



HAL
open science

The Type VI Secretion System in *Escherichia coli* and Related Species

Laure Journet, E. Cascales

► **To cite this version:**

Laure Journet, E. Cascales. The Type VI Secretion System in *Escherichia coli* and Related Species. *EcoSal Plus*, 2016, 7 (1), 10.1128/ecosalplus.ESP-0009-2015 . hal-01778564

HAL Id: hal-01778564

<https://amu.hal.science/hal-01778564>

Submitted on 25 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

The Type VI secretion system in *E. coli* and related species.

LAURE JOURNET AND ERIC CASCALES *

Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), Institut de Microbiologie de la Méditerranée (IMM), Centre National de la Recherche Scientifique (CNRS) – Aix-Marseille Université, UMR 7255, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France.

Running head : Type VI secretion

Correspondence: Eric Cascales: cascales@imm.cnrs.fr

Character count without legend to figures and references (including spaces): 42,000
Table: 1
Figures: 10

39 ABSTRACT

40
41 The Type VI secretion system (T6SS) is a multi-protein complex widespread in
42 *Proteobacteria* and dedicated to the delivery of toxins into both prokaryotic and eukaryotic
43 cells. It thus participates to inter-bacterial competition as well as pathogenesis. The T6SS is a
44 contractile weapon, related to the injection apparatus of contractile tailed bacteriophages.
45 Basically, it assembles an inner tube wrapped by a sheath-like structure and anchored to the
46 cell envelope *via* a membrane complex. The energy released by the contraction of the sheath
47 propels the inner tube through the membrane channel and towards the target cell. Although
48 the assembly and the mechanism of action are conserved across species, the repertoire of
49 secreted toxins, and the diversity of the regulatory mechanisms and of target cells make the
50 T6SS a highly versatile secretion system. The T6SS is particularly represented in *Escherichia*
51 *coli* pathotypes and *Salmonella* serotypes. In this chapter we summarize the current
52 knowledge regarding the prevalence, the assembly, the regulation and the roles of the T6SS in
53 *E. coli*, *Salmonella* and related species.
54

55 INTRODUCTION

56
57
58 The adaptation of bacterial species in their ecological niche relies on specific regulatory
59 circuits to adapt the metabolism and the growth to the extracellular conditions, but also on the
60 release of molecules – siderophores, exopolysaccharides and/or protein toxins – in the milieu.
61 To facilitate their transport through the physical barriers that the membranes represent,
62 protein toxins are specifically selected and secreted by dedicated machineries named
63 “secretion systems”. Depending on the nature of the machine itself and on the mechanism of
64 transport of the toxins, these secretory pathways are numbered I-IX. Most of these pathways,
65 including Type I (T1SS), Type II (T2SS), Type III (T3SS), Type IV (T4SS or conjugation),
66 Type V (autotransporters, intimin/invasin and two-partner pathways including contact-
67 dependent growth inhibition systems) and curli/fimbriae/chaperone-usher pathways are
68 assembled and active in *Escherichia coli* and related species such as *Salmonella*, *Shigella*,
69 *Enterobacter* and *Citrobacter*, and therefore details regarding their architecture, assembly,
70 mechanism of transport as well as the effectors they deliver are described in the
71 corresponding chapters in *EcoSalPlus*. We will describe here one of the most recently
72 identified secretion pathways, the Type VI secretion system (T6SS).
73

74 The T6SS is a multi-protein machine, widespread in Gram-negative *Proteobacteria*, with an
75 over-representation in γ -*Proteobacteria* (1-4). However, T6SS-like machines have been
76 identified and characterized in other phylum such as in Bacteroidetes (5). The assembly of
77 this secretion system requires 13 different subunits, which are usually encoded within a single
78 genetic locus on the chromosome (2, 6) (Fig. 1A). Basically, the T6SS can be viewed as a
79 syringe-like structure anchored to the cell membrane by a trans-envelope complex (3, 4, 7-9)
80 (Fig. 1B). The T6SS syringe is evolutionarily, structurally and functionally related to the
81 puncturing tails of contractile tailed bacteriophages (Fig. 1C), although it is not known
82 whether the genes encoding this structure emerged from co-option of bacteriophage genes (9,
83 10). A large diversity of toxin effectors has been identified in the recent years, from effectors
84 promoting actin modification and disabling eukaryotic cells to peptidoglycan hydrolases
85 targeting competing bacteria. Therefore the T6SS is a versatile weapon targeting cells ranging
86 from bacteria to mammalian hosts. An example of T6SS-mediated inter-bacterial killing
87 between entero-aggregative *E. coli* (EAEC) and *E. coli* K-12 is shown in Fig. 2.
88

89

90 **MECHANISM OF ACTION OF THE T6SS**

91

92 In the recent years, several aspects of the mechanism of action of the T6SS have been
93 described. Genetic, biochemical and structural characterization of the different T6SS subunits
94 or protein complexes have defined the overall architecture of this secretion apparatus. Time-
95 lapse fluorescence microscopy recordings have provided a dynamic view on how the system
96 works. These data, combined with the knowledge on similar contractile structures such as tail
97 bacteriophages or pyocins, allows to design a model for the mechanism of action (3, 4, 8, 9)
98 (Fig. 3). T6SS biogenesis starts with the assembly of the membrane complex (MC) and the
99 tail assembly platform – or baseplate complex (BC) (Fig. 3a). The syringe, composed of the
100 inner tube and capped by the needle spike and wrapped by a contractile structure, the sheath,
101 then polymerizes to form a several hundreds of nm-long tubular structure (Fig. 3b-c). During
102 T6SS assembly, toxin effectors are loaded into the tube or associate with the spike trimer.
103 Contraction of the sheath propels the inner tube/spike, allowing perforation of the target cell
104 membrane and delivery of the effectors (Fig. 3d). The contracted sheath is then disassembled
105 and recycled by a dedicated ATPase named ClpV (Fig. 3e-f).

106

107 We will describe in the next sections the genetic organization, the prevalence of T6SS gene
108 clusters, the regulatory mechanisms underlying their expression, as well as the structure,
109 assembly and roles of this secretion machine, emphasizing the current knowledge on the
110 T6SS in *E. coli*, *Salmonella* and related species.

111

112

113 **GENETIC ORGANIZATION AND PREVALENCE OF T6SS GENE** 114 **CLUSTERS IN *E. COLI*, *SALMONELLA* AND RELATED SPECIES**

115

116 Type VI secretion system genes are distributed in Gram-negative *Proteobacteria* with an
117 over-representation in γ -*proteobacteria* (1). Therefore, T6SS genes are found in most *E. coli*
118 and *Salmonella* species except the *E. coli* B and K-12 laboratory strains. The genes encoding
119 components and toxins of the Type VI secretion system are usually clustered and grouped into
120 genetic islands (1, 6). The GC content of these regions is generally different from that of the
121 core genome suggesting that they have been acquired by horizontal gene transfer (1, 2, 6).
122 These gene clusters encode the 13 core components of the T6SS, *i.e.*, all the subunits required
123 to assemble a functional apparatus. Additional genes present in these clusters encode toxins
124 and anti-toxins, adaptor proteins that binds both machine components and toxins, as well as
125 auxilliary proteins required for the assembly of the apparatus or genes of unknown function
126 that might be required for the assembly of the T6SS, the recruitment and proper delivery of
127 the toxin (3, 4, 9). According to the gene organization and to homologies/similarities, the *E.*
128 *coli* T6SS gene clusters categorize in three distinct phylogenetic groups: T6SS-1-3 (Fig. 4-5).
129 This observation suggests that these clusters were present in common ancestors or that genetic
130 exchanges occurred between all these strains that may share the same environment. However,
131 the former hypothesis is likely as (i) each of the phylogenetic group is found in both intestinal
132 (AIEC, EAEC, EHEC, EPEC,...) and non-intestinal (UPEC, APEC, MNEC,...) strains and
133 (ii) these groups are not found in bacteria that share similar environments such as *Salmonella*
134 or *Enterobacter* species (see below). Among these three phylogenetic groups, the T6SS-1
135 (Fig. 5A) and T6SS-2 (Fig. 5B) gene clusters are the most commonly found in *E. coli*
136 chromosomes. For example, the prevalence of T6SS-1, T6SS-2 and T6SS-3 in APEC
137 genomes is 14.6, 2.4 and 0.8% respectively (11, 12). Interestingly, 85% of the T6SS⁺ APEC
138 strains belong to the virulent phylogenetic groups (11, 12). It is worthy to note that, in

139 general, the T6SS-2 gene cluster is over-represented in pathogenic strains with high virulence
140 traits. This is particularly clear for entero-aggregative *E. coli* (EAEC), in which T6SS-2⁺
141 strains (e.g., 042) cause diarrhoea whereas T6SS-2⁻ strains (e.g., 17-2, 34b) fail to elicit
142 diarrhoea in human volunteers (13). However, these prevalence information should be taken
143 with care as this does not necessarily mean that T6SSs are directly involved in pathogenesis,
144 but they may prepare the ground for virulence factors by clearing the niche of potential
145 bacterial competitors. Indeed, T6SS-1 and T6SS-2 gene clusters are also found in non
146 pathogenic strains of *E. coli* such as *E. coli* W (14; Fig. 5B); however, in this strain, the
147 T6SS-1 cluster is inactivated by insertion of a mobile element (14) and it is not known
148 whether it is functional.

149
150 With the exception of *Shigella sonnei* and *S. enterica* Gallinarum, which carry T6SS-2-like
151 gene clusters (Fig. 5B), the T6SS genetic organization in *Salmonella*, *Citrobacter* or
152 *Enterobacter* species is distinct from the *E. coli* T6SS-1-3 loci. In *Salmonella enterica*
153 serotypes, T6SSs belong to five phylogenetically distinct families, named as the *Salmonella*
154 pathogenicity island (SPI) they are encoded on. T6SSs are found in SPI-6 in *S. enterica*
155 Typhimurium, SPI-19 (similar to T6SS-2) in *S. enterica* Dublin, Gallinarum or Enteridis, SPI-
156 20 and SPI-21 in *S. enterica* Arizonae and SPI-22 in *S. bongori* (15, 16) (Fig. 4 and 6).
157 However, SPI-6 T6SS remnant genes are found in serotypes Enteridis and Gallinarum
158 suggesting these genes have been lost during evolution. These T6SS loci are characterized by
159 the presence of non-core genes inserted in between the core elements (17). In *S. enterica*
160 Typhimurium LT2, the organization of the SPI-6 core genes is different from the *E. coli*
161 species but rather shares synteny with distantly strains such as *Burkholderia mallei* and
162 *Ralstonia eutropha* (17). Therefore, *Salmonella* T6SSs derive from non-*E. coli* clusters, and
163 have evolved from the original cluster(s) by the acquisition of non-core modules. These
164 modules might have been transferred between strains as a *hcp-tae4-tai4* module encoding an
165 Hcp protein and an amidase/immunity pair is found in both *S. enterica* Typhimurium and
166 *Enterobacter cloacae* although the core genes differ (18). The addition of these distinct
167 modules during evolution may confer specialized functions to these T6SSs.

168
169 A remarkable difference between the T6SSs in *Escherichia* and *Salmonella* strains is the
170 nature of the toxin effectors. T6SS-1-like clusters generally encode effector belonging to
171 phospholipases. T6SS-2 clusters have recombinant hot spots (Rhs) elements bearing putative
172 activities whereas *S. enterica* SPI-6 and *Enterobacter cloacae* T6SS gene clusters encode
173 amidases and Rhs-linked anti-bacterial activities (Table 1, Fig. 5B and 6).

174
175 In addition to the main T6SS gene clusters, additional islands encoding Hcp, VgrG, PAAR
176 and putative toxins could be found disseminated on the genome. As Hcp and VgrG have been
177 shown to be carriers for the transport of the effectors, the existence of Hcp/VgrG islands
178 suggests that they correspond to additional modules that adapt to the core machine for the
179 delivery of specific toxins. In several instances, these small islands are inserted within the
180 core gene cluster. As described above, this is particularly visible in the case of the *Salmonella*
181 *enterica* sp. Typhimurium SPI-6 gene cluster, in which additional islands are inserted within
182 core genes (17, 19) (Fig. 6). It is also very clear when comparing T6SS gene clusters from
183 distinct *E. coli* species, e.g., a *vgrG-tle-tli-paar* fragment is found in the T6SS-1-like T6SS
184 operons in EAEC 042, AIEC LF82 and UPEC UT189, but with differences in the *vgrG* gene
185 and in the effector-immunity pair (Tle effectors of families 1, 3 and 4 respectively) (Fig. 5A
186 and Table 1).

187

188 Most of the strains contain several copies of T6SS gene clusters, this multiplicity likely
189 corresponding to various lifestyles, and thus might be reflected by the regulatory mechanisms
190 and the target cells (20). However, little information are available regarding whether these
191 clusters are differently regulated, have distinct functions or similar functions in different
192 conditions. In EAEC 17-2, two T6SSs are encoded within the *pheU* pathogenicity island (21).
193 Both T6SSs have anti-bacterial activities (22) but are expressed in different conditions: while
194 the T6SS-1 cluster is under the control of the Fur repressor and hence induced during iron
195 starvation (23), the T6SS-2 cluster is under the control of AggR, the aggregation master
196 regulator and is expressed in host cells or in synthetic media mimicking the macrophages
197 environment (21). The regulatory mechanisms and function of the *E. coli* and *Salmonella*
198 T6SSs are described in more details in the corresponding paragraphs below.
199
200

201 **BIOGENESIS AND ARCHITECTURE OF THE T6SS**

202

203 The T6SS proteins assemble two modules with different evolution history. As described
204 above, the cytoplasmic syringe-like structure derives from the bacteriophage contractile tail or
205 co-evolved with it from a common ancestor. The assembly of both structures follow a similar
206 sketch : the Hcp tail tube protein polymerizes to form the inner tube, and is tipped by the
207 VgrG membrane-penetrating needle. A sheath-like structure, constituted of the TssBC
208 proteins, polymerizes in an extended, metastable conformation around this inner tube. This
209 two-layered tubular edifice, usually hundreds of nanometers long, is assembled on a platform
210 called the baseplate complex (10). The BC is tethered to the cell envelope via contacts with
211 the second module, the membrane complex (24, 25). This MC is composed of three
212 subunits distributed into the inner and outer membranes. Two of these subunits share
213 sequence homologies with two components of the Type IVb secretion system found in
214 *Legionella pneumophila* or *Coxiella burnetii* (1, 6).

215 The best characterized T6SS in *E. coli* strains and related species is the EAEC T6SS-1 (Sci-1)
216 machinery. The biogenesis of the T6SS starts with the formation of the membrane complex.
217 The MC serves as a conduit for the passage of the Hcp-VgrG needle during contraction.
218 During T6SS biogenesis, it is the first structure to be assembled. Most cells assemble 1 to 3
219 MCs that remain static (26). Fluorescence microscopy experiments showed that its
220 localization is not spatially restricted to the cell pole or to the septum but rather that it is
221 randomly distributed in the cell envelope (26). The MC is composed of three proteins (Fig.
222 7A and 7B): the outer membrane TssJ lipoprotein and the inner membrane TssL and TssM
223 proteins (27-29). The EAEC TssJ lipoprotein is tethered to the outer membrane via acyl
224 chains but faces the periplasm (28). Its tri-dimensional structure has been solved (see Fig. 7C;
225 30). TssJ has a transthyretin fold, *i.e.*, a β -sandwich of two β -sheets. Two loops, notably that
226 connecting β -strands 1 and 2, mediate contact with the TssM C-terminal domain (26, 30, 31).
227 TssM is a 130-kDa large protein, composed of three trans-membrane segments in its N-
228 terminal third, whereas the 2/3 of the protein extends through the periplasm, from the inner
229 membrane to the outer membrane-anchored TssJ lipoprotein (29, 30). Recently, the crystal
230 structure of the C-terminal portion of the periplasmic region of TssM in complex with TssJ
231 has been solved (Fig. 7C). It is composed of two domains. The structure confirmed that
232 contacts between the two partners are established by interactions of the TssM C-terminal β -
233 domain with loops L1-2 and L3-4 of TssJ (26). Interestingly, this β -domain is followed by an
234 α -helix (colored purple in Fig. 7C) that inserts into the outer membrane, the insertion being
235 facilitated by the TssJ lipoprotein (26). The 300-amino-acid loop located between TM2 and
236 TM3 shares an NTPase fold, although the presence of Walker A and B motifs is not
237 conserved among TssM homologues. TssM contacts TssL, which is composed of a ~ 200-

238 amino-acid cytoplasmic domain anchored to the inner membrane by a unique C-terminal
239 trans-membrane segment, categorizing TssL as a C-tail protein (32). The proper insertion of
240 this C-tail protein requires the YidC protein and the contribution of the DnaK cytoplasmic
241 general chaperone (32). The structure of the EAEC TssL cytoplasmic domain has been
242 reported (33). It is composed of two bundles of three α -helices, with a general shape
243 resembling a fish hook (Fig. 7D; 33). In several instances, the TssL C-terminus is fused to an
244 additional domain of the OmpA/Pal/MotB family that mediates contact with the
245 peptidoglycan (34). In the T6SS-1 of EAEC and other *E. coli* pathotypes, the TssL protein
246 interacts directly with an additional component, TagL, which carries this motif. *In vivo* and *in*
247 *vitro* studies have shown that this domain anchors the T6SS to the cell wall, and that
248 mutations preventing TagL interaction with the peptidoglycan abolish T6SS function (27).
249 The structure of the EAEC TssJLM complex has been recently solved at 11.6-Å resolution by
250 negative stain electron microscopy (Fig. 7E; 26). The TssJLM complex has an overall rocket-
251 shape structure with 5-fold symmetry. It is composed of 10 copies of each proteins, and its
252 base is composed of the TssL and TssM cytoplasmic and intra-membrane domains. The TssM
253 periplasmic domains form 10 arches starting from the base and converging to the tip of the
254 structure in two layers of 5 pillars. The assembly of the MC starts from the outer membrane
255 and progresses inward: the TssJ lipoprotein recruits TssM and then TssL is added to the
256 TssJM complex (26). The interior of the membrane complex has a size sufficient to
257 accommodate the VgrG protein. The tip complex closes the structure at the outer membrane
258 and it has been proposed that large conformational changes occur – notably a re-organization
259 of the pillars – to allow the passage of the needle upon sheath contraction (26). Indeed,
260 conformational changes in the periplasmic portion of TssM have been reported in
261 *Agrobacterium tumefaciens* (35).

262 Once assembled, the MC serves as a docking station for the BC, *i.e.* the assembly platform of
263 the T6SS tail (Fig. 8A and 8B). The T6SS BC is constituted of the TssE, -F, -G, -K and VgrG
264 subunits, which assembles independently of the MC (25). In bacteriophages, the minimal
265 baseplate is composed of six wedges (constituted of the gp6, gp53 and gp25 proteins in
266 bacteriophage T4) that assemble around the spike complex (36). TssE is the T6SS counterpart
267 of the bacteriophage gp25 wedge protein, and the structure of the EAEC TssE can be modeled
268 based on the structures of gp25 homologues (Fig. 8C; 1, 6, 37). This subunit has been
269 suggested to be connected to the sheath. Although we still lack structural information on TssF
270 and TssG, these proteins share limited homologies with the phage T4 gp6 and gp53 proteins,
271 respectively (25). In agreement with phage baseplate structure, a complex comprising TssE, -
272 F, -G and VgrG could be purified from EAEC (25). In addition, it has been shown that TssF, -
273 G and -K form a stable complex in *S. marcescens* (38). Once assembled, the BC is recruited
274 to the MC via multiple contacts including TssK-TssL, TssK-TssM and TssG-TssM
275 interactions (24, 25). The hub of the bacteriophage – and probably of the T6SS – baseplate is
276 the spike complex. The structure of the T6SS spike protein, VgrG, from uropathogenic *E. coli*
277 CFT073 has been reported (Fig. 8D; 39). This membrane-penetrating needle is a trimer with a
278 base that connects to the inner tube, followed by a region composed of repeats that form a
279 highly stable three-stranded β -helix, or β -prism. The structure of the β -prism of the *E. coli*
280 O157 VgrG protein is also known (Fig. 8D; 40). In most cases, an adaptor protein called
281 PAAR interacts at the tip of VgrG and sharpens its extremity (41). The VgrG proteins sits on
282 the Hcp inner tube. Hcp proteins assemble hexameric rings with an internal lumen of ~ 40 Å
283 and an external diameter of ~ 110 Å. The structures of several Hcp proteins from diverse
284 bacteria have been reported in the literature, including that of EAEC (Fig. 8E; 42). The Hcp
285 rings stack on each other on a head-to-tail organization, and this assembly is strictly
286 controlled *in vivo* by additional T6SS subunits (25, 43). The assembly of the inner tube is
287 coordinated with that of the sheath but the rigid tube serves as template for sheath

288 polymerization (43). The sheath, composed of the TssB and TssC proteins, is the contractile
289 structure that assembles in an extended, metastable conformation that stores the energy
290 required to propulse the inner tube (44). By using time-lapse fluorescence microscopy, it has
291 been shown that the assembly of the 600 nm-long T6SS tube/sheath is completed in ~ 20
292 seconds and could be maintained in the extended conformation for several hundreds of
293 seconds (22, 44). However, how the extended sheath is stably maintained requires further
294 investigations. The atomic structure of the T6SS sheath in its contracted form has been solved
295 by cryo-electron microscopy (Fig. 8F; 45-47). The sheath is a helical structure composed of 6-
296 TssB/TssC heterodimer strands. Contacts between the heterodimers involve the formation of a
297 4-stranded β -sheet handshake domain comprising two β -strands from TssC, one from the next
298 TssC on the same strand, and one from TssB from the neighboring strand. This assembly
299 mechanism therefore connects heterodimers of the same strand, as well as with that of the
300 next strand (47). The contraction of the sheath is a fast event that occurs in less than 5 msec
301 (44). Although the propulsion of the inner tube or the delivery of effectors has not been
302 imaged yet, the contraction of the sheath coincides with the lysis of the prey cell (Fig. 9; 22).
303 Once contracted, an N-terminal helix of the TssC subunits protrudes from the structure and
304 recruits the ClpV ATPase that will recycle the sheath subunits (44, 48-50).

307 **FUNCTION AND EFFECTORS**

309 The Type VI secretion system garnered interests due to its ability to target both eukaryotic
310 and prokaryotic cells, therefore delivering toxins with a broad range of activities including
311 nucleases, phospholipases, peptidoglycan hydrolases, NAD(P)⁺ glycohydrolases or
312 cytoskeleton remodeling enzymes (51-55). These toxins are independent polypeptides
313 confined into the Hcp tail tube lumen or displayed at the tip of the VgrG needle via adaptor
314 proteins, or covalently linked as additional C-terminal domains to VgrGs (41, 54, 56-62).

315 The Type VI secretion system recently emerged as one of the key players during bacterial
316 pathogenesis. However, beside the fact that T6SS expression is usually co-regulated with
317 virulence factors, the role of the T6SS in the infection process could be either direct or
318 indirect. In a few instances, including in pathogenic strains of *E. coli* (see below), the T6SS
319 has been shown to be directly involved in bacterial virulence such as mediating adhesion to
320 host cells or participating to the survival into macrophages or to systemic proliferation, but
321 the molecular details on how the apparatus – or specific secreted proteins – interferes with the
322 host cells have not been defined. In *V. cholerae*, it has been shown that the T6SS disables
323 macrophage by interfering with the dynamics of the cell cytoskeleton. This ability depends on
324 the C-terminal domain carried by the VgrG1 spike, which possesses actin cross-linking
325 activity, therefore curbing actin dynamics, preventing cell movement and inhibiting further
326 phagocytosis of bacterial cells (63-65). The *Aeromonas hydrophila* VgrG1 C-terminal
327 extension has been proposed to carry actin-targeting ADP-ribosyltransferase activity (66).
328 Additional T6SS effectors with anti-host activities include phospholipases in *Pseudomonas*
329 *aeruginosa*, and toxins that induce membrane fusion (such as the *B. thailandensis* and *B.*
330 *pseudomallei* VgrG5 C-terminal extensions) or interfere with microtubule dynamics (67-70).

331 The direct role of the T6SS for virulence towards mammalian models of infection has been
332 challenged by the discovery that the vast majority of T6SSs characterized so far are involved
333 in bacterial growth inhibition. The T6SS is used to deliver anti-bacterial effectors with
334 peptidoglycan hydrolase (amidase (Tae), glycoside hydrolase (Tge)) or phospholipase (Tle)
335 activities into the periplasm of the target prey cell (71-74). These amidases, muramidases and
336 phospholipases belongs to various families that hydrolyse bonds within the peptidic stems
337 (Tae1-4 families) or glucosidic chains (Tge1-3 families) of the peptidoglycan or ester bonds

338 of phospholipids (Tle1-5 families), respectively (52-54, 72, 74). Toxins with nuclease (Tde)
339 and NAD(P)⁺ glycohydrolase activities have been reported and therefore should reach the
340 cytoplasm for their action (55, 75, 76). How these toxins are transported across the inner
341 membrane is not clearly defined, but it has been proposed that the translational elongation
342 factor Tu (EF-Tu) contributes to the translocation of the NAD(P)⁺ glycohydrolase in *P.*
343 *aeruginosa* (55). Producing cells are protected from their own effectors or the effectors of
344 their siblings by the co-production of specific protein inhibitors – or immunity proteins – that
345 bind and inhibit the activity of the cognate toxins (52-54). T6SS⁺ bacteria, including closely
346 related species, carries different and unique sets of anti-bacterial effectors, supporting a
347 leading role for T6SS effectors in inter- and intra-bacterial competition and in reshaping
348 bacterial communities (52).

350 Functions and Effectors in *E. coli*, *Salmonella* and related species

351 Phenotypes associated with T6SS in *E. coli* and *Salmonella*

352
353 Most of the *E. coli* and *Salmonella* T6SSs studied so far have been shown to participate to
354 adherence to biotic and abiotic surfaces, to bacterial competition or to virulence towards
355 corresponding models of infection (Table 1). Taken together, the available information on *E.*
356 *coli* T6SSs point to a role of T6SS-1 and T6SS-3 for anti-bacterial activity, and T6SS-2 for
357 pathogenesis.

358
359 T6SS-dependent bellicose behaviors towards neighboring bacteria have been evidenced for
360 EAEC 17-2 (T6SS-1 and T6SS-3; 22), APEC TW-XM (T6SS-1; 77) and *Citrobacter*
361 *rodentium* (78). *S. enterica* Typhimurium LT2 has also been recently reported to have
362 antagonistic activities against *E. coli* and *Salmonella* species in a SPI-6 T6SS-dependent
363 manner (19). However, in *S. enterica* Typhimurium, the T6SS is up-regulated in the late
364 stages of infection, once the bacterium is internalized in eukaryotic phagocytic cells whereas
365 no up-regulation could be observed in mixed bacterial cultures (17, 19, 79). These
366 observations suggest that the anti-bacterial activity is activated inside the host. Interestingly,
367 the T6SS is under a regulatory network that also regulates virulence factors including
368 proliferation genes. Because the *S. enterica* Typhimurium population inside phagocytic cells
369 is usually clonal, Brunet et al. proposed that the T6SS may serve to eliminate disabled *S.*
370 *enterica* Typhimurium cells of the progeny, *i.e.*, the cells unable to produce the T6SS
371 immunity and proliferation genes (19).

372 A role of the T6SS for biofilm formation has been reported for EAEC 17-2 (T6SS-1; 28) and
373 APEC SEPT362 (T6SS-2; 80). In APEC, the defect in biofilm is accompanied by decreased
374 adherence to epithelial cells (80). However, it is likely that these phenotypes are caused by
375 impacts on fimbriae gene regulation or by perturbations of the biofilm structure due to the
376 anti-bacterial activity. Indeed, deletions of T6SS-2 genes in APEC SEPT362 affect the
377 expression of type 1 fimbriae and flagella, two extracellular structures required for adhesion
378 and biofilm formation (80, 81).

379 T6SS-dependent pathogenesis towards hosts has been tested for a number of strains (Table 1).
380 From the available data, and in agreement with the over-representation of T6SS-2 in virulent
381 strains, no defect in virulence have been noted for T6SS-1 or T6SS-3 mutants, whereas
382 mutations in T6SS-2 gene clusters usually impact colonization, survival or invasion. The
383 EAEC 17-2 T6SS-1 and T6SS-3 are not required for virulence towards *C. elegans* and
384 intestinal survival within BALB/c mice respectively (21, 28) and mutants in the UPEC
385 CFT073 T6SS-1 gene cluster do not present colonization defects in CBA/J mice bladders and
386 kidneys (82). By contrast to T6SS-1 and T6SS-3, the APEC DE719 and SEPT362 T6SS-2
387 display attenuated virulence and decreased systemic dissemination in chicks or ducks, and
388 reduced intracellular survival in chicken macrophage cells (12, 80). In APEC strain TW-XM,

389 T6SS-2 is necessary for cerebral infection and penetration of the blood-brain barrier (11).
390 Similarly, the MNEC K1 T6SS-2 is required for internalization in human brain microvascular
391 endothelial cells (83). In MNEC K1, T6SS-2 carries two Hcp proteins. Analyses of the
392 phenotypes of mutations in these two genes showed that Hcp1 is required for efficient binding
393 to brain endothelial cells whereas Hcp2 induces stress fiber formation, cytoskeleton re-
394 arrangements, cytokine and chemokine release and cell apoptosis *via* activation of the caspase
395 8 pathway (83). Therefore the function of this apparatus is necessary at two different stages of
396 the infection process, probably by the Hcp1- and Hcp2-specific transport of distinct effectors.
397 In *Salmonella* Typhi, the SPI-6 genes are required for systemic infection in a humanized
398 mouse model (84) whereas conflicting data have been reported for the *S. enterica*
399 Typhimurium SPI-6 T6SS regarding replication, survival and proliferation in macrophages or
400 in mice (17, 79, 85, 86). However, the most important effect on replication within phagocytic
401 cells is observed with the *tai4* mutation, a gene that encodes the immunity to the Tae4
402 amidase, suggesting that this defect is indirect and due to self-intoxication caused by the loss
403 of the immunity (17, 19). The *S. enterica* Gallinarum SPI-19 is required for survival and
404 growth within chicken macrophages and for efficient colonization of the chick gastrointestinal
405 tract and internal organs (87, 88). It is interesting to note that the phenotypes associated with
406 the deletion of the SPI-6 T6SS gene cluster in *S. enterica* Typhimurium can be rescued by the
407 expression of the *S. enterica* Gallinarum SPI-19 T6SS suggesting that both T6SSs perform
408 similar functions despite their phylogenetic differences (89).

409

410 *E. coli* and *Salmonella* T6SS toxins

411

412 In *E. coli* and *Salmonella* strains, few effectors have been characterized in details, but their
413 presence and putative activities can be easily predicted. They are organized in tandem with
414 genes encoding small proteins that likely correspond to cognate immunity proteins.
415 Furthermore, they usually co-occur with *hcp*, *vgrG* or *paar* genes.

416 *E. coli* T6SS-1 gene clusters encode putative phospholipases (Table 1, Fig. 5A). They localize
417 upstream the *vgrG* genes suggesting that, as shown for *Vibrio cholerae* (90), they use the
418 VgrG needle as carrier for their transport. Interestingly, close inspection of these
419 phospholipase genes suggest they belong to different families : while the AIEC LF82 or
420 UPEC CFT073 T6SS-1 clusters carry putative phospholipases of the Tle3 family, those
421 present on the EAEC 042 and APEC TW-XM genomes are closely related to phospholipases
422 of the Tle1 and Tle4 families respectively (Fig. 5A; 77).

423 *E. coli* T6SS-2 gene clusters, as well as the *S. enterica* Gallinarum SPI-19 T6SS-2-like T6SS,
424 usually contain genes encoding Rhs elements (Table 1), but the activity carried by these Rhs
425 proteins can not be inferred from *in silico* prediction algorithms. In addition to the main T6SS
426 gene cluster, *S. enterica* Gallinarum possesses an *hcp* island that encode an Hcp protein, an
427 amidase of the Tae3 family and its cognate Tai3 immunity (15, 73). No gene with putative
428 toxin activity is found within *E. coli* T6SS-3 gene clusters.

429 The *S. enterica* Arizonae SPI-21 T6SS carries two pairs of S-type pyocins/immunity, as well
430 as a specialized Hcp protein corresponding to a fusion between a traditional Hcp protein to an
431 effector domain (15).

432 The *S. enterica* Typhimurium SPI-6 T6SS encodes Rhs elements as well as an amidase of the
433 Tae4 family (73) (Table 1, Fig. 6). The structure of Tae4 is available, alone or in complex
434 with its cognate Tai4 immunity protein (91, 92). Tae4 is a DL-endopeptidase with a typical
435 NlpC/P60 domain. It hydrolyses the D-Glu/meso-diaminopimelic acid (mDAP) bond of
436 peptidoglycan peptidic stems. A dimer of Tai4 binds to Tae4 with a K_D of 3×10^{-10} M and
437 inhibits Tae4 activity by inserting the L4 protruding loop of one Tai4 monomer into the Tae4
438 catalytic pocket (91, 92). Contacts between Tae4 and Tai4 are stabilized by the α -helix of the
439 second Tai4 subunit (91, 92). A similar Tae4/Tai4 pair is encoded within the *Enterobacter*

440 *cloacae* SPI-6-like T6SS (73). Indeed, cross-immunity between the *S. enterica* Typhimurium
441 and *E. cloacae* Tae4/Tai4 pairs has been demonstrated (18). Finally, an original mechanism
442 has been revealed in the case of the *S. enterica* Typhimurium Rhs (93). The gene encoding the
443 full-length Rhs protein (Rhs^{main}) is followed by a non-translated region encoding an orphan C-
444 terminal extension (Rhs^{orphan}). However, serial passages in broth or within the mouse induces
445 a duplication of the region and a genetic chromosomal re-arrangement that results to the
446 production of a chimera Rhs protein constituted of the Rhs^{main} core and the Rhs^{orphan} C-
447 terminal extension with anti-bacterial activity. This elegant mechanism therefore provides a
448 selective advantage to cells of the evolved bacterial lineage as it enables to maintain the
449 immunity to Rhs^{main} and to deploy a new toxin that is active against ancestral cells (93).
450 Finally, two genes present within the *Citrobacter freundii* and *E. cloacae* T6SS gene clusters
451 encode proteins with MIX domains, an N-terminal sequence associated with several T6SS
452 toxins (94).

453 **REGULATORY MECHANISMS**

454 T6SS gene clusters are tightly regulated to adapt their expression to the environmental
455 conditions. In agreement with the broad diversity of T6SS targets and activities, T6SS gene
456 clusters are not submitted to a unique regulation but rather have hijacked most of the
457 regulatory mechanisms known in bacteria, including that at the level of transcription or post-
458 transcription (20, 95): two-component systems, transcriptional activators and repressors,
459 histone-like nucleoid associated proteins, quorum sensing, alternative sigma factors, small
460 regulatory RNAs, etc. In addition, a post-translational phosphorylation-dependent pathway
461 has been identified and characterized in Pseudomonads. This signal transmission involves
462 sensing of specific stimuli and activation of a trans-envelope transducing cascade comprising
463 the TagFQRST proteins and leading to the PpkA-dependent phosphorylation of the forkhead-
464 associated FHA protein (96-100). The reversibility of the activation is secured by the de-
465 phosphorylation of FHA by the PppA phosphatase (96).

466 Regulation of T6SS gene clusters in *E. coli*, *Salmonella* and related species

467 Transcriptional regulation

468 T6SSs have been studied in details in *Pseudomonas*, *Agrobacterium* and *Vibrio* species.
469 Hence, we have a comprehensive picture of the regulatory mechanisms underlying expression
470 of the T6SS gene clusters in these bacteria. By contrast, except for the entero-aggregative *E.*
471 *coli* and *S. enterica* Typhimurium T6SSs, only very few is know on the regulation of T6SSs
472 in other *E. coli* and *Salmonella* serotypes.

473 Enteroaggregative *E. coli*. The EAEC 17-2 strain genome encodes two complete sets of T6SS
474 genes (families T6SS-1 and T6SS-3). The T6SS-1 family cluster (also called *sci-1*) is under
475 the control of the Fur repressor (23, Fig. 10). Fur – for Ferric uptake regulator – is the main
476 regulator that couples iron homeostasis to gene expression (101). In presence of iron, Fur
477 binds to two Fur boxes present in tandem in the promoter sequence of this cluster,
478 overlapping with the transcriptional -10 (Fur⁻¹⁰) and -35 (Fur⁻³⁵), preventing RNA polymerase
479 recruitment and therefore turning OFF the T6SS-1 genes (23). This very simple mechanism is
480 complexified by an epigenetic circuit depending on the action of the Dam adenosine
481 methyltransferase, which couples T6SS expression to the replication state. The Fur⁻¹⁰ box
482 contains a GATC motif that is recognized and methylated by Dam. When Fur is bound to the
483 Fur⁻¹⁰ box, the site is not methylated. However, under low iron conditions, Fur is dislodged,
484 the T6SS-1 genes are turned ON. If cells replicate, the Fur⁻¹⁰ box is methylated after the first
485
486
487
488
489
490

491 replication, preventing Fur to bind back, therefore turning the T6SS genes under a constitutive
492 ON state. Fur therefore controls the passage between the OFF and ON states whereas Dam is
493 a sensor of replication and controls the passage between the reversible and constitutive ON
494 states (23) (Fig. 10). In agreement with these data, the EAEC T6SS-1 is activated in minimal
495 media or in iron depletion conditions (24). However, this mechanism is unlikely to be
496 conserved between the T6SS-1 clusters shared by pathogenic *E. coli* as no Fur box could be
497 readily identified in their promoter regions.

498 The EAEC T6SS-3 gene cluster (also called *sci-2* or *aai*) is activated in synthetic media
499 mimicking the macrophage environment such as Eagle's medium (21, 22). DNA microarrays
500 and quantitative RT-PCR have demonstrated that this activation depends on an AraC-like
501 transcriptional regulator called AggR (21, 102). Although no consensus binding site has been
502 defined for AggR, this activator regulates other EAEC virulence factors such as the Aaf
503 fimbriae, the dispersin and the dispersin transporter (102).

504 No data are available yet for the T6SS-2 gene cluster found in the EAEC 042 strain. Although
505 it remains to be experimentally tested, it has been proposed that regulation of the T6SS-2
506 *EC042_0229* gene involves the synergistical action of the cyclic AMP receptor protein (CRP)
507 and the nucleoid-associated protein Fis by an original mechanism requiring Fis-dependent
508 compensation of the non-optimal spacing between the CRP and RNA polymerase binding
509 sites (103).

510
511 *Salmonella enterica*. In *S. enterica* Typhimurium LT2, the SPI-6 T6SS gene cluster is
512 controlled by the SsrA/B Two-component system, one of the major regulatory pathways of
513 *Salmonella* virulence (79). The SsrB response regulator binds to and positively regulates most
514 SPI-2 promoters including those controlling expression of the T3SS genes (104). By contrast,
515 SsrA/B exerts a negative control on the SPI-6 T6SS gene cluster (17, 79), probably by direct
516 SsrB binding on distinct promoter regions (105). The expression of T6SS genes encoded
517 within the SPI-6 pathogenicity island are not detected under laboratory *in vitro* conditions (79,
518 106); however, promoter reporter and transcriptional profiling studies showed that the
519 expression of these genes is activated in the late stages of macrophage and epithelial cells
520 infection (17, 107). In addition to SsrB, the SPI-6 T6SS genes are silenced by the histone-like
521 nucleoid structuring H-NS protein (19, 108, 109). H-NS binds to A/T-rich motifs and
522 polymerizes to spread and silence the genes by preventing access to the RNA polymerase or
523 activators. H-NS is thus a xenogenic silencer that usually represses horizontally-acquired
524 genes and islands (110). Because T6SS genes are clustered in these islands, H-NS is probably
525 involved in the regulation of many T6SS gene clusters in pathogenic *E. coli* but this needs to
526 be addressed.

527 In *S. enterica* Typhi Ty2, several regulators have been identified such as RcsB, PmrA and
528 Hfq, but their contribution for the activation of the T6SS genes is relatively weak (111). In
529 *Enterobacter cloacae*, several genes of the T6SS cluster are under the control of a
530 LuxR/acylhomoserine lactone-dependent quorum sensing mechanism and therefore respond
531 to the population density (112).

532
533 In addition to the EAEC T6SS-3 cluster, shown to be induced in media mimicking the
534 macrophage environment, most of the *E. coli* and *Salmonella* T6SS genes are induced in *in*
535 *vivo* conditions. This has been reported for the *S. enterica* Typhimurium SPI-6 T6SS genes,
536 which are upregulated during macrophage infection (79, 107) and for the *S. enterica*
537 Gallinarum SPI-19 T6SS genes which are upregulated after internalization by murine or avian
538 macrophages (87). These data suggest that these T6SS gene clusters might play a direct role
539 during infection or that host-mediated activation of the anti-bacterial activity might help to
540 clear the niche and to enable efficient colonization.

541

542 Transcriptional frameshifting and post-translational activation
543

544 In *Citrobacter rodentium*, the *tssM* gene is subjected to transcriptional frameshifting. The
545 *tssM* gene is interrupted by a premature stop codon, but a poly-A slippery tract located
546 upstream the stop codon induces incorporation of additional adenosines in the RNA and
547 hence the synthesis of TssM length variants (113). Yet, whether the frameshifting efficiency
548 is influenced by environmental cues or by regulatory factors is unknown.
549

550 Finally, it is interesting to note that genes encoding the phosphorylation-dependent post-
551 translational pathway found in *Pseudomonas* and *Agrobacterium* species are present in none
552 of the *E. coli*, *Salmonella*, *Citrobacter* or *Enterobacter* strain genomes sequenced so far,
553 except for those encoding FHA proteins which are found associated with *E. coli* T6SS-2-like
554 and *Salmonella* SPI-19 clusters (Fig. 5B and Fig. 6). However, the contribution of FHA for
555 the assembly or the activation of the system has not been reported in these strains.
556

557

558 **CLOSING REMARKS AND FUTURE DIRECTIONS** 559

560 This chapter summarizes the current knowledge on the T6SSs present in *E. coli* and related
561 species. It is clear that the recent years have provided a detailed view on the architecture and
562 mechanism of assembly of this apparatus. However, the regulatory mechanisms underlying
563 the expression of these gene clusters, the effectors delivered by this machinery and the
564 function of the T6SS during host infection remain enigmatic for most enterobacterial
565 pathogens. Further studies will provide a better understanding of the T6SS contribution in the
566 ecological niche of these strains or for pathogenesis. Similarly, although a number of anti-
567 bacterial effectors with amidase, peptidoglycan hydrolase, phospholipase and DNase
568 activities have been identified or predicted, it remains to determine whether phospholipases
569 and DNases might be targeted into eukaryotic host cells and to identify anti-eukaryotic-
570 specific effectors. These toxins would be therefore interesting targets for the development of
571 drugs that will interfere with these toxins, not only for human health, but also in the cases in
572 which the bacterial pathogen targets poultry or cattle.
573

574

575 **ACKNOWLEDGMENTS** 576

577 We thank Yannick R. Brunet for generating the time-lapse recordings shown in Fig. 2, the
578 past and present members of the Cascales group for insightful discussions and Andy
579 Vojeambont for encouragements. We apologize for omissions of primary works owing to
580 space constraints. Work in E.C. laboratory is supported by the Centre National de la
581 Recherche Scientifique, the Aix-Marseille Université, doctoral and post-doctoral fellowships
582 from the French Ministère de la Recherche and the Fondation pour la Recherche Médicale,
583 and grants from the Agence Nationale de la Recherche (ANR-10-JCJC-1303-03 and ANR-14-
584 CE14-0006-02).
585

586

587

588 **REFERENCES**

588 **1. Bingle LE, Bailey CM, Pallen MJ.** 2008. Type VI secretion: a beginner's guide. *Curr Opin*
589 *Microbiol.* 11:3-8.

590 **2. Boyer F, Fichant G, Berthod J, Vandenbrouck Y, Attree I.** 2009. Dissecting the bacterial type

- 591 VI secretion system by a genome wide in silico analysis: what can be learned from available microbial
592 genomic resources? *BMC Genomics*. **10**:104.
- 593 **3. Coulthurst SJ.** 2013. The Type VI secretion system - a widespread and versatile cell targeting
594 system. *Res Microbiol*. **164**:640-54.
- 595 **4. Zoued A, Brunet YR, Durand E, Aschtgen MS, Logger L, Douzi B, Journet L, Cambillau C,**
596 **Cascales E.** 2014. Architecture and assembly of the Type VI secretion system. *Biochim Biophys Acta*.
597 **1843**:1664-73.
- 598 **5. Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ, Goo YA, Tran BQ, Barry NA,**
599 **Zheng H, Peterson SB, Chou S, Gonen T, Goodlett DR, Goodman AL, Mougous JD.** 2014. A
600 type VI secretion-related pathway in Bacteroidetes mediates interbacterial antagonism. *Cell Host*
601 *Microbe*. **16**:227-36.
- 602 **6. Cascales E.** 2008. The type VI secretion toolkit. *EMBO Rep*. **9**:735-41.
- 603 **7. Silverman JM, Brunet YR, Cascales E, Mougous JD.** 2012. Structure and regulation of the type
604 VI secretion system. *Annu Rev Microbiol*. **66**:453-72.
- 605 **8. Ho BT, Dong TG, Mekalanos JJ.** 2014. A view to a kill: the bacterial type VI secretion system.
606 *Cell Host Microbe*. **15**:9-21.
- 607 **9. Basler M.** 2015. Type VI secretion system: secretion by a contractile nanomachine. *Philos Trans R*
608 *Soc Lond B Biol Sci*. **370**:1679-1691.
- 609 **10. Cascales E, Cambillau C.** 2012. Structural biology of type VI secretion systems. *Philos Trans R*
610 *Soc Lond B Biol Sci*. **367**:1102-11.
- 611 **11. Ma, J., M. Sun, Y. Bao, Z. Pan, W. Zhang, C. Lu, and H. Yao.** 2013. Genetic diversity and
612 features analysis of type VI secretion systems loci in avian pathogenic *Escherichia coli* by wide
613 genomic scanning. *Infect Genet Evol* **20**:454-64.
- 614 **12. Wang S, Dai J, Meng Q, Han X, Han Y, Zhao Y, Yang D, Ding C, Yu S.** 2014. DotU
615 expression is highly induced during *in vivo* infection and responsible for virulence and Hcp1 secretion
616 in avian pathogenic *Escherichia coli*. *Front Microbiol*. **5**:588.
- 617 **13. Nataro JP, Deng Y, Cookson S, Cravioto A, Savarino SJ, Guers LD, Levine MM, Tacket CO.**
618 1995. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. *J*
619 *Infect Dis*. **171**:465-8.
- 620 **14. Archer, C. T., J. F. Kim, H. Jeong, J. H. Park, C. E. Vickers, S. Y. Lee, and L. K. Nielsen.**
621 2011. The genome sequence of *E. coli* W (ATCC 9637): comparative genome analysis and an
622 improved genome-scale reconstruction of *E. coli*. *BMC Genomics* **12**:9.
- 623 **15. Blondel CJ, Jiménez JC, Contreras I, Santiviago CA.** 2009. Comparative genomic analysis
624 uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella*
625 serotypes. *BMC Genomics*. **10**:354.
- 626 **16. Fookes M, Schroeder GN, Langridge GC, Blondel CJ, Mammina C, Connor TR, Seth-Smith**
627 **H, Vernikos GS, Robinson KS, Sanders M, Petty NK, Kingsley RA, Bäumlér AJ, Nuccio SP,**
628 **Contreras I, Santiviago CA, Maskell D, Barrow P, Humphrey T, Nastasi A, Roberts M, Frankel**
629 **G, Parkhill J, Dougan G, Thomson NR.** 2011. *Salmonella bongori* provides insights into the

- 630 evolution of the *Salmonellae*. *PLoS Pathog.* **7**:e1002191.
- 631 **17. Mulder DT, Cooper CA, Coombes BK.** 2012. Type VI secretion system-associated gene clusters
632 contribute to pathogenesis of *Salmonella enterica* serovar Typhimurium. *Infect Immun.* **80**:1996-2007.
- 633 **18. Zhang H, Gao ZQ, Wei Y, Xu JH, Dong YH.** 2013. Insights into the cross-immunity mechanism
634 within effector families of bacteria type VI secretion system from the structure of StTae4-EcTai4
635 complex. *PLoS One.* **8**:e73782.
- 636 **19. Brunet YR, Khodr A, Logger L, Aussel L, Mignot T, Rimsky S, Cascales E.** 2015. H-NS
637 silencing of the *Salmonella* pathogenicity island 6-encoded type VI secretion system limits *Salmonella*
638 *enterica* serovar Typhimurium interbacterial killing. *Infect Immun.* **83**:2738-50.
- 639 **20. Bernard CS, Brunet YR, Gueguen E, Cascales E.** 2010. Nooks and crannies in type VI
640 secretion regulation. *J Bacteriol.* **192**:3850-60.
- 641 **21. Dudley EG, Thomson NR, Parkhill J, Morin NP, Nataro JP.** 2006. Proteomic and microarray
642 characterization of the AggR regulon identifies a *pheU* pathogenicity island in enteroaggregative
643 *Escherichia coli*. *Mol Microbiol.* **61**:1267-82.
- 644 **22. Brunet YR, Espinosa L, Harchouni S, Mignot T, Cascales E.** 2013. Imaging type VI secretion-
645 mediated bacterial killing. *Cell Rep.* **3**:36-41.
- 646 **23. Brunet YR, Bernard CS, Gavioli M, Lloubès R, Cascales E.** 2011. An epigenetic switch
647 involving overlapping *fur* and DNA methylation optimizes expression of a type VI secretion gene
648 cluster. *PLoS Genet.* **7**:e1002205.
- 649 **24. Zoued A, Durand E, Bebeacua C, Brunet YR, Douzi B, Cambillau C, Cascales E, Journet L.**
650 2013. TssK is a trimeric cytoplasmic protein interacting with components of both phage-like and
651 membrane anchoring complexes of the type VI secretion system. *J Biol Chem.* **288**:27031-41.
- 652 **25. Brunet YR, Zoued A, Boyer F, Douzi B, Cascales E.** 2015. The Type VI secretion TssEFGK-
653 VgrG phage-like baseplate is recruited to the TssJLM membrane complex via multiple contacts and
654 serves as assembly platform for tail tube/sheath polymerization. *PLOS Genetics.* **11**:e1005545.
- 655 **26. Durand E, Nguyen VS, Zoued A, Logger L, Péhau-Arnaudet G, Aschtgen MS, Spinelli S,
656 Desmyter A, Bardiaux B, Dujancourt A, Roussel A, Cambillau C, Cascales E, Fronzes R.** 2015.
657 Biogenesis and structure of a type VI secretion membrane core complex. *Nature.* **523**:555-60.
- 658 **27. Aschtgen MS, Gavioli M, Dessen A, Lloubès R, Cascales E.** 2010. The SciZ protein anchors the
659 enteroaggregative *Escherichia coli* Type VI secretion system to the cell wall. *Mol Microbiol.* **75**:886-
660 99.
- 661 **28. Aschtgen MS, Bernard CS, de Bentzmann S, Lloubès R, Cascales E.** 2008. SciN is an outer
662 membrane lipoprotein required for type VI secretion in enteroaggregative *Escherichia coli*. *J*
663 *Bacteriol.* **190**:7523-31.
- 664 **29. Ma LS, Lin JS, Lai EM.** 2009. An IcmF family protein, ImpL_M, is an integral inner membrane
665 protein interacting with ImpK_L, and its walker a motif is required for type VI secretion system-
666 mediated Hcp secretion in *Agrobacterium tumefaciens*. *J Bacteriol.* **191**:4316-29.
- 667 **30. Felisberto-Rodrigues C, Durand E, Aschtgen MS, Blangy S, Ortiz-Lombardia M, Douzi B,
668 Cambillau C, Cascales E.** 2011. Towards a structural comprehension of bacterial type VI secretion

- 669 systems: characterization of the TssJ-TssM complex of an *Escherichia coli* pathovar. *PLoS Pathog.*
670 7:e1002386.
- 671 **31. Nguyen VS, Logger L, Spinelli S, Desmyter A, Le TT, Kellenberger C, Douzi B, Durand E,**
672 **Roussel A, Cascales E, Cambillau C.** 2015. Inhibition of type VI secretion by an anti-TssM llama
673 nanobody. *PLoS One.* **10**:e0122187.
- 674 **32. Aschtgen MS, Zoued A, Lloubès R, Journet L, Cascales E.** 2012. The C-tail anchored TssL
675 subunit, an essential protein of the enteroaggregative *Escherichia coli* Sci-1 Type VI secretion system,
676 is inserted by YidC. *Microbiologyopen.* **1**:71-82.
- 677 **33. Durand E, Zoued A, Spinelli S, Watson PJ, Aschtgen MS, Journet L, Cambillau C, Cascales**
678 **E.** 2012. Structural characterization and oligomerization of the TssL protein, a component shared by
679 bacterial type VI and type IVb secretion systems. *J Biol Chem.* **287**:14157-68.
- 680 **34. Aschtgen MS, Thomas MS, Cascales E.** 2010. Anchoring the type VI secretion system to the
681 peptidoglycan: TssL, TagL, TagP... what else? *Virulence.* **1**:535-40.
- 682 **35. Ma LS, Narberhaus F, Lai EM.** 2012. IcmF family protein TssM exhibits ATPase activity and
683 energizes type VI secretion. *J Biol Chem.* **287**:15610-21.
- 684 **36. Leiman PG, Arisaka F, van Raaij MJ, Kostyuchenko VA, Aksyuk AA, Kanamaru S,**
685 **Rossmann MG.** 2010. Morphogenesis of the T4 tail and tail fibers. *Virology.* **7**:355.
- 686 **37. Lossi NS, Dajani R, Freemont P, Filloux A.** 2011. Structure-function analysis of HsiF, a gp25-
687 like component of the type VI secretion system, in *Pseudomonas aeruginosa*. *Microbiology.*
688 **157**:3292-305.
- 689 **38. English G, Byron O, Cianfanelli FR, Prescott AR, Coulthurst SJ.** 2014. Biochemical analysis
690 of TssK, a core component of the bacterial Type VI secretion system, reveals distinct oligomeric states
691 of TssK and identifies a TssK-TssFG subcomplex. *Biochem J.* **461**:291-304.
- 692 **39. Leiman PG, Basler M, Ramagopal UA, Bonanno JB, Sauder JM, Pukatzki S, Burley SK,**
693 **Almo SC, Mekalanos JJ.** 2009. Type VI secretion apparatus and phage tail-associated protein
694 complexes share a common evolutionary origin. *Proc Natl Acad Sci USA.* **106**:4154-9.
- 695 **40. Uchida K, Leiman PG, Arisaka F, Kanamaru S.** 2014. Structure and properties of the C-
696 terminal β -helical domain of VgrG protein from *Escherichia coli* O157. *J Biochem.* **155**:173-82.
- 697 **41. Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ, Leiman PG.** 2013. PAAR-repeat
698 proteins sharpen and diversify the type VI secretion system spike. *Nature.* **500**:350-3.
- 699 **42. Douzi B, Spinelli S, Blangy S, Roussel A, Durand E, Brunet YR, Cascales E, Cambillau C.**
700 2014. Crystal structure and self-interaction of the type VI secretion tail-tube protein from
701 enteroaggregative *Escherichia coli*. *PLoS One.* **9**:e86918.
- 702 **43. Brunet YR, Hénin J, Celia H, Cascales E.** 2014. Type VI secretion and bacteriophage tail tubes
703 share a common assembly pathway. *EMBO Rep.* **15**:315-21.
- 704 **44. Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ.** 2012. Type VI secretion
705 requires a dynamic contractile phage tail-like structure. *Nature.* **483**:182-6.
- 706 **45. Kube S, Kapitein N, Zimniak T, Herzog F, Mogk A, Wendler P.** 2014. Structure of the VipA/B

- 707 type VI secretion complex suggests a contraction-state-specific recycling mechanism. *Cell Rep.* **8**:20-
708 30.
- 709 **46. Clemens DL, Ge P, Lee BY, Horwitz MA, Zhou ZH.** 2015. Atomic structure of T6SS reveals
710 interlaced array essential to function. *Cell.* **160**:940-51.
- 711 **47. Kudryashev M, Wang RY, Brackmann M, Scherer S, Maier T, Baker D, DiMaio F,**
712 **Stahlberg H, Egelman EH, Basler M.** 2015. Structure of the type VI secretion system contractile
713 sheath. *Cell.* **160**:952-62.
- 714 **48. Bönemann G, Pietrosiuk A, Diemand A, Zentgraf H, Mogk A.** 2009. Remodelling of
715 VipA/VipB tubules by ClpV-mediated threading is crucial for type VI protein secretion. *EMBO J.*
716 **28**:315-25.
- 717 **49. Pietrosiuk A, Lenherr ED, Falk S, Bönemann G, Kopp J, Zentgraf H, Sinning I, Mogk A.**
718 2011. Molecular basis for the unique role of the AAA+ chaperone ClpV in type VI protein secretion. *J*
719 *Biol Chem.* **286**:30010-21.
- 720 **50. Kapitein N, Bönemann G, Pietrosiuk A, Seyffer F, Hausser I, Locker JK, Mogk A.** 2013.
721 ClpV recycles VipA/VipB tubules and prevents non-productive tubule formation to ensure efficient
722 type VI protein secretion. *Mol Microbiol.* **87**:1013-28.
- 723 **51. Schwarz S, Hood RD, Mougous JD.** 2010. What is type VI secretion doing in all those bugs?
724 *Trends Microbiol.* **18**:531-7.
- 725 **52. Russell AB, Peterson SB, Mougous JD.** 2014. Type VI secretion system effectors: poisons with a
726 purpose. *Nat Rev Microbiol.* **12**:137-48.
- 727 **53. Benz J, Meinhart A.** 2014. Antibacterial effector/immunity systems: it's just the tip of the iceberg.
728 *Curr Opin Microbiol.* **17**:1-10.
- 729 **54. Durand E, Cambillau C, Cascales E, Journet L.** 2014. VgrG, Tae, Tle, and beyond: the
730 versatile arsenal of Type VI secretion effectors. *Trends Microbiol.* **22**:498-507.
- 731 **55. Whitney JC, Quentin D, Sawai S, LeRoux M, Harding BN, Ledvina HE, Tran BQ, Robinson**
732 **H, Goo YA, Goodlett DR, Raunser S, Mougous JD.** 2015. An interbacterial NAD(P)⁺
733 glycohydrolase toxin requires elongation factor Tu for delivery to target cells. *Cell.* in press
- 734 **56. Alcoforado Diniz J, Liu YC, Coulthurst SJ.** 2015. Molecular weaponry : diverse effectors
735 delivered by the Type VI secretion system. *Cell Microbiol.* in press.
- 736 **57. Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ.** 2007. Type VI secretion system
737 translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proc Natl Acad*
738 *Sci USA.* **104**:15508-13.
- 739 **58. Silverman JM, Agnello DM, Zheng H, Andrews BT, Li M, Catalano CE, Gonen T, Mougous**
740 **JD.** 2013. Haemolysin coregulated protein is an exported receptor and chaperone of type VI secretion
741 substrates. *Mol Cell.* **51**:584-93.
- 742 **59. Whitney JC, Beck CM, Goo YA, Russell AB, Harding BN, De Leon JA, Cunningham DA,**
743 **Tran BQ, Low DA, Goodlett DR, Hayes CS, Mougous JD.** 2014. Genetically distinct pathways
744 guide effector export through the type VI secretion system. *Mol Microbiol.* **92**:529-42.

- 745 **60. Alcoforado Diniz J, Coulthurst SJ.** 2015. Intraspecies competition in *Serratia marcescens* is
746 mediated by type VI-secreted Rhs effectors and a conserved effector-associated accessory protein. *J*
747 *Bacteriol.* **197**:2350-60.
- 748 **61. Liang X, Moore R, Wilton M, Wong MJ, Lam L, Dong TG.** 2015. Identification of divergent
749 type VI secretion effectors using a conserved chaperone domain. *Proc Natl Acad Sci USA.* **112**:9106-
750 11.
- 751 **62. Unterweger D, Kostiuk B, Ötjengerdes R, Wilton A, Diaz-Satizabal L, Pukatzki S.** 2015.
752 Chimeric adaptor proteins translocate diverse type VI secretion system effectors in *Vibrio cholerae*.
753 *EMBO J.* in press.
- 754 **63. Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ.** 2007. Type VI secretion system
755 translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proc Natl Acad*
756 *Sci USA.* **104**:15508-13.
- 757 **64. Ma AT, McAuley S, Pukatzki S, Mekalanos JJ.** 2009. Translocation of a *Vibrio cholerae* type
758 VI secretion effector requires bacterial endocytosis by host cells. *Cell Host Microbe.* **5**:234-43.
- 759 **65. Durand E, Derrez E, Audoly G, Spinelli S, Ortiz-Lombardia M, Raoult D, Cascales E,**
760 **Cambillau C.** 2012. Crystal structure of the VgrG1 actin cross-linking domain of the *Vibrio cholerae*
761 type VI secretion system. *J Biol Chem.* **287**:38190-9.
- 762 **66. Suarez G, Sierra JC, Erova TE, Sha J, Horneman AJ, Chopra AK.** 2010. A type VI secretion
763 system effector protein, VgrG1, from *Aeromonas hydrophila* that induces host cell toxicity by ADP
764 ribosylation of actin. *J Bacteriol.* **192**:155-68.
- 765 **67. Jiang F, Waterfield NR, Yang J, Yang G, Jin Q.** 2014. A *Pseudomonas aeruginosa* type VI
766 secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. *Cell Host Microbe.*
767 **15**:600-10.
- 768 **68. Schwarz S, Singh P, Robertson JD, LeRoux M, Skerrett SJ, Goodlett DR, West TE,**
769 **Mougous JD.** 2014. VgrG-5 is a *Burkholderia* type VI secretion system-exported protein required for
770 multinucleated giant cell formation and virulence. *Infect Immun.* **82**:1445-52.
- 771 **69. Toesca IJ, French CT, Miller JF.** 2014. The Type VI secretion system spike protein VgrG5
772 mediates membrane fusion during intercellular spread by pseudomallei group *Burkholderia* species.
773 *Infect Immun.* **82**:1436-44.
- 774 **70. Sana TG, Baumann C, Merdes A, Soscia C, Rattei T, Hachani A, Jones C, Bennett KL,**
775 **Filloux A, Superti-Furga G, Voulhoux R, Bleves S.** 2015. Internalization of *Pseudomonas*
776 *aeruginosa* strain PAO1 into epithelial cells is promoted by interaction of a T6SS effector with the
777 microtubule network. *MBio.* **6**:e00712.
- 778 **71. Hood RD, Singh P, Hsu F, Güvener T, Carl MA, Trinidad RR, Silverman JM, Ohlson BB,**
779 **Hicks KG, Plemel RL, Li M, Schwarz S, Wang WY, Merz AJ, Goodlett DR, Mougous JD.** 2010.
780 A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe.*
781 **7**:25-37.
- 782 **72. Russell AB, Hood RD, Bui NK, LeRoux M, Vollmer W, Mougous JD.** 2011. Type VI secretion
783 delivers bacteriolytic effectors to target cells. *Nature.* **475**:343-7.
- 784 **73. Russell AB, Singh P, Brittnacher M, Bui NK, Hood RD, Carl MA, Agnello DM, Schwarz S,**

- 785 **Goodlett DR, Vollmer W, Mougous JD.** 2012. A widespread bacterial type VI secretion effector
786 superfamily identified using a heuristic approach. *Cell Host Microbe*. **11**:538-49.
- 787 **74. Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai SN, Mougous**
788 **JD.** 2013. Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors.
789 *Nature*. **496**:508-12.
- 790 **75. Koskiniemi S, Lamoureux JG, Nikolakakis KC, t'Kint de Roodenbeke C, Kaplan MD, Low**
791 **DA, Hayes CS.** 2013. Rhs proteins from diverse bacteria mediate intercellular competition. *Proc Natl*
792 *Acad Sci USA*. **110**:7032-7.
- 793 **76. Ma LS, Hachani A, Lin JS, Filloux A, Lai EM.** 2014. *Agrobacterium tumefaciens* deploys a
794 superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta.
795 *Cell Host Microbe*. **16**:94-104.
- 796 **77. Ma J, Bao Y, Sun M, Dong W, Pan Z, Zhang W, Lu C, Yao H.** 2014. Two functional type VI
797 secretion systems in avian pathogenic *Escherichia coli* are involved in different pathogenic pathways.
798 *Infect Immun*. **82**:3867-79.
- 799 **78. Gueguen E, Cascales E.** 2013. Promoter swapping unveils the role of the *Citrobacter rodentium*
800 CTS1 type VI secretion system in interbacterial competition. *Appl Environ Microbiol*. **79**:32-8.
- 801 **79. Parsons DA, Heffron F.** 2005. *sciS*, an *icmF* homolog in *Salmonella enterica* serovar
802 Typhimurium, limits intracellular replication and decreases virulence. *Infect Immun*. **73**:4338-45.
- 803 **80. de Pace F, Nakazato G, Pacheco A, de Paiva JB, Sperandio V, da Silveira WD.** 2010. The type
804 VI secretion system plays a role in type 1 fimbria expression and pathogenesis of an avian pathogenic
805 *Escherichia coli* strain. *Infect Immun*. **78**:4990-8.
- 806 **81. de Pace F, Boldrin de Paiva J, Nakazato G, Lancellotti M, Sircili MP, Guedes Stehling E,**
807 **Dias da Silveira W, Sperandio V.** 2011. Characterization of IcmF of the type VI secretion system in
808 an avian pathogenic *Escherichia coli* (APEC) strain. *Microbiology*. **157**:2954-62.
- 809 **82. Lloyd AL, Henderson TA, Vigil PD, Mobley HL.** 2009. Genomic islands of uropathogenic
810 *Escherichia coli* contribute to virulence. *J Bacteriol*. **191**:3469-81.
- 811 **83. Zhou Y, Tao J, Yu H, Ni J, Zeng L, Teng Q, Kim KS, Zhao GP, Guo X, Yao Y.** 2012. Hcp
812 family proteins secreted via the type VI secretion system coordinately regulate *Escherichia coli* K1
813 interaction with human brain microvascular endothelial cells. *Infect Immun*. **80**:1243-51.
- 814 **84. Libby SJ, Brehm MA, Greiner DL, Shultz LD, McClelland M, Smith KD, Cookson BT,**
815 **Karlinsey JE, Kinkel TL, Porwollik S, Canals R, Cummings LA, Fang FC.** 2010. Humanized
816 nonobese diabetic-scid IL2r gammanull mice are susceptible to lethal *Salmonella* Typhi infection.
817 *Proc Natl Acad Sci USA*. **107**:15589-94.
- 818 **85. Klumpp J, Fuchs TM.** 2007. Identification of novel genes in genomic islands that contribute to
819 *Salmonella typhimurium* replication in macrophages. *Microbiology*. **153**:1207-20.
- 820 **86. Liu J, Guo JT, Li YG, Johnston RN, Liu GR, Liu SL.** 2013. The type VI secretion system gene
821 cluster of *Salmonella typhimurium*: required for full virulence in mice. *J Basic Microbiol*. **53**:600-7.
- 822 **87. Blondel CJ, Jiménez JC, Leiva LE, Alvarez SA, Pinto BI, Contreras F, Pezoa D, Santiviago**
823 **CA, Contreras I.** 2013. The type VI secretion system encoded in *Salmonella* pathogenicity island 19

- 824 is required for *Salmonella enterica* serotype Gallinarum survival within infected macrophages. *Infect*
825 *Immun.* **81**:1207-20.
- 826 **88. Blondel CJ, Yang HJ, Castro B, Chiang S, Toro CS, Zaldívar M, Contreras I, Andrews-**
827 **Polymenis HL, Santiviago CA.** 2010. Contribution of the type VI secretion system encoded in SPI-
828 19 to chicken colonization by *Salmonella enterica* serotypes Gallinarum and Enteritidis. *PLoS One.*
829 **5**:e11724.
- 830 **89. Pezoa D, Yang HJ, Blondel CJ, Santiviago CA, Andrews-Polymenis HL, Contreras I.** 2013.
831 The type VI secretion system encoded in SPI-6 plays a role in gastrointestinal colonization and
832 systemic spread of *Salmonella enterica* serovar Typhimurium in the chicken. *PLoS One.* **8**:e63917.
- 833 **90. Dong TG, Ho BT, Yoder-Himes DR, Mekalanos JJ.** Identification of T6SS-dependent effector
834 and immunity proteins by Tn-seq in *Vibrio cholerae*. *Proc Natl Acad Sci USA.* **110**:2623-8.
- 835 **91. Zhang H, Zhang H, Gao ZQ, Wang WJ, Liu GF, Xu JH, Su XD, Dong YH.** 2013. Structure of
836 the type VI effector-immunity complex (Tae4-Tai4) provides novel insights into the inhibition
837 mechanism of the effector by its immunity protein. *J Biol Chem.* **288**:5928-39.
- 838 **92. Benz J, Reinstein J, Meinhart A.** 2013. Structural insights into the effector-immunity system
839 Tae4/Tai4 from *Salmonella typhimurium*. *PLoS One.* **8**:e67362.
- 840 **93. Koskiniemi S, Garza-Sánchez F, Sandegren L, Webb JS, Braaten BA, Poole SJ, Andersson**
841 **DI, Hayes CS, Low DA.** 2014. Selection of orphan Rhs toxin expression in evolved *Salmonella*
842 *enterica* serovar Typhimurium. *PLoS Genet.* **10**:e1004255.
- 843 **94. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K.**
844 2014. Marker for type VI secretion system effectors. *Proc. Natl. Acad. Sc. USA.* **111**:9271-76.
- 845 **95. Leung KY, Siame BA, Snowball H, Mok YK.** 2011. Type VI secretion regulation: crosstalk and
846 intracellular communication. *Curr Opin Microbiol.* **14**:9-15.
- 847 **96. Mougous JD, Gifford CA, Ramsdell TL, Mekalanos JJ.** 2007. Threonine phosphorylation post-
848 translationally regulates protein secretion in *Pseudomonas aeruginosa*. *Nat Cell Biol.* **9**:797-803.
- 849 **97. Hsu F, Schwarz S, Mougous JD.** 2009. TagR promotes PpkA-catalysed type VI secretion
850 activation in *Pseudomonas aeruginosa*. *Mol Microbiol.* **72**:1111-25.
- 851 **98. Silverman JM, Austin LS, Hsu F, Hicks KG, Hood RD, Mougous JD.** 2011. Separate inputs
852 modulate phosphorylation-dependent and -independent type VI secretion activation. *Mol Microbiol.*
853 **82**:1277-90.
- 854 **99. Casabona MG, Silverman JM, Sall KM, Boyer F, Couté Y, Poirel J, Grunwald D, Mougous**
855 **JD, Elsen S, Attree I.** 2013. An ABC transporter and an outer membrane lipoprotein participate in
856 posttranslational activation of type VI secretion in *Pseudomonas aeruginosa*. *Environ Microbiol.*
857 **15**:471-86.
- 858 **100. Lin JS, Wu HH, Hsu PH, Ma LS, Pang YY, Tsai MD, Lai EM.** 2014. Fha interaction with
859 phosphothreonine of TssL activates type VI secretion in *Agrobacterium tumefaciens*. *PLoS Pathog.*
860 **10**:e1003991.
- 861 **101. Escolar L, Pérez-Martín J, de Lorenzo V.** 1999. Opening the iron box: transcriptional
862 metalloregulation by the Fur protein. *J Bacteriol.* **181**:6223-9.

- 863 **102. Morin N, Santiago AE, Ernst RK, Guillot SJ, Nataro JP.** 2013. Characterization of the AggR
864 regulon in enteroaggregative *Escherichia coli*. *Infect Immun.* **81**:122-32.
- 865 **103. Rossiter AE, Browning DF, Leyton DL, Johnson MD, Godfrey RE, Wardius CA, Desvaux**
866 **M, Cunningham AF, Ruiz-Perez F, Nataro JP, Busby SJ, Henderson IR.** 2011. Transcription of
867 the plasmid-encoded toxin gene from enteroaggregative *Escherichia coli* is regulated by a novel co-
868 activation mechanism involving CRP and Fis. *Mol Microbiol.* **81**:179-91.
- 869 **104. Fass E, Groisman EA.** 2009. Control of *Salmonella* pathogenicity island-2 gene expression.
870 *Curr Opin Microbiol.* **12**:199-204.
- 871 **105. Tomljenovic-Berube AM, Mulder DT, Whiteside MD, Brinkman FS, Coombes BK.** 2010.
872 Identification of the regulatory logic controlling *Salmonella* pathoadaptation by the SsrA-SsrB two-
873 component system. *PLoS Genet.* **6**:e1000875.
- 874 **106. Kröger C, Dillon SC, Cameron AD, Papenfort K, Sivasankaran SK, Hokamp K, Chao Y,**
875 **Sittka A, Hébrard M, Händler K, Colgan A, Leekitcharoenphon P, Langridge GC, Lohan AJ,**
876 **Loftus B, Lucchini S, Ussery DW, Dorman CJ, Thomson NR, Vogel J, Hinton JC.** 2012. The
877 transcriptional landscape and small RNAs of *Salmonella enterica* serovar Typhimurium. *Proc Natl*
878 *Acad Sci USA.* **109**:E1277-86.
- 879 **107. Hautefort I, Thompson A, Eriksson-Ygberg S, Parker ML, Lucchini S, Danino V,**
880 **Bongaerts RJ, Ahmad N, Rhen M, Hinton JC.** 2008. During infection of epithelial cells *Salmonella*
881 *enterica* serovar Typhimurium undergoes a time-dependent transcriptional adaptation that results in
882 simultaneous expression of three type 3 secretion systems. *Cell Microbiol.* **10**:958-84.
- 883 **108. Navarre WW, Porwollik S, Wang Y, McClelland M, Rosen H, Libby SJ, Fang FC.** 2006.
884 Selective silencing of foreign DNA with low GC content by the H-NS protein in *Salmonella*. *Science.*
885 **313**:236-8.
- 886 **109. Lucchini S, Rowley G, Goldberg MD, Hurd D, Harrison M, Hinton JC.** 2006. H-NS
887 mediates the silencing of laterally acquired genes in bacteria. *PLoS Pathog.* **2**:e81.
- 888 **110. Navarre WW, McClelland M, Libby SJ, Fang FC.** 2007. Silencing of xenogeneic DNA by H-
889 NS-facilitation of lateral gene transfer in bacteria by a defense system that recognizes foreign DNA.
890 *Genes Dev.* **21**:1456-71.
- 891 **111. Wang M, Luo Z, Du H, Xu S, Ni B, Zhang H, Sheng X, Xu H, Huang X.** 2011. Molecular
892 characterization of a functional type VI secretion system in *Salmonella enterica* serovar Typhi. *Curr*
893 *Microbiol.* **63**:22-31.
- 894 **112. Sabag-Daigle A, Dyszel JL, Gonzalez JF, Ali MM, Ahmer BM.** 2015. Identification of *sdjA*-
895 regulated genes in a mouse commensal strain of *Enterobacter cloacae*. *Front Cell Infect Microbiol.*
896 **5**:47.
- 897 **113. Gueguen E, Wills NM, Atkins JF, Cascales E.** 2014. Transcriptional frameshifting rescues
898 *Citrobacter rodentium* type VI secretion by the production of two length variants from the
899 prematurely interrupted *tssM* gene. *PLoS Genet.* **10**:e1004869.
- 900 **114. Li J, Yao Y, Xu HH, Hao L, Deng Z, Rajakumar K, Ou HY.** 2015. SecReT6: a web-based
901 resource for type VI secretion systems found in bacteria. *Environ Microbiol.* **17**:2196-202.
- 902

903 LEGEND TO FIGURES

904 **Figure 1. Genetic organization and general architecture of the T6SS.** (A) Schematic representation
905 of the T6SS core genes. Genes are specified by a letter corresponding to the Tss nomenclature (“A”
906 corresponding to “TssA”) or by their vernacular, usual names (Hcp, VgrG, PAAR and ClpV). The
907 color code is shared with panels B and C. (B) Architecture of the T6SS. The membrane complex,
908 composed of the TssJ lipoprotein (orange) and the TssM (blue) and TssL (red) inner membrane
909 proteins, is indicated (OM, outer membrane; PG, cell wall; IM, inner membrane). The different
910 regions of the tail (spike, tube, sheath & baseplate) are shown. (C) Architecture of a contractile tailed
911 bacteriophage. Components that are shared with the T6SS (spike, tube, sheath & baseplate) are
912 depicted with the same color code (LTF, long tail fibers).

913 **Figure 2. Interbacterial competition between *E. coli* cells.** Time-lapse fluorescence microscopy
914 recordings of GFP-labeled EAEC T6SS⁺ cells (green) in presence of mCherry-labeled T6SS⁻ prey
915 bacterial cells (red) in T6SS-3 inducing conditions (one image every 7.5 min). Prey cells that are
916 killed and not present in the next frame are indicated by white arrows. Scale bar is 5 μm.

917 **Figure 3. Mechanism of action of the T6SS.** The biogenesis of the T6SS starts with the assembly of
918 the TssJLM membrane complex (MC) and recruitment of the baseplate complex (BC) (a), which
919 serves as platform for polymerization of the tail tube/sheath structure (b, c). During elongation of the
920 tail structure, effectors (red balls) can be loaded inside the inner tube lumen or attached to the VgrG
921 spike. Following contact with a prey cell, the sheath contracts and propels the inner tube/spike towards
922 the target, allowing penetration and delivery of the effectors (d). Once contracted, the ClpV AAA+
923 ATPase is recruited to the apparatus for recycling sheath subunits (e, f). The MC (and BC ?) might be
924 re-used for a new round of assembly.

925 **Figure 4. Phylogenetic tree of selected T6SS gene clusters.** T6SS gene clusters catagorize in 5
926 phylogenetic groups (A-E) (1, 2). The distribution of the *E. coli*-associated T6SSs (T6SS-1-3, red)
927 and *Salmonella*-associated SPI T6SSs (green) is shown, as well as that of *E. cloacae* and *C. rodentium*
928 (blue) and the model T6SSs from *P. aeruginosa*, *V. cholerae* and *F. tularensis* (black).

929 **Figure 5. Organization of T6SS-1-3 gene clusters.** Genes encoding the T6SS-1 (A), T6SS-2 (B) and
930 T6SS-3 (C) in the indicated *E. coli* strains are shown schematically. Homologous genes are colored
931 similarly (see box below). When predictable, putative phospholipase effector/immunity pairs
932 (Tle1/Tli1, Tle3/Tli3 or Tle4/Tli4) or *rhs* genes are indicated. ORFs with unknown function are shown
933 in white. Genes into brackets are not present or not identical in all the strains listed. Genes were
934 identified using the SecReT6 database (114).

935 **Figure 6. Organization of T6SS gene clusters in *Salmonella*, *Enterobacter* and *Citrobacter*.** Genes
936 encoding the T6SS in the indicated strains are shown schematically. Homologous genes are colored
937 similarly (see box in Fig. 5). When predictable, *rhs* genes are indicated. The *rhs*^{main} and *rhs*^{orphan} ORF
938 shown to undergoes re-arrangements (93) are indicated in the *S. enterica* Typhimurium SPI-6 gene
939 cluster, as well as the Tae4/Tai4 effector/immunity pairs in *S. enterica* Typhimurium SPI-6 and *E.*
940 *cloacae*. ORFs with unknown function are shown in white. Genes were identified using the SecReT6
941 database (114). Note that the transcription of the *C. rodentium* *tssM* gene, interrupted by an early stop
942 codon, is rescued by frameshifting (113).

943 **Figure 7. Architecture and structure of the T6SS membrane complex.** (A) The *tssJ*, *tssL* and *tssM*
944 genes which encode the components of the membrane complex. (B) Schematic representation of the
945 TssJ, -L and -M proteins : TssJ is an outer membrane (OM)-tethered lipoprotein whereas TssL and
946 TssM are inner membrane (IM)-embedded proteins. In T6SS-1, the membrane complex comprises an
947 additional protein, TagL, which binds to the peptidoglycan (PG) layer (not depicted here) (27). (C)
948 Crystal structure of the complex between the soluble fragment of TssJ (orange) and the two C-terminal
949 domains of the TssM periplasmic segment (light and dark blue) including the C-terminal helix that

950 inserts into the outer membrane (in purple) from EAEC T6SS-1 (PDB: 4Y7O) (Reprinted from
951 reference 26 with permissions). (D) Crystal structure of the cytoplasmic domain of TssL from EAEC
952 T6SS-1 (PDB: 3U66) (33). (E) Negative stain electron microscopy structure reconstruction of the
953 EAEC TssJLM complex (lower panel, EMDB: 2927) (adapted from reference 26 with permissions)
954 (scale bar is 50 nm). The position of the outer (OM) and inner (IM) membranes are predicted based on
955 the presence of detergent micelle and the putative location of the trans-membrane segments of TssM,
956 respectively. In the upper panel is shown a top view of the TssJLM complex in which crystal
957 structures of the TssJ-M complex (panel C) are docked, highlighting the presence of two concentric
958 layers closing the channel at the outer membrane.

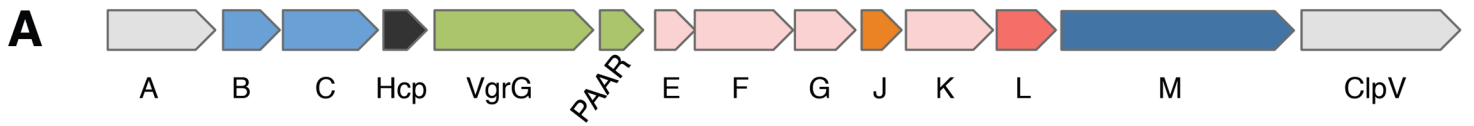
959 **Figure 8. Architecture and structure of the T6SS tail complex.** (A) The *tssA*, *tssB*, *tssC*, *tssE*, *tssF*,
960 *tssG*, *tssK*, *hcp*, *vgrG* and *paar* genes which encode the components of the tail complex (blue, sheath
961 subunits; black, inner tube subunit; green, spike subunits; pink, putative baseplate subunits). (B)
962 Schematic representation of the T6SS tail complex (same color code as panel A). (C) Structural model
963 of EAEC T6SS-1 TssE based on the bacteriophage gp25 crystal structure (PDB: 4HRZ). (D)
964 Composite structure made with the crystal structures (from bottom to top) of the UPEC CTF073
965 VgrG1 protein (PDB : 2P57) (39), the *E. coli* O157 EDL933 β -helical prism (PDB: 3WIT) (40) and
966 the *E. coli* O6 PAAR protein (PDB: 4JIW) (41). (E) Crystal structure of the EAEC T6SS-1 Hcp
967 hexamer (left, top view; right, side view) (PDB: 4HKH) (42). (F) Cryo-electron micrograph of a
968 contracted T6SS sheath from *V. cholerae* (left panel, scale bar is 100 nm) and atomic-resolution cryo-
969 electron structure of the TssB-C complex (PDB: 3J9G) (47) (adapted from reference 47 with
970 permissions).

971 **Figure 9. T6SS sheath contraction coincides with target cell lysis.** Time-lapse fluorescence
972 microscopy recordings of EAEC producing fluorescently-labeled sheath subunits (TssB-sfGFP) in
973 presence of mCherry-labeled T6SS⁻ *E. coli* K-12 prey cells (one image every 7.5 min). The time-lapse
974 highlights the assembly and the contraction (white arrow) of the T6SS sheath, followed by the lysis of
975 the target cell. Scale bar is 1 μ m. Adapted from reference 22 with permissions.

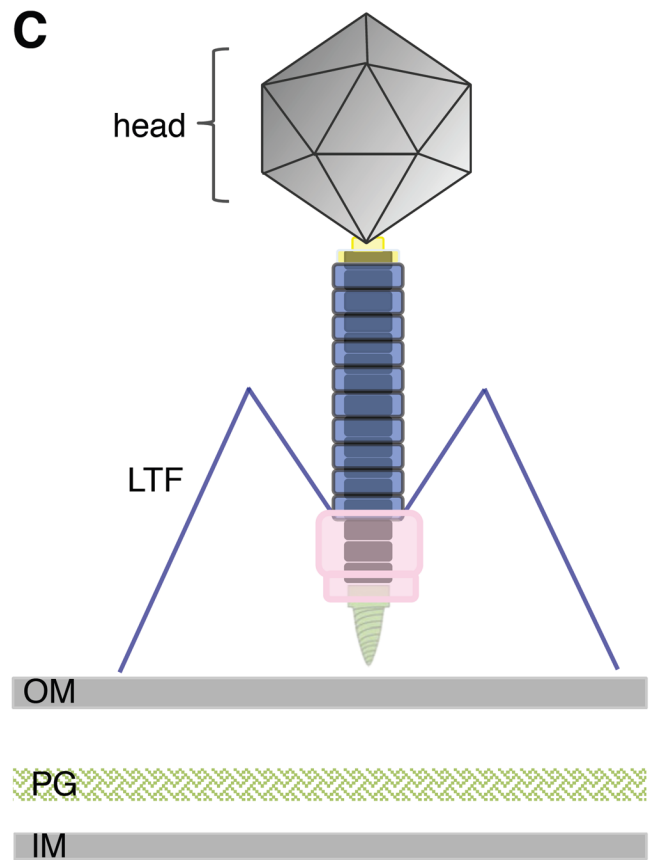
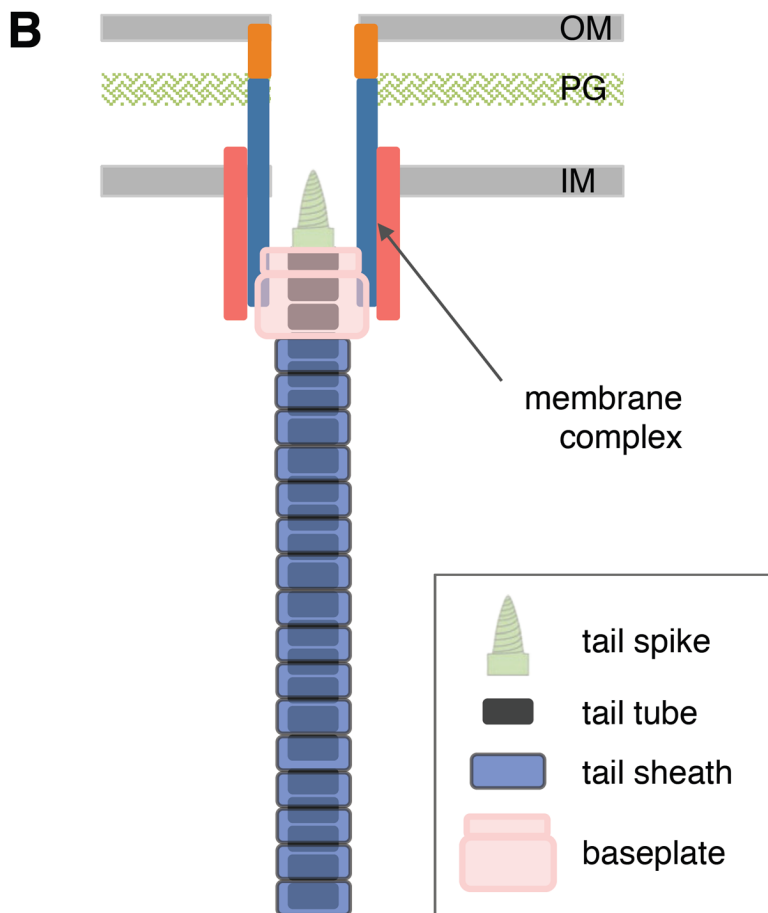
976 **Figure 10. Regulation of the EAEC T6SS-1 gene cluster.** (A) Schematic representation of the
977 promoter organization of the EAEC T6SS-1 gene cluster. The location of the -10 and -35
978 transcriptional elements (blue), of the Fur-binding sequences (red) and of one of the GATC site
979 (green) are shown. (B) Regulatory mechanism of the EAEC T6SS-1 gene cluster (23). In iron-replete
980 conditions, a Fur dimer (red balls) represses the expression of the T6SS-1 gene cluster by binding to
981 the Fur⁻¹⁰ box, which overlaps with the -10 element (OFF). When iron is limiting, the -10 element is
982 available for the RNA polymerase allowing expression of the T6SS-1 genes (ON). Upon replication,
983 the GATC site is methylated (CH₃) and by preventing Fur binding allows Fur-independent,
984 constitutive expression of the T6SS-1 gene cluster.

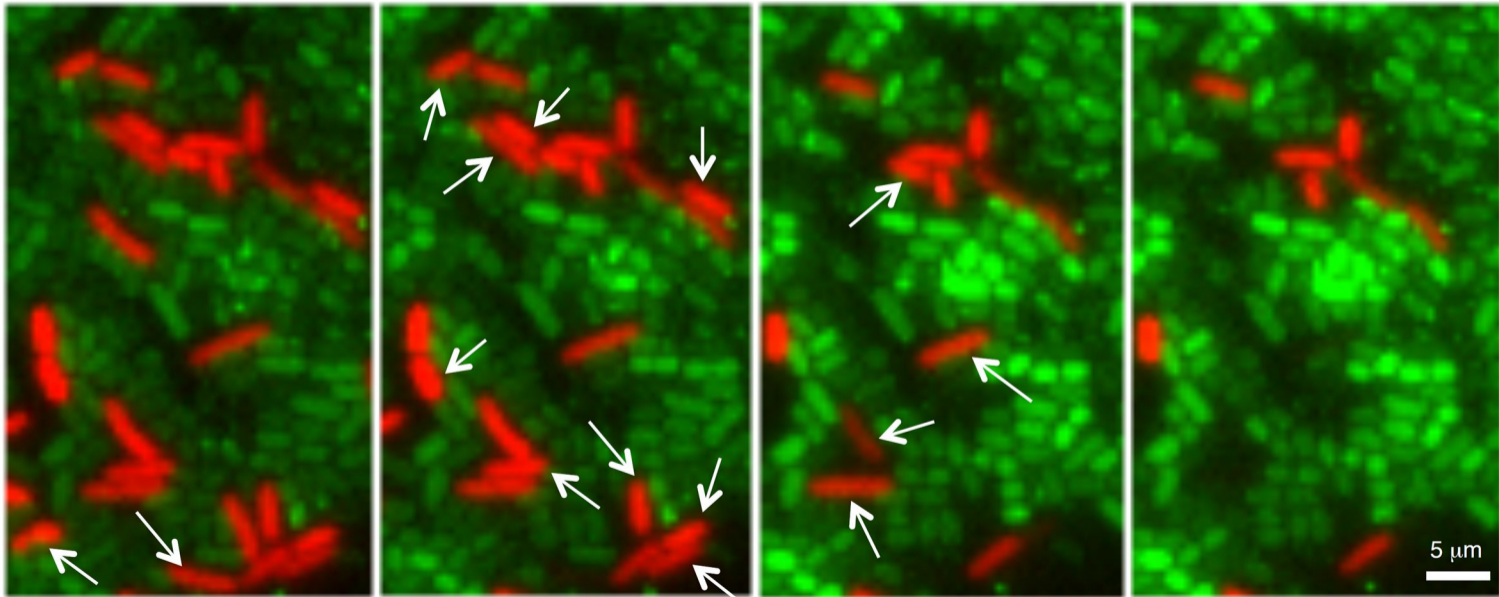
T6SS	pathotype/ serotype	strain	activity	effector	references
T6SS-1	EAEC	17-2	biofilm	-	28
			anti-bacterial	Tle1 (putative)	-
	APEC	TW-XM	biofilm	-	77
			anti-bacterial	Tle4	77
T6SS-2	APEC	DE719	attenuated virulence in ducks, reduced intracellular in chicken macrophages	-	12
		SEPT362	attenuated virulence in chicks	-	80
		TW-XM	penetration of the blood-brain barrier during cerebral infection	-	77
	MNEC	K1	invasion of human brain microvascular endothelial cells	-	83
T6SS-3	EAEC	17-2	anti-bacterial	-	22
SPI-6	<i>S. Typhimurium</i>	LT2	affect replication in macrophage and systemic dissemination in mice and in chicks	-	17, 79, 85, 86, 89
			anti-bacterial	Tae4	19, 92
			anti-bacterial	Rhs ^{orphan}	93
	<i>S. Typhi</i>	Ty2	systemic infection in mice	-	84
	<i>E. cloacae</i>	ATCC13047	anti-bacterial (putative)	Tae4	18, 73
SPI-19	<i>S. Gallinarum</i>	287/91	colonization of the gastrointestinal tract and systemic dissemination in chicks	-	88
CTS1	<i>C. rodentium</i>	IC68	anti-bacterial	-	78

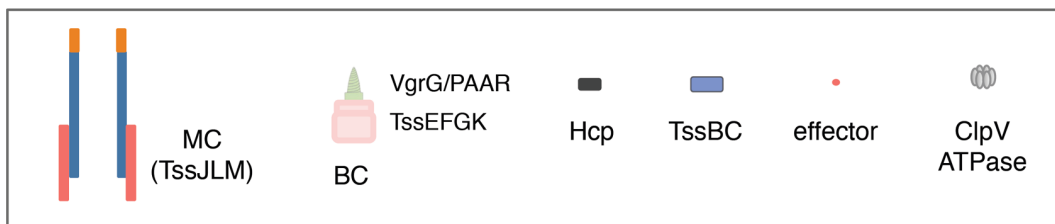
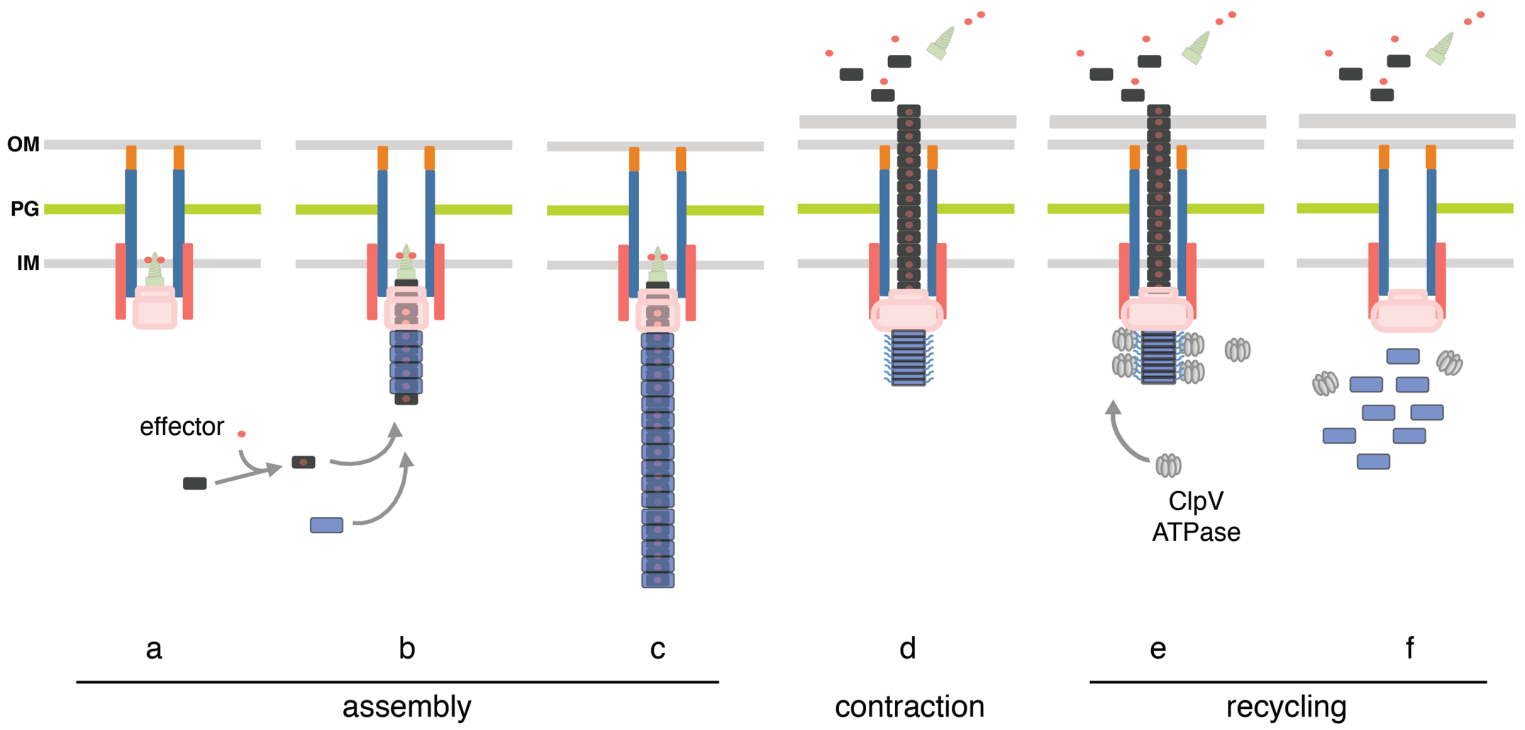
Table 1. Phenotypes and effectors associated with T6SS in *E. coli*, *Salmonella* and related species.

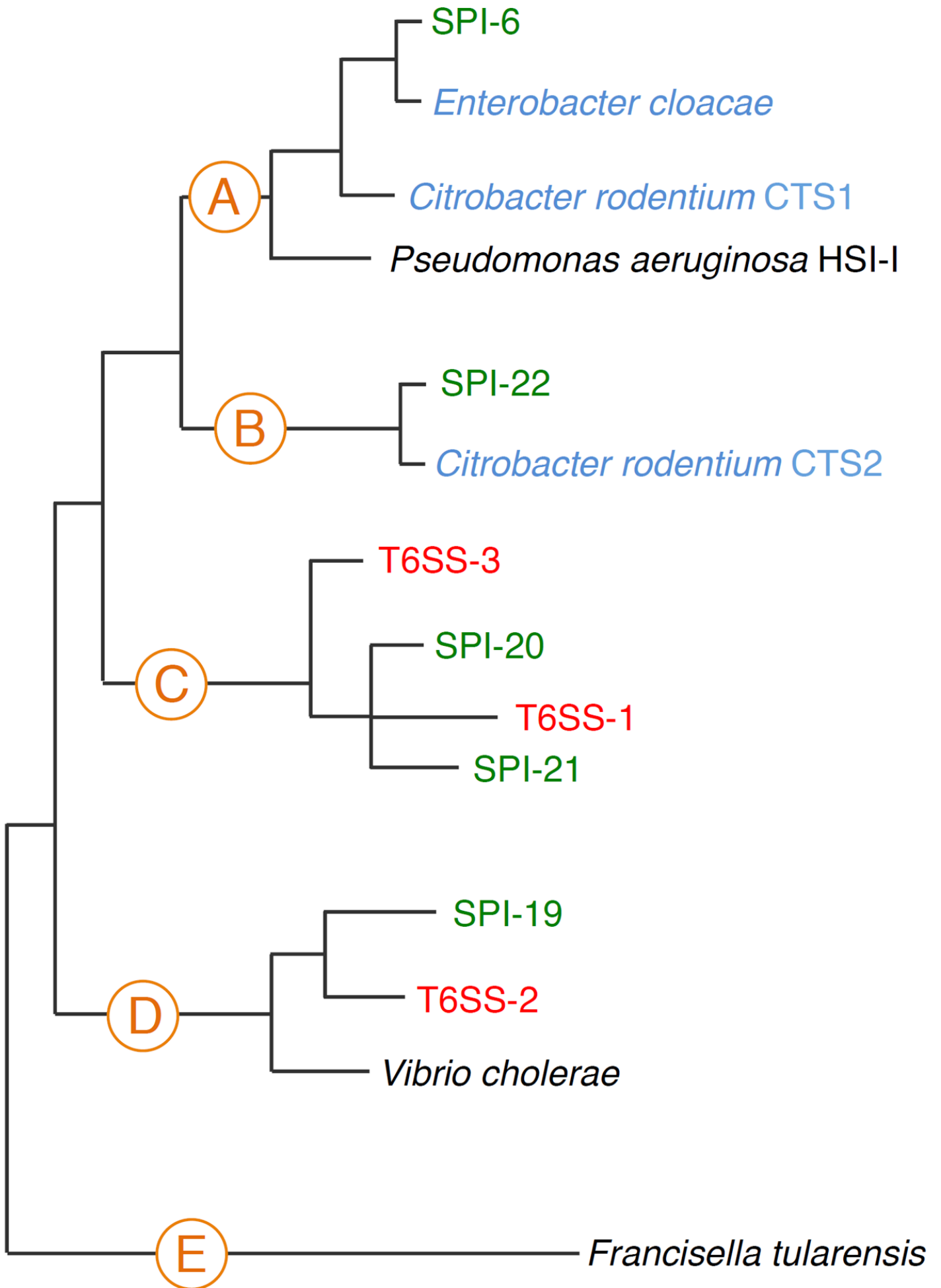


1 kb



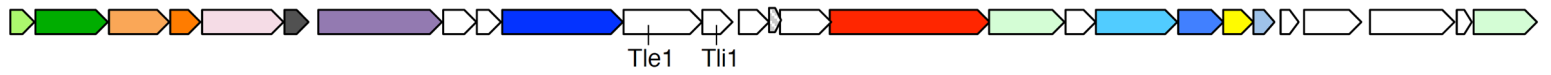




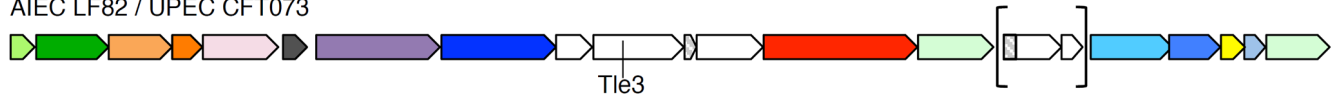


A T6SS-1

EAEC 042



AIEC LF82 / UPEC CFT073

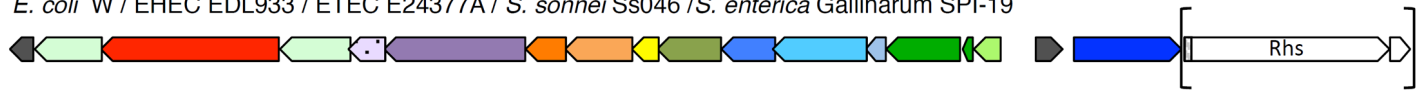


UPEC UT189 / APEC TW-XM / APEC ED205

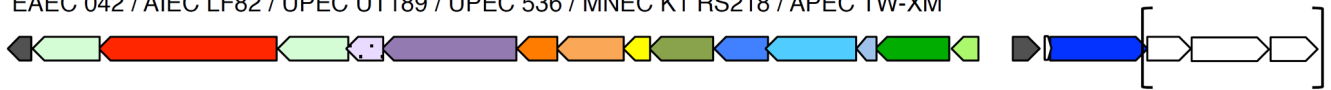


B T6SS-2

E. coli W / EHEC EDL933 / ETEC E24377A / *S. sonnei* Ss046 / *S. enterica* Gallinarum SPI-19



EAEC 042 / AIEC LF82 / UPEC UT189 / UPEC 536 / MNEC K1 RS218 / APEC TW-XM

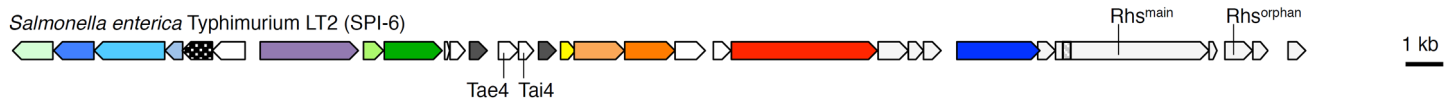


C T6SS-3

EAEC 042 / APEC ED205 / STEC/EAEC O104:H4



Salmonella enterica Typhimurium LT2 (SPI-6)



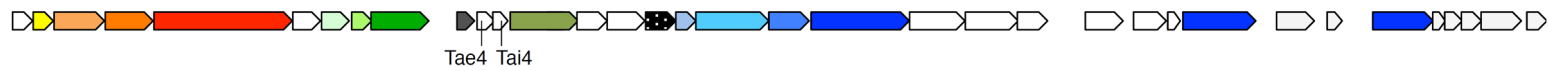
Salmonella enterica Typhi GIFU100007 (SPI-6)



Salmonella enterica Gallinarum (SPI-19)

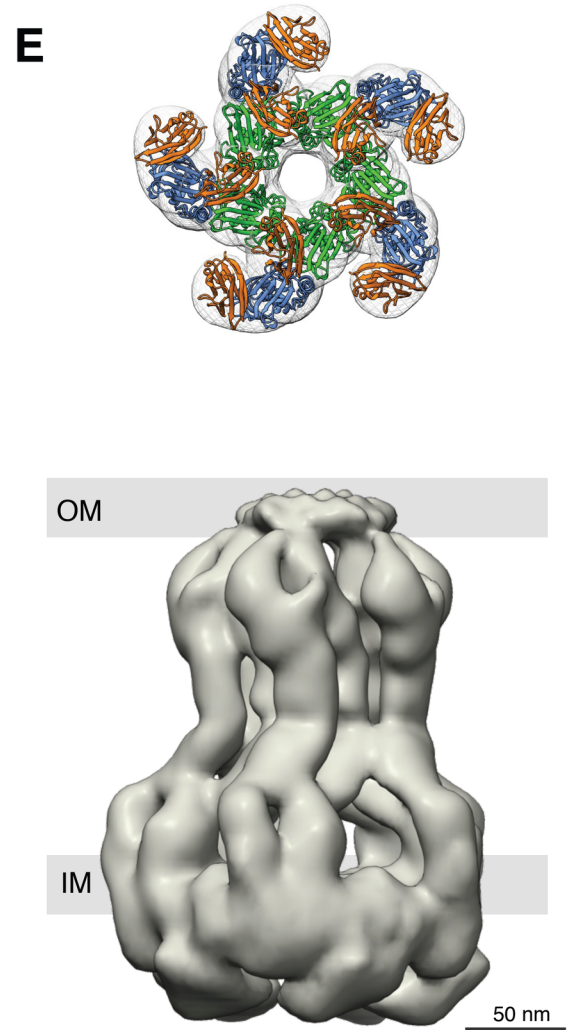
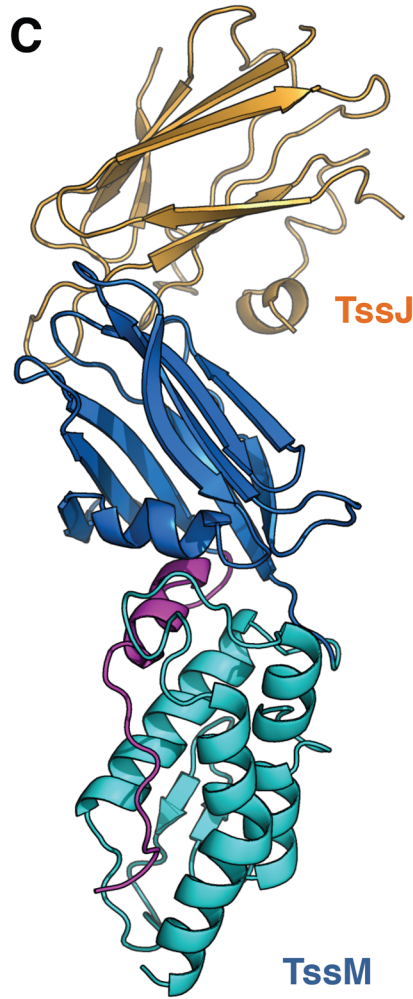
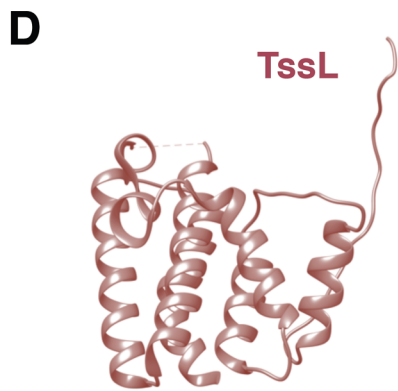
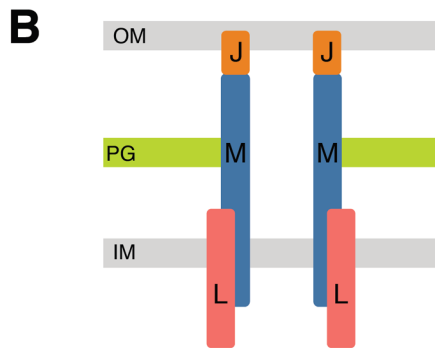


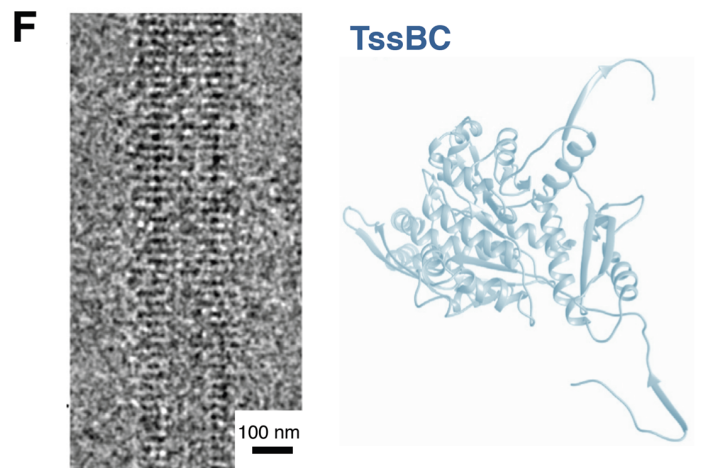
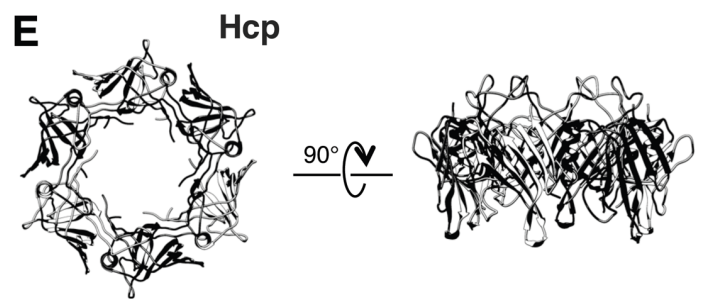
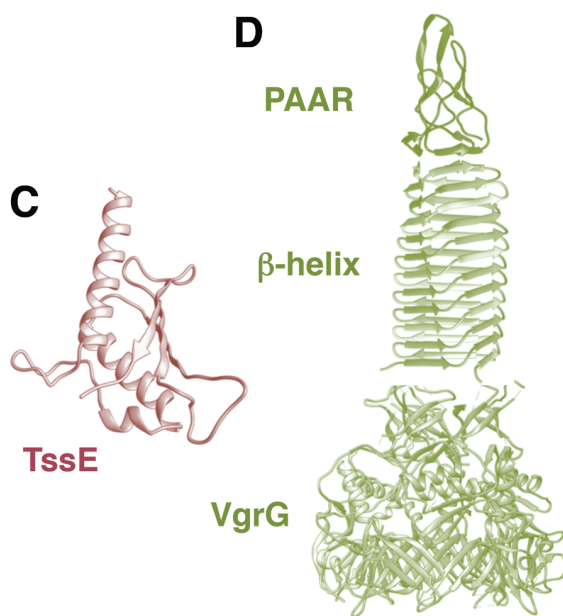
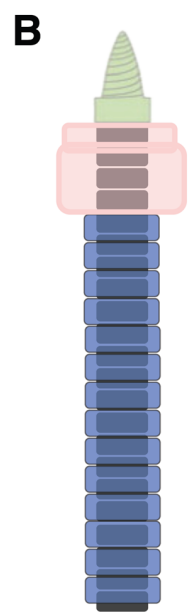
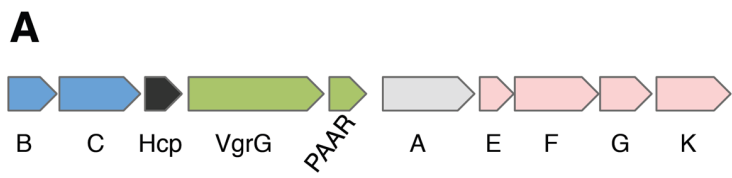
Enterobacter cloacae ATCC13047



Citrobacter rodentium CTS1



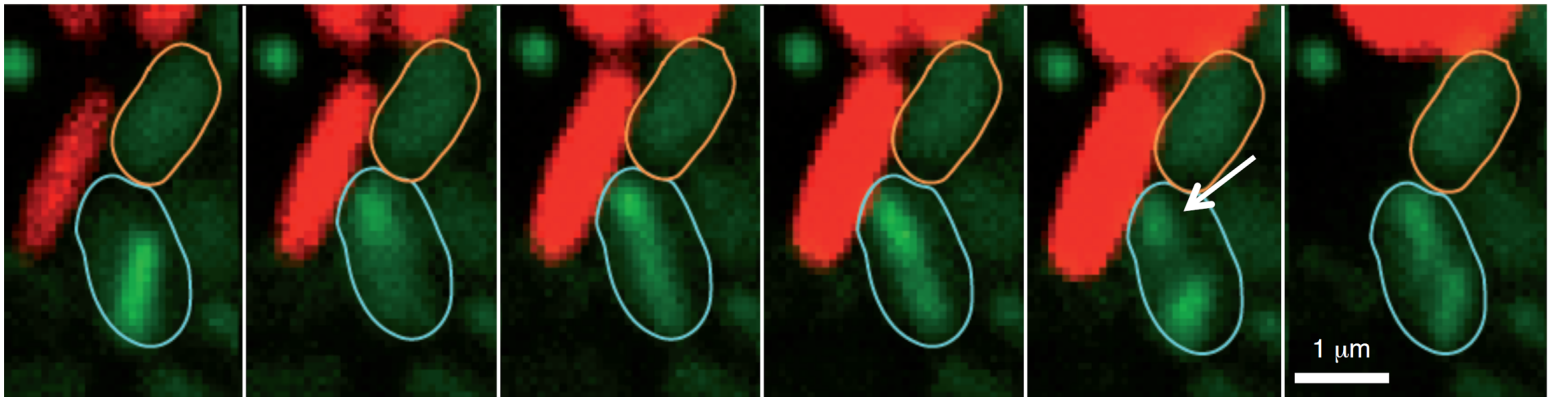




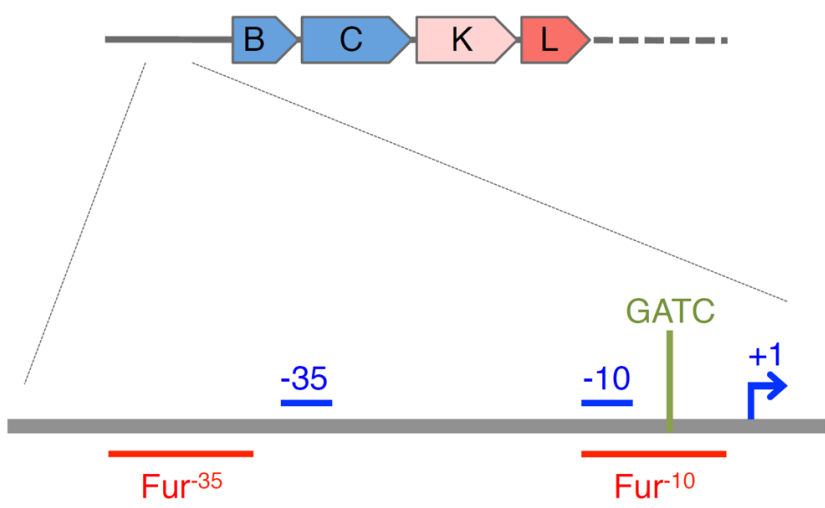
assembly

contraction

prey lysis



A



B

