Science* **347**, 63-67.
546
547   **Boyer, F., Fichant, G., Berthod, J., Vandenbrouck, Y. & Attree, I. (2009).** Dissecting the bacterial type
548   VI secretion system by a genome wide in silico analysis: what can be learned from available microbial
549   genomic resources? *BMC Genomics* **10**, 104.
550
551   **Brackmann, M., Nazarov, S., Wang, J. & Basler, M. (2017).** Using Force to Punch Holes: Mechanics
552   of Contractile Nanomachines. *Trends in Cell Biology* **27**, 623-632.
553
554   **Brodmann, M., Dreier, R. F., Broz, P. & Basler, M. (2017).** *Francisella* requires dynamic type VI
555   secretion system and ClpB to deliver effectors for phagosomal escape. *Nature Communications* **8**,
556   15853.
557
558   **Brooks, T. M., Unterweger, D., Bachmann, V., Kostiuk, B. & Pukatzki, S. (2013).** Lytic activity of the
559   *Vibrio cholerae* type VI secretion toxin VgrG-3 is inhibited by the antitoxin TsaB. *The Journal of*
560   *Biological Chemistry* **288**, 7618-7625.
561
562   **Burkinshaw, B. J., Liang, X., Wong, M., Le, A. N. H., Lam, L. & Dong, T. G. (2018).** A type VI secretion
563   system effector delivery mechanism dependent on PAAR and a chaperone-co-chaperone complex.
564   *Nature Microbiology* **3**, 632-640.
565
566   **Chatzidaki-Livanis, M., Geva-Zatorsky, N. & Comstock, L. E. (2016).** *Bacteroides fragilis* type VI
567   secretion systems use novel effector and immunity proteins to antagonize human gut *Bacteroidales*
568   species. *Proc Natl Acad Sci U S A* **113**, 3627-3632.
569
570   **Chen, H., Yang, D., Han, F., Tan, J., Zhang, L., Xiao, J., Zhang, Y. & Liu, Q. (2017).** The Bacterial T6SS
571   Effector EvpP Prevents NLRP3 Inflammasome Activation by Inhibiting the Ca(2+)-Dependent MAPK-
572   Jnk Pathway. *Cell Host Microbe* **21**, 47-58.
573
574   **Cherrak, Y., Rapisarda, C., Pellarin, R. & other authors (2018).** Biogenesis and structure of a type VI
575   secretion baseplate. *Nature Microbiology* **3**, 1404-1416.
576
577   **Cianfanelli, F. R., Alcoforado Diniz, J., Guo, M., De Cesare, V., Trost, M. & Coulthurst, S. J. (2016a).**
578   VgrG and PAAR Proteins Define Distinct Versions of a Functional Type VI Secretion System. *PLoS*
579   *Pathogens* **12**, e1005735.
580
581   **Cianfanelli, F. R., Monlezun, L. & Coulthurst, S. J. (2016b).** Aim, Load, Fire: The Type VI Secretion
582   System, a Bacterial Nanoweapon. *Trends Microbiol* **24**, 51-62.
583
584   **Clemens, D. L., Ge, P., Lee, B. Y., Horwitz, M. A. & Zhou, Z. H. (2015).** Atomic structure of T6SS
585   reveals interlaced array essential to function. *Cell* **160**, 940-951.
586
587   **Clemens, D. L., Lee, B. Y. & Horwitz, M. A. (2018).** The *Francisella* Type VI Secretion System.
588   *Frontiers in Cellular and Infection Microbiology* **8**, 121.
589

590   **Cooper, R. M., Tsimring, L. & Hasty, J. (2017).** Inter-species population dynamics enhance microbial
591   horizontal gene transfer and spread of antibiotic resistance. *eLife* **6**.

593   **Coyne, M. J., Roelofs, K. G. & Comstock, L. E. (2016).** Type VI secretion systems of human gut
594   *Bacteroidales* segregate into three genetic architectures, two of which are contained on mobile
595   genetic elements. *BMC Genomics* **17**, 58.

597   **Dix, S. R., Owen, H. J., Sun, R. & other authors (2018).** Structural insights into the function of type VI
598   secretion system TssA subunits. *Nature Communications* **9**, 4765.

600   **Durand, E., Cambillau, C., Cascales, E. & Journet, L. (2014).** VgrG, Tae, Tle, and beyond: the versatile
601   arsenal of Type VI secretion effectors. *Trends Microbiol* **22**, 498-507.

603   **Durand, E., Nguyen, V. S., Zoued, A. & other authors (2015).** Biogenesis and structure of a type VI
604   secretion membrane core complex. *Nature* **523**, 555-560.

606   **Eshraghi, A., Kim, J., Walls, A. C. & other authors (2016).** Secreted Effectors Encoded within and
607   outside of the Francisella Pathogenicity Island Promote Intramacrophage Growth. *Cell Host Microbe*
608   **20**, 573-583.

610   **Fast, D., Kostiuk, B., Foley, E. & Pukatzki, S. (2018).** Commensal pathogen competition impacts host
611   viability. *Proc Natl Acad Sci U S A* **115**, 7099-7104.

613   **Fitzsimons, T. C., Lewis, J. M., Wright, A., Kleifeld, O., Schittenhelm, R. B., Powell, D., Harper, M. &
614   Boyce, J. D. (2018).** Identification of Novel *Acinetobacter baumannii* Type VI Secretion System
615   Antibacterial Effector and Immunity Pairs. *Infect Immun* **86**.

617   **Flaugnatti, N., Le, T. T., Canaan, S. & other authors (2016).** A phospholipase A1 antibacterial Type VI
618   secretion effector interacts directly with the C-terminal domain of the VgrG spike protein for
619   delivery. *Mol Microbiol* **99**, 1099-1118.

621   **Forster, A., Planamente, S., Manoli, E., Lossi, N. S., Freemont, P. S. & Filloux, A. (2014).** Coevolution
622   of the ATPase ClpV, the sheath proteins TssB and TssC, and the accessory protein TagJ/HsiE1
623   distinguishes type VI secretion classes. *J Biol Chem* **289**, 33032-33043.

625   **Fu, Y., Ho, B. T. & Mekalanos, J. J. (2018).** Tracking *Vibrio cholerae* Cell-Cell Interactions during
626   Infection Reveals Bacterial Population Dynamics within Intestinal Microenvironments. *Cell Host
627   Microbe* **23**, 274-281 e272.

629   **Gong, Y., Zhang, Z., Liu, Y., Zhou, X. W., Anwar, M. N., Li, Z. S., Hu, W. & Li, Y. Z. (2018).** A nuclease-
630   toxin and immunity system for kin discrimination in *Myxococcus xanthus*. *Environ Microbiol* **20**,
631   2552-2567.

633   **Haapalainen, M., Mosorin, H., Dorati, F. & other authors (2012).** Hcp2, a Secreted Protein of the
634   Phytopathogen *Pseudomonas syringae* pv. Tomato DC3000, Is Required for Fitness for Competition
635   against Bacteria and Yeasts. *Journal of Bacteriology* **194**, 4810-4822.

637   **Hachani, A., Allsopp, L. P., Oduko, Y. & Filloux, A. (2014).** The VgrG proteins are "a la carte" delivery
638   systems for bacterial type VI effectors. *J Biol Chem* **289**, 17872-17884.

640 **Hachani, A., Wood, T. E. & Filloux, A. (2016).** Type VI secretion and anti-host effectors. *Curr Opin*
641 *Microbiol* **29**, 81-93.
642

643 **Hecht, A. L., Casterline, B. W., Earley, Z. M., Goo, Y. A., Goodlett, D. R. & Bubeck Wardenburg, J.**
644 **(2016).** Strain competition restricts colonization of an enteric pathogen and prevents colitis. *EMBO*
645 *Rep* **17**, 1281-1291.
646

647 **Hood, R. D., Singh, P., Hsu, F. & other authors (2010).** A type VI secretion system *of Pseudomonas*
648 *aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* **7**, 25-37.
649

650 **Jiang, F., Waterfield, N. R., Yang, J., Yang, G. & Jin, Q. (2014).** A *Pseudomonas aeruginosa* type VI
651 secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. *Cell Host Microbe*
652 **15**, 600-610.
653

654 **Jiang, F., Wang, X., Wang, B., Chen, L., Zhao, Z., Waterfield, N. R., Yang, G. & Jin, Q. (2016).** The
655 *Pseudomonas aeruginosa* Type VI Secretion PGAP1-like Effector Induces Host Autophagy by
656 Activating Endoplasmic Reticulum Stress. *Cell Rep* **16**, 1502-1509.
657

658 **Kirchberger, P. C., Unterweger, D., Provenzano, D., Pukatzki, S. & Boucher, Y. (2017).** Sequential
659 displacement of Type VI Secretion System effector genes leads to evolution of diverse immunity
660 gene arrays in *Vibrio cholerae*. *Scientific reports* **7**, 45133.
661

662 **Koskiniemi, S., Lamoureux, J. G., Nikolakakis, K. C., t'Kint de Roodenbeke, C., Kaplan, M. D., Low,**
663 **D. A. & Hayes, C. S. (2013).** Rhs proteins from diverse bacteria mediate intercellular competition.
664 *Proc Natl Acad Sci U S A* **110**, 7032-7037.
665

666 **Koskiniemi, S., Garza-Sanchez, F., Sandegren, L., Webb, J. S., Braaten, B. A., Poole, S. J., Andersson,**
667 **D. I., Hayes, C. S. & Low, D. A. (2014).** Selection of orphan Rhs toxin expression in evolved
668 *Salmonella enterica* serovar Typhimurium. *PLoS Genetics* **10**, e1004255.
669

670 **Kudryashev, M., Wang, R. Y., Brackmann, M. & other authors (2015).** Structure of the type VI
671 secretion system contractile sheath. *Cell* **160**, 952-962.
672

673 **LaCourse, K. D., Peterson, S. B., Kulasekara, H. D., Radey, M. C., Kim, J. & Mougous, J. D. (2018).**
674 Conditional toxicity and synergy drive diversity among antibacterial effectors. *Nature Microbiology* **3**,
675 440-446.
676

677 **Ledvina, H. E., Kelly, K. A., Eshraghi, A. & other authors (2018).** A Phosphatidylinositol 3-Kinase
678 Effector Alters Phagosomal Maturation to Promote Intracellular Growth of *Francisella*. *Cell Host*
679 *Microbe* **24**, 285-295 e288.
680

681 **LeRoux, M., Kirkpatrick, R. L., Montauti, E. I. & other authors (2015).** Kin cell lysis is a danger signal
682 that activates antibacterial pathways of *Pseudomonas aeruginosa*. *eLife* **4**.
683

684 **Lin, J., Zhang, W., Cheng, J. & other authors (2017).** A Pseudomonas T6SS effector recruits PQS-
685 containing outer membrane vesicles for iron acquisition. *Nature Communications* **8**, 14888.
686

687 **Lin, J. S., Wu, H. H., Hsu, P. H., Ma, L. S., Pang, Y. Y., Tsai, M. D. & Lai, E. M. (2014).** Fha interaction
688 with phosphothreonine of TssL activates type VI secretion in *Agrobacterium tumefaciens*. *PLoS*
689 *Pathogens* **10**, e1003991.
690

691   **Logan, S. L., Thomas, J., Yan, J., Baker, R. P., Shields, D. S., Xavier, J. B., Hammer, B. K. &**
692   **Parthasarathy, R. (2018).** The *Vibrio cholerae* type VI secretion system can modulate host intestinal
693   mechanics to displace gut bacterial symbionts. *Proc Natl Acad Sci U S A* **115**, E3779-E3787.
694
695   **Ma, J., Pan, Z., Huang, J., Sun, M., Lu, C. & Yao, H. (2017a).** The Hcp proteins fused with diverse
696   extended-toxin domains represent a novel pattern of antibacterial effectors in type VI secretion
697   systems. *Virulence* **8**, 1189-1202.
698
699   **Ma, J., Sun, M., Dong, W., Pan, Z., Lu, C. & Yao, H. (2017b).** PAAR-Rhs proteins harbor various C-
700   terminal toxins to diversify the antibacterial pathways of type VI secretion systems. *Environ*
701   *Microbiol* **19**, 345-360.
702
703   **Ma, J., Sun, M., Pan, Z., Lu, C. & Yao, H. (2018).** Diverse toxic effectors are harbored by vgrG islands
704   for interbacterial antagonism in type VI secretion system. *Biochimica et Biophysica Acta* **1862**, 1635-
705   1643.
706
707   **Ma, L. S., Hachani, A., Lin, J. S., Filloux, A. & Lai, E. M. (2014).** *Agrobacterium tumefaciens* deploys a
708   superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta.
709   *Cell Host Microbe* **16**, 94-104.
710
711   **MacIntyre, D. L., Miyata, S. T., Kitaoka, M. & Pukatzki, S. (2010).** The *Vibrio cholerae* type VI
712   secretion system displays antimicrobial properties. *Proc Natl Acad Sci U S A* **107**, 19520-19524.
713
714   **Majerczyk, C., Schneider, E. & Greenberg, E. P. (2016).** Quorum sensing control of Type VI secretion
715   factors restricts the proliferation of quorum-sensing mutants. *eLife* **5**.
716
717   **Marchi, M., Boutin, M., Gazengel, K. & other authors (2013).** Genomic analysis of the biocontrol
718   strain *Pseudomonas fluorescens* Pf29Arp with evidence of T3SS and T6SS gene expression on plant
719   roots. *Environmental microbiology reports* **5**, 393-403.
720
721   **Mariano, G., Monlezun, L. & Coulthurst, S. J. (2018).** Dual Role for DsbA in Attacking and Targeted
722   Bacterial Cells during Type VI Secretion System-Mediated Competition. *Cell Rep* **22**, 774-785.
723
724   **McNally, L., Bernardy, E., Thomas, J., Kalziqi, A., Pentz, J., Brown, S. P., Hammer, B. K., Yunker, P. J.**
725   **& Ratcliff, W. C. (2017).** Killing by Type VI secretion drives genetic phase separation and correlates
726   with increased cooperation. *Nature Communications* **8**, 14371.
727
728   **Miyata, S. T., Kitaoka, M., Brooks, T. M., McAuley, S. B. & Pukatzki, S. (2011).** *Vibrio cholerae*
729   requires the type VI secretion system virulence factor VasX to kill *Dictyostelium discoideum*. *Infection*
730   *and Immunity* **79**, 2941-2949.
731
732   **Miyata, S. T., Unterweger, D., Rudko, S. P. & Pukatzki, S. (2013).** Dual expression profile of type VI
733   secretion system immunity genes protects pandemic Vibrio cholerae. *PLoS Pathogens* **9**, e1003752.
734
735   **Mougous, J. D., Cuff, M. E., Raunser, S. & other authors (2006).** A virulence locus of *Pseudomonas*
736   *aeruginosa* encodes a protein secretion apparatus. *Science* **312**, 1526-1530.
737
738   **Nazarov, S., Schneider, J. P., Brackmann, M., Goldie, K. N., Stahlberg, H. & Basler, M. (2018).** Cryo-
739   EM reconstruction of Type VI secretion system baseplate and sheath distal end. *EMBO J* **37**.
740

741   **Nguyen, V. S., Douzi, B., Durand, E., Roussel, A., Cascales, E. & Cambillau, C. (2018).** Towards a
742   complete structural deciphering of Type VI secretion system. *Curr Opin Structural Biology* **49**, 77-84.
743

744   **Ostrowski, A., Cianfanelli, F. R., Porter, M., Mariano, G., Peltier, J., Wong, J. J., Swedlow, J. R.,**
745   **Trost, M. & Coulthurst, S. J. (2018).** Killing with proficiency: Integrated post-translational regulation
746   of an offensive Type VI secretion system. *PLoS Pathogens* **14**, e1007230.
747

748   **Peleg, A. Y., Hogan, D. A. & Mylonakis, E. (2010).** Medically important bacterial-fungal interactions.
749   *Nat Rev Microbiol* **8**, 340-349.
750

751   **Poole, S. J., Diner, E. J., Aoki, S. K., Braaten, B. A., t'Kint de Roodenbeke, C., Low, D. A. & Hayes, C.**
752   **S. (2011).** Identification of functional toxin/immunity genes linked to contact-dependent growth
753   inhibition (CDI) and rearrangement hotspot (Rhs) systems. *PLoS Genetics* **7**, e1002217.
754

755   **Pukatzki, S., Ma, A. T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W. C., Heidelberg, J. F. &**
756   **Mekalanos, J. J. (2006).** Identification of a conserved bacterial protein secretion system in *Vibrio*
757   *cholerae* using the *Dictyostelium* host model system. *Proc Natl Acad Sci U S A* **103**, 1528-1533.
758

759   **Pukatzki, S., Ma, A. T., Revel, A. T., Sturtevant, D. & Mekalanos, J. J. (2007).** Type VI secretion
760   system translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proc*
761   *Natl Acad Sci U S A* **104**, 15508-15513.
762

763   **Quentin, D., Ahmad, S., Shanthamoorthy, P., Mougous, J. D., Whitney, J. C. & Raunser, S. (2018).**
764   Mechanism of loading and translocation of type VI secretion system effector Tse6. *Nature*
765   *Microbiology* **3**, 1142-1152.
766

767   **Ray, A., Schwartz, N., de Souza Santos, M., Zhang, J., Orth, K. & Salomon, D. (2017).** Type VI
768   secretion system MIX-effectors carry both antibacterial and anti-eukaryotic activities. *EMBO Rep* **18**,
769   1978-1990.
770

771   **Renault, M. G., Zamarreno Beas, J., Douzi, B., Chabalier, M., Zoued, A., Brunet, Y. R., Cambillau, C.,**
772   **Journet, L. & Cascales, E. (2018).** The gp27-like Hub of VgrG Serves as Adaptor to Promote Hcp Tube
773   Assembly. *J Mol Biol* **430**, 3143-3156.
774

775   **Ringel, P. D., Hu, D. & Basler, M. (2017).** The Role of Type VI Secretion System Effectors in Target
776   Cell Lysis and Subsequent Horizontal Gene Transfer. *Cell Rep* **21**, 3927-3940.
777

778   **Russell, A. B., Hood, R. D., Bui, N. K., LeRoux, M., Vollmer, W. & Mougous, J. D. (2011).** Type VI
779   secretion delivers bacteriolytic effectors to target cells. *Nature* **475**, 343-347.
780

781   **Russell, A. B., Singh, P., Brittnacher, M. & other authors (2012).** A Widespread Bacterial Type VI
782   Secretion Effector Superfamily Identified Using a Heuristic Approach. *Cell host & microbe* **11**, 538-
783   549.
784

785   **Russell, A. B., LeRoux, M., Hathazi, K., Agnello, D. M., Ishikawa, T., Wiggins, P. A., Wai, S. N. &**
786   **Mougous, J. D. (2013).** Diverse type VI secretion phospholipases are functionally plastic antibacterial
787   effectors. *Nature* **496**, 508-512.
788

789   **Russell, A. B., Wexler, A. G., Harding, B. N. & other authors (2014).** A type VI secretion-related
790   pathway in *Bacteroidetes* mediates interbacterial antagonism. *Cell Host Microbe* **16**, 227-236.
791

792 **Salomon, D., Kinch, L. N., Trudgian, D. C., Guo, X., Klimko, J. A., Grishin, N. V., Mirzaei, H. & Orth, K.**
793 **(2014).** Marker for type VI secretion system effectors. *Proc Natl Acad Sci U S A* **111**, 9271-9276.
794
795 **Sana, T. G., Flaugnatti, N., Lugo, K. A. & other authors (2016).** *Salmonella* Typhimurium utilizes a
796 T6SS-mediated antibacterial weapon to establish in the host gut. *Proc Natl Acad Sci U S A* **113**,
797 E5044-5051.
798
799 **Santin, Y. G. & Cascales, E. (2017).** Domestication of a housekeeping transglycosylase for assembly
800 of a Type VI secretion system. *EMBO Rep* **18**, 138-149.
801
802 **Santin, Y. G., Doan, T., Lebrun, R., Espinosa, L., Journet, L. & Cascales, E. (2018).** In vivo TssA
803 proximity labelling during type VI secretion biogenesis reveals TagA as a protein that stops and holds
804 the sheath. *Nature Microbiology* **3**, 1304-1313.
805
806 **Schell, M. A., Ulrich, R. L., Ribot, W. J. & other authors (2007).** Type VI secretion is a major virulence
807 determinant in *Burkholderia mallei*. *Mol Microbiol* **64**, 1466-1485.
808
809 **Schwarz, S., West, T. E., Boyer, F. & other authors (2010).** Burkholderia type VI secretion systems
810 have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathog* **6**.
811
812 **Shneider, M. M., Buth, S. A., Ho, B. T., Basler, M., Mekalanos, J. J. & Leiman, P. G. (2013).** PAAR-
813 repeat proteins sharpen and diversify the type VI secretion system spike. *Nature* **500**, 350-353.
814
815 **Si, M., Wang, Y., Zhang, B. & other authors (2017a).** The Type VI Secretion System Engages a Redox-
816 Regulated Dual-Functional Heme Transporter for Zinc Acquisition. *Cell Rep* **20**, 949-959.
817
818 **Si, M., Zhao, C., Burkinshaw, B., Zhang, B., Wei, D., Wang, Y., Dong, T. G. & Shen, X. (2017b).**
819 Manganese scavenging and oxidative stress response mediated by type VI secretion system in
820 *Burkholderia thailandensis*. *Proc Natl Acad Sci U S A* **114**, E2233-E2242.
821
822 **Silverman, J. M., Agnello, D. M., Zheng, H., Andrews, B. T., Li, M., Catalano, C. E., Gonen, T. &**
823 **Mougous, J. D. (2013).** Haemolysin Coregulated Protein Is an Exported Receptor and Chaperone of
824 Type VI Secretion Substrates. *Mol Cell* **51**, 584-593.
825
826 **Speare, L., Cecere, A. G., Guckes, K. R., Smith, S., Wollenberg, M. S., Mandel, M. J., Miyashiro, T. &**
827 **Septer, A. N. (2018).** Bacterial symbionts use a type VI secretion system to eliminate competitors in
828 their natural host. *Proc Natl Acad Sci U S A* **115**, E8528-E8537.
829
830 **Srikannathasan, V., English, G., Bui, N. K., Trunk, K., O'Rourke, P. E. F., Rao, V. A., Vollmer, W.,**
831 **Coulthurst, S. J. & Hunter, W. N. (2013).** Structural basis for Type VI secreted peptidoglycan DL-
832 endopeptidase function, specificity and neutralization in *Serratia marcescens*. *Acta Crystallogr D* **69**,
833 2468-2482.
834
835 **Steele, M. I., Kwong, W. K., Whiteley, M. & Moran, N. A. (2017).** Diversification of Type VI Secretion
836 System Toxins Reveals Ancient Antagonism among Bee Gut Microbes. *MBio* **8**.
837
838 **Tang, J. Y., Bullen, N. P., Ahmad, S. & Whitney, J. C. (2018).** Diverse NADase effector families
839 mediate interbacterial antagonism via the type VI secretion system. *J Biol Chem* **293**, 1504-1514.
840
841 **Thomas, J., Watve, S. S., Ratcliff, W. C. & Hammer, B. K. (2017).** Horizontal Gene Transfer of
842 Functional Type VI Killing Genes by Natural Transformation. *MBio* **8**.

843
844 **Ting, S. Y., Bosch, D. E., Mangiameli, S. M. & other authors (2018).** Bifunctional Immunity Proteins
845 Protect Bacteria against FtsZ-Targeting ADP-Ribosylating Toxins. *Cell* **175**, 1380-1392 e1314.
846
847 **Toska, J., Ho, B. T. & Mekalanos, J. J. (2018).** Exopolysaccharide protects *Vibrio cholerae* from
848 exogenous attacks by the type 6 secretion system. *Proc Natl Acad Sci U S A* **115**, 7997-8002.
849
850 **Troselj, V., Treuner-Lange, A., Sogaard-Andersen, L. & Wall, D. (2018).** Physiological Heterogeneity
851 Triggers Sibling Conflict Mediated by the Type VI Secretion System in an Aggregative Multicellular
852 Bacterium. *MBio* **9**.
853
854 **Trunk, K., Peltier, J., Liu, Y. C. & other authors (2018).** The type VI secretion system deploys
855 antifungal effectors against microbial competitors. *Nature Microbiology* **3**, 920-931.
856
857 **Unterweger, D., Kostiuk, B., Otjengerdes, R., Wilton, A., Diaz-Satizabal, L. & Pukatzki, S. (2015).**
858 Chimeric adaptor proteins translocate diverse type VI secretion system effectors in *Vibrio cholerae*.
859 *EMBO J* **34**, 2198-2210.
860
861 **Verster, A. J., Ross, B. D., Radey, M. C., Bao, Y., Goodman, A. L., Mougous, J. D. & Borenstein, E.**
862 **(2017).** The Landscape of Type VI Secretion across Human Gut Microbiomes Reveals Its Role in
863 Community Composition. *Cell Host Microbe* **22**, 411-419 e414.
864
865 **Vettiger, A. & Basler, M. (2016).** Type VI Secretion System Substrates Are Transferred and Reused
866 among Sister Cells. *Cell* **167**, 99-110 e112.
867
868 **Vettiger, A., Winter, J., Lin, L. & Basler, M. (2017).** The type VI secretion system sheath assembles at
869 the end distal from the membrane anchor. *Nature Communications* **8**, 16088.
870
871 **Wan, B., Zhang, Q., Ni, J. & other authors (2017).** Type VI secretion system contributes to
872 Enterohemorrhagic *Escherichia coli* virulence by secreting catalase against host reactive oxygen
873 species (ROS). *PLoS Pathogens* **13**, e1006246.
874
875 **Wang, J., Brackmann, M., Castano-Diez, D., Kudryashev, M., Goldie, K. N., Maier, T., Stahlberg, H.**
876 **& Basler, M. (2017).** Cryo-EM structure of the extended type VI secretion system sheath-tube
877 complex. *Nature Microbiology* **2**, 1507-1512.
878
879 **Wang, T., Si, M., Song, Y. & other authors (2015).** Type VI Secretion System Transports Zn2+ to
880 Combat Multiple Stresses and Host Immunity. *PLoS Pathogens* **11**, e1005020.
881
882 **Weber, B. S., Hennon, S. W., Wright, M. S., Scott, N. E., de Berardinis, V., Foster, L. J., Ayala, J. A.,**
883 **Adams, M. D. & Feldman, M. F. (2016).** Genetic Dissection of the Type VI Secretion System in
884 *Acinetobacter* and Identification of a Novel Peptidoglycan Hydrolase, TagX, Required for Its
885 Biogenesis. *MBio* **7**, e01253-16.
886
887 **Wenren, L. M., Sullivan, N. L., Cardarelli, L., Septer, A. N. & Gibbs, K. A. (2013).** Two independent
888 pathways for self-recognition in *Proteus mirabilis* are linked by type VI-dependent export. *MBio* **4**,
889 e00374-13.
890
891 **Wexler, A. G., Bao, Y., Whitney, J. C. & other authors (2016).** Human symbionts inject and
892 neutralize antibacterial toxins to persist in the gut. *Proc Natl Acad Sci U S A* **113**, 3639-3644.
893

894 **Whitney, J. C., Chou, S., Russell, A. B., Biboy, J., Gardiner, T. E., Ferrin, M. A., Brittnacher, M.,**
895 **Vollmer, W. & Mougous, J. D. (2013).** Identification, structure, and function of a novel type VI
896 secretion peptidoglycan glycoside hydrolase effector-immunity pair. *J Biol Chem* **288**, 26616-26624.
897
898 **Whitney, J. C., Beck, C. M., Goo, Y. A. & other authors (2014).** Genetically distinct pathways guide
899 effector export through the type VI secretion system. *Mol Microbiol* **92**, 529-542.
900
901 **Whitney, J. C., Quentin, D., Sawai, S. & other authors (2015).** An interbacterial NAD(P)$^+$
902 glycohydrolase toxin requires elongation factor Tu for delivery to target cells. *Cell* **163**, 607-619.
903
904 **Willett, J. L., Gucinski, G. C., Fatherree, J. P., Low, D. A. & Hayes, C. S. (2015).** Contact-dependent
905 growth inhibition toxins exploit multiple independent cell-entry pathways. *Proc Natl Acad Sci U S A*
906 **112**, 11341-11346.
907
908 **Zhang, D., de Souza, R. F., Anantharaman, V., Iyer, L. M. & Aravind, L. (2012).** Polymorphic toxin
909 systems: Comprehensive characterization of trafficking modes, processing, mechanisms of action,
910 immunity and ecology using comparative genomics. *Biology Direct* **7**, 18.
911
912 **Zhao, W., Caro, F., Robins, W. & Mekalanos, J. J. (2018).** Antagonism toward the intestinal
913 microbiota and its effect on *Vibrio cholerae* virulence. *Science* **359**, 210-213.
914
915 **Zheng, J., Ho, B. & Mekalanos, J. J. (2011).** Genetic Analysis of Anti-Amoebae and Anti-Bacterial
916 Activities of the Type VI Secretion System in *Vibrio cholerae*. *PloS One* **6**, e23876.
917
918 **Zoued, A., Durand, E., Brunet, Y. R. & other authors (2016).** Priming and polymerization of a
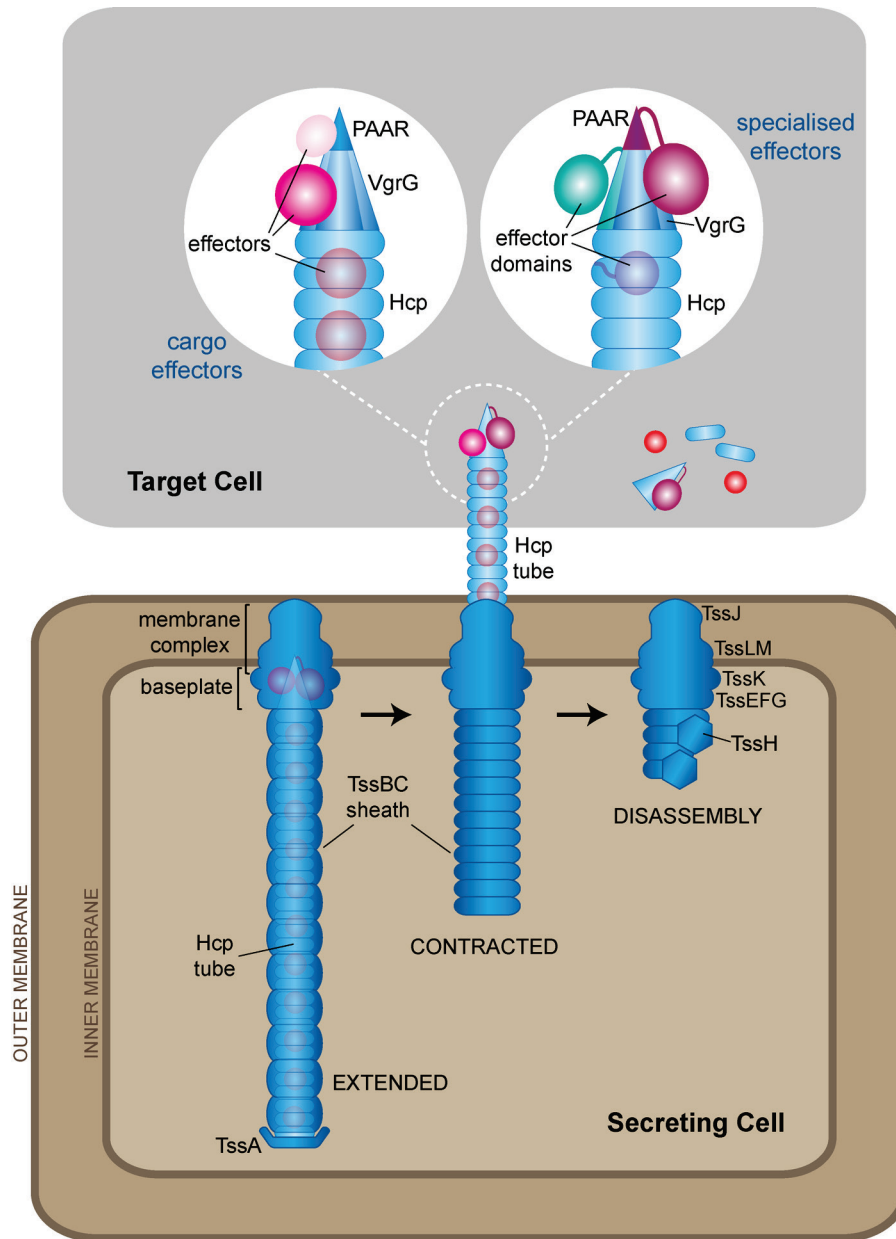919 bacterial contractile tail structure. *Nature* **531**, 59-63.
920
921

Figure 1. Effector delivery by the Type VI Secretion system of a Gram-negative bacterium.
Schematic illustration of the current model for the contraction-based 'firing' mechanism of the T6SS. The T6SS assembles with the contractile TssBC sheath in an 'extended' conformation. Contraction of this sheath, which is anchored to a cytoplasmic baseplate docked on an envelope-spanning membrane complex, drives the Hcp-VgrG-PAAR puncturing structure out of the cell. An adjacent target cell can also be breached by this tube-spike structure. Following contraction, TssH depolymerises the contracted sheath and the T6SS disassembles at least partially, ready for a new round of firing. The insets illustrate the different ways in which effectors can interact with the expelled Hcp-VgrG-PAAR puncturing structure in order to be translocated out of the secreting cell and into a target cell. 'Cargo' effectors non-covalently interact with Hcp, VgrG or PAAR proteins, whilst 'specialised' effectors consist of an effector domain covalently fused to a VgrG or Hcp protein or a protein containing a PAAR-repeat containing domain. For further details, see the text.
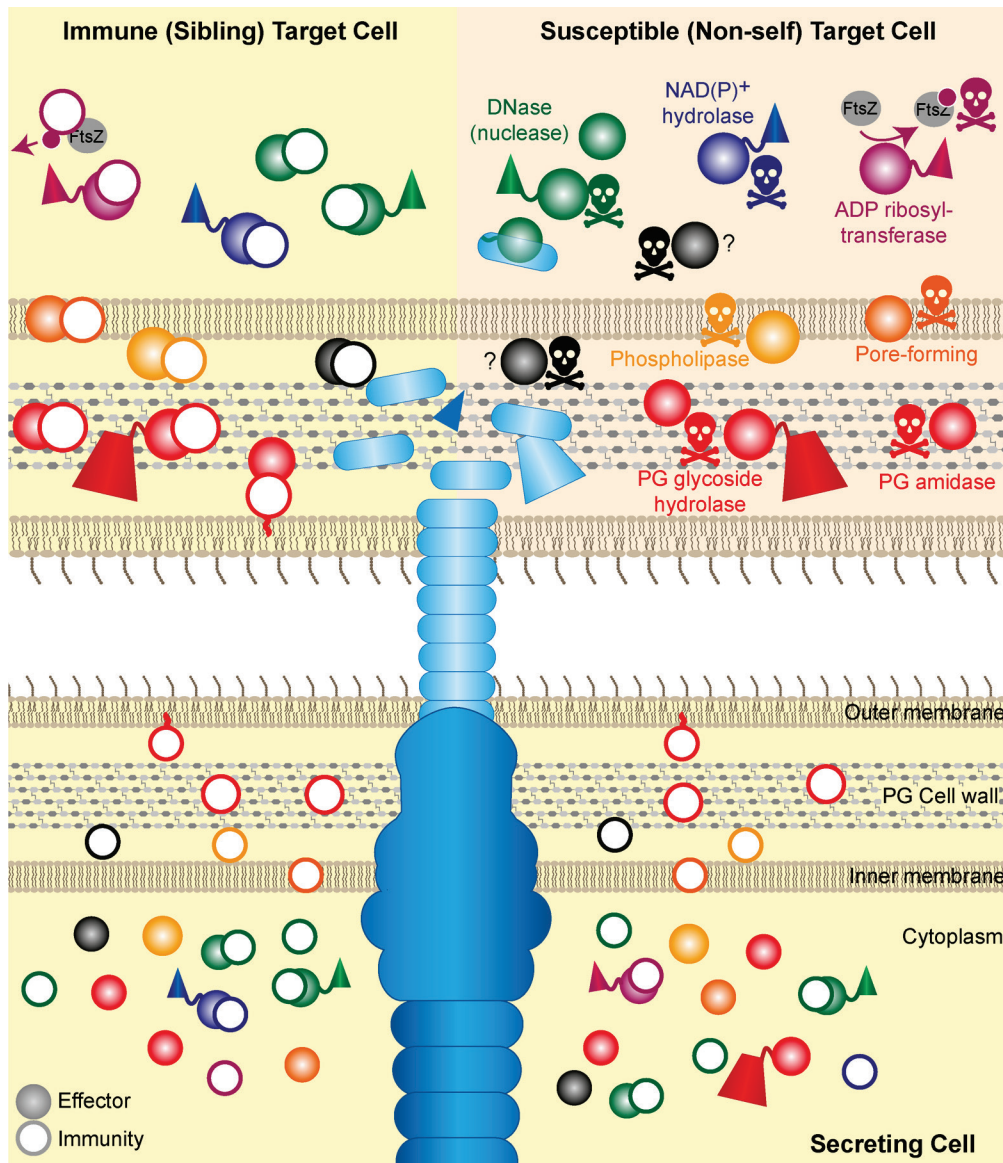
Figure 2. Anti-bacterial effectors and cognate immunity proteins.
Schematic illustration of modes of action of T6SS-delivered anti-bacterial effector proteins and their neutralisation by self-protecting immunity proteins. The T6SS can deliver a variety of toxic effector proteins into a target cell, which may act on different cellular targets including the peptidoglycan cell wall, cellular nucleic acids and the inner membrane. Immunity proteins specific for each individual effector are localised at the site of action of their cognate effector and typically neutralise the toxin by direct binding and physical inhibition. Immunity proteins protect the secreting cell from its own toxins prior to secretion, if they act in the cytoplasm and are not shielded by another structure such as an Rhs repeat domain (this possibility is not depicted here). Immunity proteins also protect genetically identical cells from the action of all the effectors delivered by their neighbouring sibling. Note that only one PAAR-containing protein and one VgrG trimer can be delivered in a single firing event, although several are included here for illustrative purposes. PG, peptidoglycan.
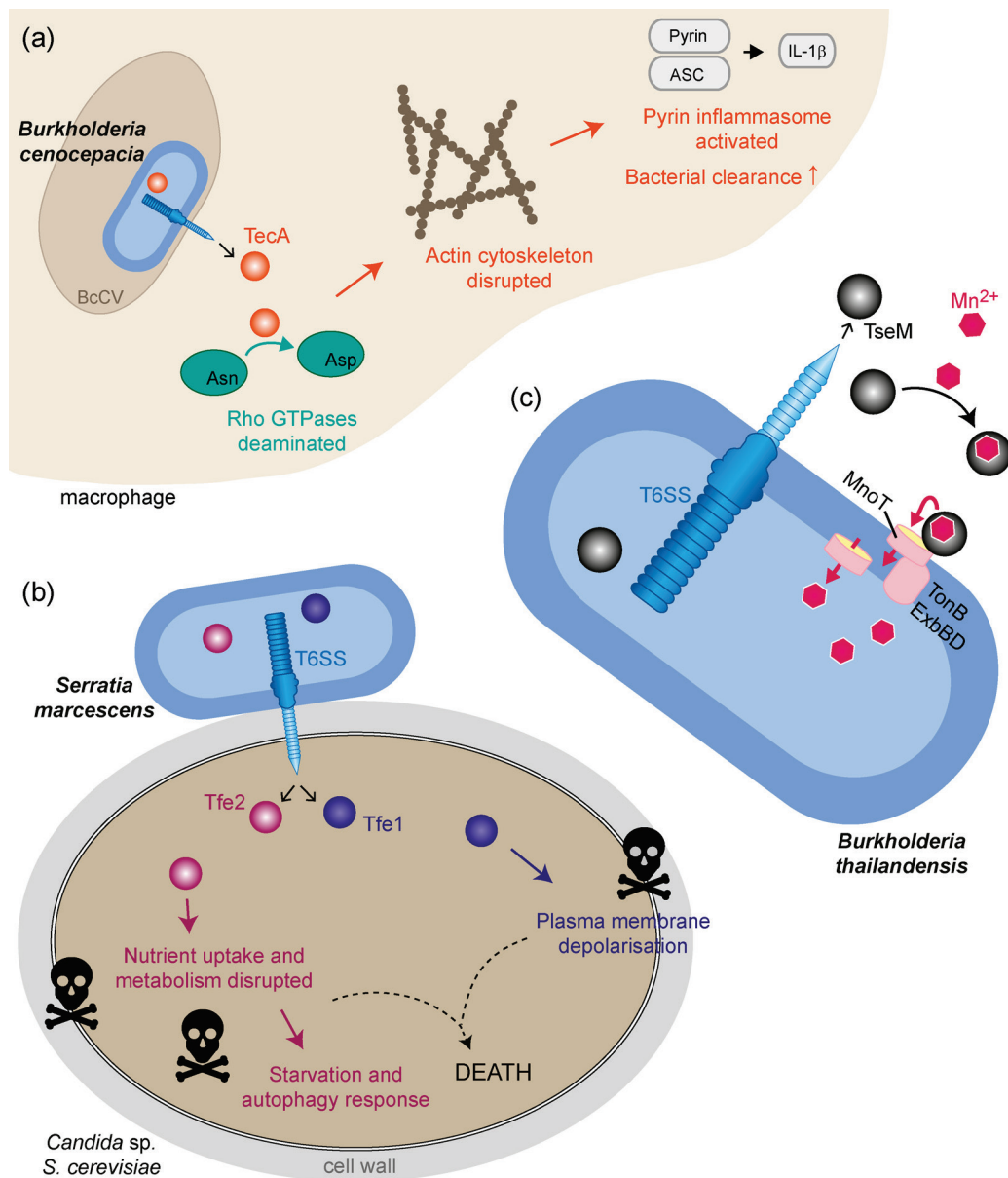
Figure 3. Examples of Type VI secreted effectors with roles distinct from anti-bacterial toxins. Schematic illustration of current models for the action of the anti-host effector TecA (a), anti-fungal effectors Tfe1 and Tfe2 (b), and metallophore effector TseM (c). (a) *B. cenocepacia* delivers TecA from within the B. cenocepacia containing vacuole (BcCV), causing deamidation of specific asparagine residues in Rho family GTPases RhoA and Rac1, leading to disruption of the actin cytoskeleton, activation of the pyrin inflammasome and pyroptosis and increasing bacterial clearance (Aubert *et al.*, 2016). (b) *S. marcescens* delivers Tfe1 and Tfe2 into fungal cells, including *C. albicans* and *S. cerevisiae*. The action of Tfe1 leads to depolarisation of the fungal plasma membrane without formation of large aspecific pores. The action of Tfe2 disrupts inter-related pathways involved in sulfate assimilation, plasma membrane nutrient transport and amino acid metabolism, leading to a starvation response including induction of autophagy. Intoxication by Tfe1 and Tfe2 can eventually cause cell death (Trunk *et al.*, 2018). (c) *B. thailandensis* uses its T6SS-4 to translocate TseM to the extracellular milieu, where it binds Mn^{2+}. TseM loaded with Mn^{2+} interacts with the outer membrane TonB-dependent receptor MnoT, which is associated with a TonB-ExbD-ExbB complex, transferring Mn^{2+} from TseM to MnoT and allowing its active import across the outer membrane. Either the SitABCD or MntH transporters may then be utilised to import Mn^{2+} across the inner membrane (Si *et al.*, 2017b).