Type VI secretion system effectors: poisons with a purpose

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Abstract | The type VI secretion system (T6SS) mediates interactions between a broad range of Gram-negative bacterial species. Recent studies have led to a substantial increase in the number of characterized T6SS effector proteins and a more complete and nuanced view of the adaptive importance of the system. Although the T6SS is most often implicated in antagonism, in this Review, we consider the case for its involvement in both antagonistic and non-antagonistic behaviours. Clarifying the roles that type VI secretion has in microbial communities will contribute to broader efforts to understand the importance of microbial interactions in maintaining human and environmental health, and will inform efforts to manipulate these interactions for therapeutic or environmental benefit.

Haemolysin co-regulated protein
(Hcp). A ring-shaped substrate and structural component of the type VI secretion system. It is structurally related to the T4 bacteriophage tail tube protein gp19.

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Bacteria colonize almost every imaginable habitat on earth, and many environments, ranging from soil to vertebrate digestive tracts, harbour a wide range of bacterial species[1,2]. In other habitats, such as in the light organ of the bobtail squid[3] and in certain acute infections, single species of bacteria can dominate; however, even when the diversity of bacterial species is low, individual bacterial cells rarely live in isolation. Instead, they typically grow, divide and die in close proximity to other bacterial cells. Accordingly, every aspect of bacterial growth and physiology has the potential to be influenced by interbacterial interactions. New mechanisms by which bacteria interact continue to be discovered, and range from simple competition for nutrients to highly evolved symbioses, as in the formation of metabolically interdependent structured consortia[3–6]. Evidence from a variety of habitats now suggests that the outcome of interbacterial interactions can have important consequences for ecosystem function as well as for human health[7–9].

It was recently discovered that one mechanism by which Gram-negative cells that are in close proximity to each other can interact is by contact-dependent transport of proteins from a donor cell to a recipient cell via the activity of an apparatus known as the type VI secretion system (T6SS)[10] (BOX 1). This system was initially found to deliver effector proteins into eukaryotic cells; however, it has since been shown to more often mediate interbacterial interactions[11] (BOX 2). The T6SS, which is typically encoded by clusters of contiguous genes, is a complex structure that is composed of 13 conserved proteins and a variable complement of accessory elements. T6SSs are widely distributed in the genomes of Proteobacteria — in free-living and in eukaryote-associated species, including both pathogens and symbionts of animals and plants — and some species encode as many as six phylogenetically and functionally divergent systems[12–14].

The first indication that type VI secretion (T6S) could be involved in interbacterial interactions came from the identification of three effector proteins that are secreted by the haemolysin co-regulated protein (Hcp) secretion island-I-encoded T6SS of *Pseudomonas aeruginosa* (H1-T6SS)[10]. Each of these effectors has toxicity towards bacteria and is encoded adjacent to a gene encoding a product that provides immunity to the toxin, thereby preventing self-intoxication[15]. Growth competition assays between a donor strain that was capable of toxin secretion and a recipient strain that was engineered to lack one or more effector–immunity (E–I) gene pairs showed that effectors are translocated between bacteria through the T6SS and that this process confers a significant fitness advantage to donor strains. As the T6SSs of additional bacteria are studied and their effectors are identified, it is becoming evident that the delivery of toxic effectors to other bacterial cells is a fundamental activity of the system.

Although we now know that many bacterial species, including the pathogenic organisms *Serratia marcescens*, *Vibrio cholerae* and *P. aeruginosa*, are equipped to deliver effector proteins to other bacteria via T6S under laboratory conditions, the importance of this activity in natural communities remains unclear[10,16,17]. The toxicity of the bacteria-targeted effectors that have been identified so far suggests that T6S is important for bacterial

Box 1 | **Structure and function of the T6SS**

The type VI secretion system (T6SS) is thought to consist of two main complexes in association with additional bridging and cytoplasmic elements: a membrane-associated assembly, which includes two proteins that are homologous to elements of bacterial type IV secretion systems, and an assembly with components that structurally resemble the bacteriophage sheath, tube and tail spike proteins[129–131]. These two subassemblies work together by an unknown mechanism to translocate effector proteins across the envelope of the donor cell and then through the outer membrane of a recipient cell. Although the superstructure of the T6SS remains unsolved, analyses of the individual components have produced a theoretical model by which the system might function (reviewed in REFS 132,133).

Current hypotheses on the function of the T6SS predominantly focus on the constituents of the phage-like subassembly, as most available structure–function data concern this putative complex. There are several phage-like elements that constitute an active T6SS (and analogous phage proteins): TssB and TssC (bacteriophage contractile sheath), haemolysin co-regulated protein (Hcp) (gp19 tail–tube protein), TssE (gp25 baseplate assembly protein) and valine–glycine repeat protein G (VgrG) (a fusion of the gp5 and gp27 tip proteins)[129,130]. By analogy with their phage counterparts, these components of the T6SS are thought to resemble an inverted bacteriophage, in which VgrG forms a cell-puncturing tip, Hcp forms a tail–tube structure through which effector proteins might travel, and TssB and TssC form a sheath that contracts to provide energy for effector translocation. Notably, although a dynamic TssB–TssC sheath has been directly observed, the remainder of the inverted-phage hypothesis still requires *in vivo* confirmation[131].

An additional complexity of the structure of the T6SS is that Hcp and VgrG are not only essential components of the system but are also shed into the extracellular milleu on activation of the system, which indicates that these proteins have dual roles as both structural components and substrates of the T6SS[132]. Moreover, although most of the 13 core T6SS genes are found in a single copy within a given secretion locus, multiple Hcp and VgrG homologues are often associated with, and secreted by, a single T6SS[10,46,134]. This leads to the hypothesis that Hcp and VgrG proteins function as adaptors that interact with effector proteins and recruit them to the apparatus. This has been supported by the presence of *vgrG* and *hcp* genes in operons that contain type VI secretion (T6) effectors, the observation that effector fusions to both Hcp and VgrG can occur, the finding that effector-linked PAAR (Pro-Ala-Ala-Arg) domains can directly interact with VgrG and, most directly, by the finding that allele-specific interactions with the pore of Hcp are required for the stabilization and secretion of certain effectors[17,19,36,108,135,136].

competition, but it is not known to what extent toxin translocation facilitates the invasion of bacteria into new habitats or protects established populations from invasion by incoming competitors. It is also not understood to what extent T6SS-mediated antagonism facilitates competition between individual bacteria within the same species versus between species. As we discuss, there are also indications that the function of T6S can extend beyond simple antagonism. Clarifying the role that the system has in a natural setting will further our understanding of the mechanisms that shape microbial community structure and the resulting consequences for ecosystem function. In this Review, we consider the many functions that the T6SS might have in mediating interactions between diverse bacteria, focusing on the possibilities that are suggested by the activities of known interbacterial effector proteins.

T6SS effector proteins

T6SS effectors take many forms, ranging from relatively simple single-domain proteins to large multi-domain proteins that are composed of apparent fusions between toxins and secreted structural elements of the T6SS[10,18]. Despite this diversity, common themes that probably reflect highly conserved mechanistic aspects of the T6 interbacterial targeting system have emerged. Perhaps most pervasive is the co-occurrence of effectors with cognate immunity proteins[19]. These proteins are generally encoded by genes that are adjacent to cognate effector genes, and effector inactivation is achieved by a direct binding mechanism[20,21]. As most immunity proteins are only required for defence against effectors that are delivered by neighbouring cells, this co-occurrence highlights the capacity of the T6SS to attack both friend and foe.

Cell wall-degrading effectors. As the major structural component of the bacterial cell wall, peptidoglycan is a common target for antibacterial strategies. This is perhaps most convincingly shown by the convergent evolution of many interorganismal competitive strategies that target peptidoglycan[22–26]. It is therefore not surprising that enzymes that target peptidoglycan are a major component of the T6SS effector 'arsenal' (FIG. 1a). Indeed, T6S exported 1 (Tse1) and Tse3, which were the first antibacterial T6SS effectors to be biochemically characterized, both have peptidoglycan-degrading activity[15]. By targeting the peptide and glycan moieties of the molecule, respectively, these enzymes have the potential to digest the macromolecular sacculus of a typical Gram-negative bacterium into small, soluble fragments.

P. aeruginosa Tse1 is arguably the most thoroughly characterized T6SS effector. Soon after its discovery and characterization, several groups reported X-ray crystal structures, which showed that, like certain housekeeping amidases, Tse1 is a compact molecule that has a papain-like cysteine protease fold[20,27–29]. However, structural studies have so far fallen short of providing an explanation for its context-specific activity. Peptidoglycan consists of a glycan backbone that is composed of alternating β1,4-linked *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) subunits. The MurNAc residues are further modified by the addition of peptides that can vary both in length and composition, which provide structural support for the molecule by engaging in crosslinks to adjacent strands. Peptidoglycan crosslinks are asymmetric, occurring between the fourth amino acid (D-Ala) of the acyl donor peptide and the third amino acid (*meso*-diaminopimelic acid) of the acceptor peptide. Interestingly, Tse1 cleaves between the second and third residues of the peptide stem, but it shows a strong preference for this site within pentapeptide strands or for the donor strand within tetrapeptide crosslinks[15,27]. Given that peptidoglycan is first incorporated into the sacculus in the pentapeptide form, this specificity could direct Tse1 to sites of peptidoglycan synthesis, the disruption of which has catastrophic effects on the cell[24–26]. In support of this hypothesis is the observation that Tse1 is responsible for most H1-T6SS-dependent lysis of competing organisms[30].

Tse1 belongs to a larger group of T6SS effectors, which are known as the type VI amidase effector (Tae) superfamily[19]. Tae proteins comprise at least four highly divergent families (FIG. 1). Although the evolutionary relationship between these groups has not yet been

Sacculus
The total cell wall structure (which contains peptidoglycan and associated molecules) of a bacterium; the term derives from the appearance of isolated cell wall superstructures as meshwork bags.

Type IV secretion systems
Secretion systems involved in the transfer of both DNA and proteins to bacterial and eukaryotic targets.

Box 2 | **The type VI secretion system before 'T6'**

What we now know as the type VI secretion system (T6SS) was independently discovered to be a secretion system by two groups before the term 'type VI secretion system' was coined[137]. The system was first described as a cluster of impaired in nitrogen fixation (*imp*) genes in *Rhizobium leguminosarum*, as mutations in several *imp* genes, which are now known to encode core components of the secretory apparatus, were shown to influence the symbiotic host range of the organism[138]. It was further noted that *imp*-like gene clusters were conserved in assorted bacteria, that certain Imp proteins showed similarity to those that were involved in characterized secretion pathways and that *imp* mutants are defective in protein secretion. Remarkably, Spaink and colleagues concluded that the observed phenotypes in *imp* mutants, were "caused by genes that are most likely involved in the temperature-dependent secretion of proteins"[138]. Temperature-dependent type VI secretion (T6S) activation has since been observed by several groups and is now a well-studied phenomenon[49,59,61,62].

Shortly after the groundbreaking work on the *imp* genes of *R. leguminosarum*, Leung and co-workers identified the T6SS of *Edwardsiella tarda* in a mass spectrometric screen for secreted virulence factors[139]. The authors identified two secreted proteins, which they termed *E. tarda* virulence protein A (EvpA) and EvpC, and mapped the ORFs that encode these proteins to a large conserved gene cluster, *evpA–H*, that is now recognized to be a T6SS[140]. On the basis of the requirement that they observed for *evpA* and *evpB* in the export of EvpC — which is a haemolysin co-regulated protein (Hcp) homologue — the authors accurately concluded that Evp encodes a novel protein secretion system.

ascertained, all available experimental evidence indicates that the Tae proteins are functional analogues. For example, *tae* genes are invariably located adjacent to ORFs that encode periplasmic immunity determinants, known as type VI secretion amidase immunity (Tai) proteins. Further uniting the Tae superfamily is the observation that each of the members that has been characterized so far — including representatives from each family — function as an amidase that catalyses the hydrolysis of Gram-negative peptidoglycan[19, 31]. The activity of only a few Tae proteins has been examined in interbacterial competition; however, the consensus that was reached from studies that examined members of Tae1, Tae2 and Tae4 is that the proteins also show a common reliance on the T6SS in order to act effectively on target cells[16,19]. As Tae proteins are exported by multiple T6SS clades across a range of T6SS-positive organisms, the presence of shared properties of the Tae superfamily suggests that there is major functional and mechanistic conservation throughout T6S.

As mentioned above, Tse3 targets the glycan backbone of peptidoglycan rather than the peptide crosslinks. The β1,4 bonds between MurNAc and GlcNAc and between GlcNAc and MurNAc are both targets of antimicrobial enzymes, and cleavage of these bonds by muramidases and glucosaminidases, respectively, has the potential to impair the integrity of the peptidoglycan sacculus[32]. Although Tse3, which is a T6SS glycoside hydrolase that has a characterized cleavage site specificity, functions as a muramidase, this protein belongs to a larger superfamily of T6SS glycoside hydrolase effector (Tge) proteins that may additionally include glucosaminidase enzymes[15,33] (FIG. 1). Three sequence-divergent groups of Tge proteins have been identified (known as Tge1–3), and the first (Tge1) is solely composed of Tse3. Although the Tge3 family has yet to be experimentally validated, a Tge2 family member from *Pseudomonas protegens* ($Tge2^{PP}$) was shown to transit the T6SS pathway and function as an antibacterial effector. The bond cleavage specificity of $Tge2^{PP}$ has not been determined; however, the X-ray crystal structure of the protein places it in glycoside hydrolase family 73. These enzymes share the lysozyme muramidase fold, but are distinguished by a conserved active site tyrosine residue and generally have *N*-acetylglucosaminidase activity.

Beyond its posited structural role in the T6SS apparatus, the valine–glycine repeat protein G (VgrG) protein seems to directly facilitate the translocation of certain effectors and effector domains. The carboxy-terminal effector domain of *V. cholerae* VgrG3 has the predicted fold of a muramidase, causes lysis when directed to the periplasm and can degrade peptidoglycan[18] (FIG. 1a). Furthermore, the presence of a *vgrG3* cognate immunity gene is only essential in strains that have an active T6SS, which indicates that the VgrG3 protein participates in T6S-mediated intercellular competition[17]. Given that the VgrG3 effector domain is unrelated to Tge family proteins and is likely to be exported by a distinct mechanism, it seems to represent a distinct family of muramidase effectors. VgrG3 also provides the first example of a VgrG effector domain that participates in interbacterial T6S, as all previously characterized VgrG effector domains mediate microorganism–host interactions[34,35]. The finding that effectors that belong to the same catalytic class and are functional in the same compartment of the cell can be both fusion proteins and independent effectors has important implications. It suggests that the effector domains of specialized VgrG proteins can be derived from pre-existing effectors and that the evolutionary pressure that drives the formation of VgrG–effector fusions is not necessarily related to unique delivery requirements or target organisms. Instead, the selective pressure that drives VgrG–effector fusions is more commonplace and may be related to achieving efficient co-regulation and accurate stoichiometry.

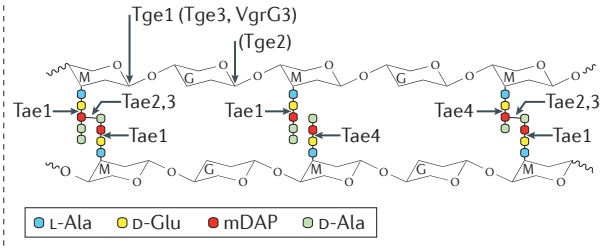
Cell membrane targeting. The cell membrane, like the cell wall, is a conserved and essential component of the bacterial cell. It is therefore not surprising that this structure is also a target of T6SS effectors (FIG. 1b). A group of T6SS phospholipase effectors, known as the type VI lipase effector (Tle) proteins, directly target the bacterial membrane by hydrolysing its component lipids[36]. There are five known Tle families, Tle1–5, that further distribute into two catalytic classes: those that seem to use a nucleophilic serine in a Gly-X-Ser-X-Gly (where X means any amino acid) motif (such as Tle1–4), and those that use dual catalytic histidine residues in His-X-Lys-X-X-X-X-Asp motifs (such as Tle5). The Tle immunity proteins known as type VI lipase immunity 1–5 (Tli1–5) localize to the periplasmic space and directly inactivate their cognate effectors, which indicates that Tle effectors are likely to initiate membrane destruction from the periplasm, similarly to cell wall-degrading effector proteins.

Two Tle families (Tle3 and Tle4) have not been confirmed beyond their bioinformatic identification, whereas representatives from Tle1, Tle2 and Tle5 have

Valine–glycine repeat protein G
(VgrG). A substrate and structural component of the type VI secretion system (T6SS). It is structurally related to the T4 bacteriophage tail spike apparatus, gp27 and gp5.

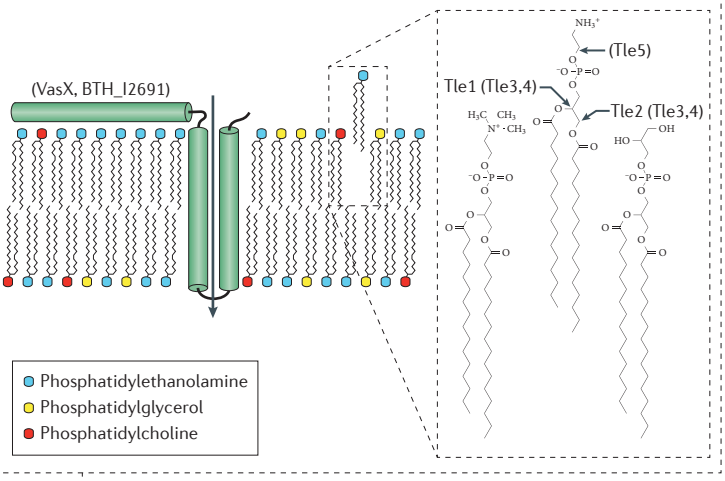
a Cell wall-targeting

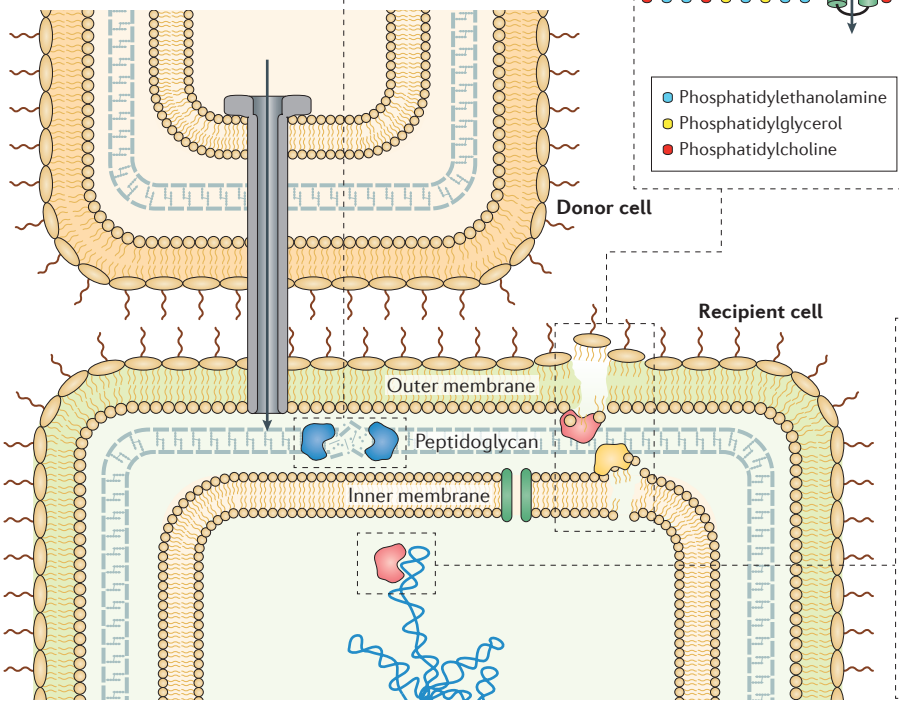
Effector class	Groups	Activity	Distribution	Number
Tae	1-4	Amidase	Beta-, Gamma-and Deltaproteobacteria	53
Tge	1-3	Glycosidase	Beta- and Gammaproteobacteria	13
VgrG3	NA	Muramidase	Gammaproteobacteria	NA



b Membrane-targeting

Effector class	Groups	Activity	Distribution	Number
Tle	1-5	Phospholipase	Beta-, Gamma-and Deltaproteobacteria	377
VasX and BTH_I2691	NA	Pore forming	Beta- and Gammaproteobacteria	NA





c Nucleic acid-targeting or cytoplasmic

Effector class	Groups	Activity	Distribution	Number
RhsA and RhsB	NA	DNase	Gammaproteo-bacteria	NA
Tse2	NA	Cyto-plasmic	Beta- and Gammaproteo-bacteria	5

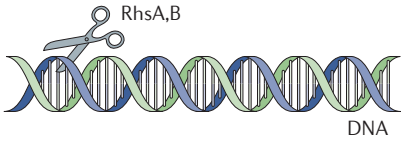


Figure 1 | **T6SS effectors target varying aspects of bacterial physiology.** Localization and activity of interbacterial T6S effectors delivered by an attacking donor cell to a recipient cell via the T6S apparatus. Effector targets include the cell wall peptidoglycan (part **a**), the inner and outer membrane (part **b**) and nucleic acids or other unknown cytoplasmic targets (part **c**). Precise enzymatic specificity is indicated, where known; parentheses indicate enzymatic activities predicted from structure–function and/or nonspecific enzymatic analyses that have yet to be biochemically confirmed. In the tables, group refers to the number of evolutionarily distinct families of effector proteins within an enzymatic class, and number to the unique instances of homologues in those groups. The numbers presented are limited to those reported in the literature; groups and numbers are provided only when a systematic effort has been made to characterize a class of effectors[10,15,16,18,19,33,36,44,136]. G, N-acetylglucosamine; M, N-acetylmuramic acid; mDAP, *meso*-diaminopimelic acid; NA, not applicable.

been both biochemically and phenotypically validated. Interestingly, these families degrade membranes by attacking different bonds in phospholipids (FIG. 1b). In addition to showing bond specificity within a given phospholipid, Tle proteins also seem to display phospholipid headgroup preference. Phospholipid analysis of cells that were intoxicated with Tle5 from *P. aeruginosa* showed that this phospholipase D enzyme has a preference for phosphatidylethanolamine, which is the major phospholipid constituent of the bacterial membrane[36].

The intact structure of peptidoglycan and even most of its subunit constituents (such as D-amino acids and MurNAc), are strictly associated with bacteria, leaving little doubt that cell wall-targeting effectors function exclusively against bacterial targets. However, the membranes of both bacteria and eukaryotes primarily consist of phospholipids, with certain constituents only differing in their relative concentrations between the two types of organisms. This fact raises the intriguing possibility that the Tle effectors could participate both

in interbacterial interactions and, perhaps opportunistically, in mediating interactions with eukaryotic cells. In support of this idea, the disruption of *P. aeruginosa tle5* and *V. cholerae tle2* attenuates these organisms in eukaryotic infection models[17,37]. The potential of Tle-based interdomain targeting by the T6SS will require additional study; however, it is worth noting that the invariable association of Tle effectors and Tli immunity proteins suggests that the basal role of these proteins lies in interbacterial interactions.

The barrier that is provided by the cellular membrane is not only susceptible to hydrolysis by phospholipases but can also be disrupted by the insertion of pore-forming proteins, which create channels that dissipate essential chemiosmotic gradients. This strategy is used by bacteria that secrete bacteriocins and has been well-studied for colicin Ia[38]. An antibacterial T6SS effector in *V. cholerae*, VasX, and a protein that is secreted by an interbacterial T6SS in *Burkholderia thailandensis* (Uniprot code BTH_I2691), both have predicted structures that closely match that of colicin Ia[17,19,39,40] (FIG. 1b). Consistent with this observation, a recent report found that VasX disrupts the inner membrane of target cells that lack the immunity protein TsiV2 (REF. 41). The relatedness of pore-forming bacteriocins and VasX-like T6S effectors may extend to the mechanism of immunity; TsiV2 and the predicted immunity proteins of VasX homologues have a membrane topology and localization that mirrors that of pore-forming bacteriocin immunity proteins[38,42].

Nucleic acid targeting. The cell envelope does not seem to contain all of the targets of the vast T6SS effector repertoire; nucleases and predicted nucleases are also represented in the T6SS interbacterial effector arsenal (FIG. 1c). Recent studies of recombination hotspot proteins (Rhs proteins) suggest that a subset of these proteins are T6S nuclease effectors[43,44]. The *Dickeya dadantii* Rhs proteins, RhsA and RhsB, contain C-terminal endonuclease-related effector domains and are transferred between bacterial cells in a manner that is dependent on closely linked *vgrG* genes. When expressed in *Escherichia coli*, these domains result in chromosomal and plasmid DNA degradation, and growth inhibition and a loss of DAPI staining accompanies their delivery to target cells. T6S-dependent export of Rhs proteins in *S. marcescens* and *Proteus mirabilis*[45] suggests that this type of effector may have a broad role in T6S-mediated bacterial interactions[46]. Whether Rhs proteins and previously described T6SS effector classes share the same genetic requirements for export, or whether Rhs proteins might usurp components of the T6SS pathway to achieve intercellular transfer via a distinct mechanism, remains to be determined.

The general study of both the form and the function of effector proteins has greatly inproved our understanding of interbacterial T6S. This increased knowledge of the theoretical capabilities of the T6SS has provided a framework for a sophisticated understanding of this pathway. With this in mind, in the following sections of this Review, we further explore the role of the T6SS in interbacterial competition and, moreover, the potential of this system to mediate interactions beyond simple antagonism.

T6SS-mediated interbacterial antagonism

The role of T6S in mediating interbacterial antagonism has received considerable attention. Systems in *B. thailandensis*, *P. aeruginosa*, *V. cholerae*, *Vibrio parahaemolyticus*, *S. marcescens*, *Citrobacter rodentium*, *Pseudomonas syringae* and *Acinetobacter baumanii* have been shown to increase fitness when these organisms are grown in competition with other bacteria in the laboratory[10,14,47–52]. Interbacterial T6S seems to enable bacteria to attack one another as they compete for space and resources. However, it is important to note that bacteria live in dynamic communities that often consist of many species in close association both with each other and with additional biotic and abiotic elements. As it is in these complex surroundings that T6SSs evolved, it is difficult to define the adaptive importance of the pathway, based solely on laboratory competition experiments. Nevertheless, in combination with growth competition studies, the results of biochemical, genomic and regulatory analyses have — as outlined below — produced evidence to support the argument that many T6SSs do have a direct role in interbacterial competition.

As previously mentioned, T6SS effectors target bacterial structures that are both essential and highly conserved. Therefore, these proteins seem to have evolved to exert deleterious effects on a broad array of competitors. The mechanisms by which these proteins attack and disrupt essential pathways further supports their role in antagonism. The structures of amidase-type T6SS effectors have shown that they have open active sites that are devoid of the inhibitory regulatory domains that are present in similar housekeeping enzymes[20,21,27–29,53]. The measured activities of effectors support the predictions that these proteins lack stringent regulation, as many that have been studied so far show *in vitro* activity in the absence of co-activators[15,18,19,27]. Mirroring their *in vitro* activity, T6S-dependent intoxication of cells by amidase and phospholipase effector proteins results in rapid lysis owing to cell wall and membrane damage, respectively[27,31,36]. Overall, biochemical analyses of T6SS effectors have provided evidence that is consistent with the participation of these proteins in bacterial antagonism.

Genomic and evolutionary evidence further supports the involvement of T6S in interbacterial antagonism. First, immunity proteins are maintained in organisms that lack cognate effector proteins[19]. Although these orphan immunity proteins have not been shown to be functional, their persistence suggests that there is a selective pressure derived from attack by other organisms. Second, active effector proteins are, without exception, found in the presence of immunity proteins. This highlights the fitness cost that is associated with effector intoxication in the absence of immunity. Finally, the role of T6S in interbacterial antagonism is supported by the fact that bacteria-targeting T6SSs have not been identified in organisms that live in privileged sites, protected

Bacteriocins
Proteins released by bacteria that exerts toxic effects on related organisms. Bacteriocins and specific immunity determinants that protect the producing cell are typically encoded by adjacent genes.

Recombination hotspot proteins
(Rhs proteins). Large multi-domain proteins that have a central element consisting of a repeating motif and a toxin domain at their carboxyl terminus. They were initially identified as sites of frequent recombination in the *Escherichia coli* genome.

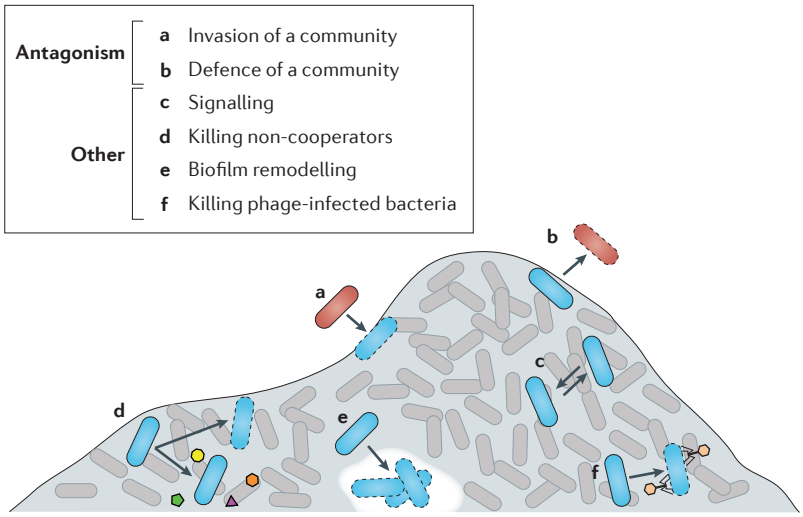


Figure 2 | **Multiple roles for interbacterial T6S.** The figure shows the potential activities of type VI secretion (T6S) in an established bacterial community, both antagonistic activities (between red and blue competitor cells; **a** and **b**) and non-antagonistic activities (between two blue cells; **c**–**f**). Dashed outlines indicate cells that are being targeted by the type VI secretion system. The arrows show the directionality of T6S interactions.

from competition from other bacteria. For example, in *Burkholderia mallei*, the loss of interbacterial T6SS was concomitant with its evolution from a free-living saprophyte to an obligate pathogen[14].

Observations regarding bacterial antagonism have led Cornforth and Foster[54] to postulate that bacterially encoded antibacterial toxins could be preferentially produced in response to competition-specific stresses. Interestingly, it has been shown in both intra- and inter-species interactions that the activation of the T6SS in one cell can stimulate the system in neighbouring cells[31,55]. This recognition is sufficiently integral to the activity of the *P. aeruginosa* H1-T6SS that inactivation of the T6SS of a competitor organism confers resistance to intoxication[31]. Therefore, the intercellular positive regulatory behaviour of some T6SSs seems to conform to the predictions of an antagonistic pathway, although it should be noted that not all T6SSs seem to use this form of regulation[55].

The conditions that regulate the production of a pathway can provide valuable insights into its physiological function. Some T6SSs, such as the H1-T6SS of *P. aeruginosa* and the *vas* system of *V. cholerae* O1 strains, are repressed by quorum sensing[56,57]. This indicates that these systems are active when cells have not established a dense community and, therefore, if they operate antagonistically, their function might be to aid in the colonization of surfaces via the displacement of competing bacteria (FIG. 2a). Conversely, other T6SSs, such as the H2-T6SS of *P. aeruginosa*, are induced under conditions of high cell density[56]. These T6SSs could be involved in the defence of communities from invading organisms or, alternatively, in the invasion of communities of organisms that produce compatible quorum signals (FIG. 2a,b). In addition, some T6SSs are regulated by environmental cues, such as temperature, pH or iron availability[58–62]. With respect to interbacterial antagonism, these signals may indicate the passage of organisms to an environment in which competitor bacteria are present.

Quorum sensing
An interbacterial signalling system in which signal concentration correlates with cell density, thus providing a measure of the local concentration of signal-producing organisms.

Implications of intraspecies antagonism

The more that niche requirements overlap between two organisms, the more probable it is that they will compete in the environment. Therefore, organisms that are related at the species level are likely to be in fierce competition with each other to occupy mutually favourable niches[63]. Although strains sometimes cooperate and form mixed communities, in many cases, growth of one strain precludes growth of another, both in the laboratory and in the environment[64–67]. Indeed, antagonism between strains has been studied in depth, and various mechanisms that reinforce competition have been identified[68–70]. Some of these, such as contact-dependent growth inhibition (CDI), closely resemble T6S in that they require close association with target cells and that they use polymorphic toxin–immunity pairs[71] (BOX 3).

T6SS-dependent interstrain competition has been directly observed between natural isolates of *S. marcescens* and *V. cholerae*[48,72]. In addition, some observed E–I pairs are variably present between different strains of a species, such as Tle5–Tli5 in *P. aeruginosa,* which suggests that intraspecies competition might be widespread[36,37]. A striking example of the contribution of T6S to interstrain competition is found in the cooperative swarming behaviour of *P. mirabilis*. Boundaries of dead and dying cells are typically observed when the swarm fronts of non-isogenic *P. mirabilis* strains encounter each other. This phenomenon involves a self-recognition activity that has been linked to three genetic loci, identification of self (*ids*), identity recognition (*idr*) and type VI secretion (*tss*)[45,67]. The *tss* gene cluster encodes a T6SS apparatus, and within the *ids* and *idr* loci are genes that encode VgrG proteins and VasX- and Rhs-like proteins, respectively. Consistent with a T6S-like mechanism underlying self-recognition in *P. mirabilis*, the process depends on the activity of the *tss*-encoded T6SS, and self-recognition activity correlates with variability in the *ids* and *idr* loci. Therefore, recognition that is facilitated by the T6SS seems to directly contribute to the capacity of *P. mirabilis* strains to cooperate.

Interstrain competition might explain the diverse repertoire of T6SS E–I pairs in many organisms. Selection to maintain diversity could be due to an arms race, in which a cell that lacks an E–I pair that is present in its neighbour, is rapidly displaced. This further provides an explanation for why a given organism would translocate closely related effector proteins — although these are likely to be redundant in enzymatic function, they can differ in immune recognition and would be non-redundant with respect to interstrain competition[16,19]. Such processes can ultimately drive speciation. Whereas within contacting populations the acquisition of an E–I pair might lead to the sweeping success of that genotype, kin that contact one another less often might acquire E–I loci that render them at a standoff. This would affect genetic exchange between organisms, not only by limiting their spatial contact but also by preventing contact-dependent mechanisms of genetic exchange.

Box 3 | **T6S and CDI — analogous, but not functionally redundant**

Although versatile, the type VI secretion system (T6SS) does not typically provide bacteria with the full complement of contact-dependent competitive and cooperative mechanisms. Gram-negative bacteria therefore encode additional pathways that may, in certain cases, share properties with type VI secretion (T6S); however, these pathways possess distinct, specialized capabilities.

One such pathway, contact-dependent inhibition (CDI), uses a two-partner secretion mechanism to present filamentous toxins, termed CdiA proteins, at the cell surface (reviewed in REF. 71). According to the prevailing model, this positions the carboxy-terminal toxin domain distal to the donor cell such that it is poised to engage with and enter the target cell. Like T6SS effectors, the toxin domain of CdiA is highly polymorphic — even within families that exhibit the same enzymatic activity[141–143]. Also similarly to the T6SS, the CDI system self-targets; therefore, equally polymorphic cognate immunity proteins protect against self-intoxication.

CDI systems are common in Proteobacteria, in which they are often found in co-occurrence with T6S. Why then might an organism possess these seemingly functionally redundant pathways? Whereas the T6SS targets Gram-negative bacteria in a mostly indiscriminate manner, CDI is so far only known to occur between highly related bacteria — probably owing to its requirement for outer membrane receptors that vary between bacterial species[144]. Therefore, CDI does not directly function as a broad defence mechanism, but instead seems to be more functionally restricted. Data also suggest that the physical and temporal constraints on CDI function might be more permissive than those of T6S, enabling CDI to function under circumstances that are not favourable to T6S. For example, CDI-dependent fitness differences can be observed between bacterial populations that are cultivated in liquid medium, whereas even constitutive, highly active T6SS effector donor strains seem to show no capacity to target sensitive recipients under these conditions[10,145]. A possible explanation for this difference is that transient contacts that are brought about by cellular collisions do not provide the time that is needed for the assembly of an appropriately oriented, complex secretory apparatus such as the T6SS. It is currently difficult to directly compare the kinetics of intoxication by these two systems. However, the toxin domain of CdiA is readily observed in the cytoplasm of target cells within one hour of co-cultivation with donor cells, whereas, under similar conditions, the lysis of recipient cells by the T6SS does not occur robustly until well after one hour[30,146]. As we further unravel the intricacies of these two pathways, it is probable that additional insights into their divergent functions and evolutionary importance will be made.

Potential roles for T6S beyond antagonism

Although there is substantial evidence for an antagonistic role for many T6SSs, there remains the possibility that some of these systems might have roles beyond competition. One of the most compelling pieces of evidence that indicates additional roles for the T6SS is that it is often activated during conditions of high cell density[56,57,73,74]. As clonal expansion can lead to the spatial segregation of cells as clusters of closely related groups, these T6S-activated cells are likely to primarily contact immediate progeny[75]. Under such conditions, T6S could be used to defend the assemblage; however, the considerable amount of kin-targeting that must also occur suggests that additional hypotheses should be considered. It is worth noting that diffusible antibiotics are also generally produced in the presence of a quorum[54]. Nevertheless, unlike the T6SS, these products can affect competitors that are located at a distance and thus are not restricted to targeting their probably isogenic neighbours.

An instructive frame of reference to generate hypotheses about isogenic intoxication are toxin–antitoxin systems (TA systems)[76,77], and, indeed, analogies have been made between TA systems and T6SS E–I pairs[78]. Although TA systems are not generally thought to function in *trans* nor to depend on additional machinery to exert their effects, when only considering isogenic intoxication there is the possibility of considerable functional overlap between TA systems and antibacterial T6SS E–I pairs. In this respect, it is important to note that TA systems, which were once considered to mostly function in the context of plasmid addiction and selfish genetic elements, have recently received much attention for their roles in metabolic regulation, biofilm formation, phage defence and stress response, such as persistence in the presence of antibiotics[79–84]. It is in light of these, and other findings, that we discuss alternative roles for the T6SS below.

Signalling. Many bacteria seem to only transiently exist as independent cells and, instead, form complex multicellular communities, punctuated by dispersal events[85]. Communication is essential to the establishment and maintenance of these communities; however, previous studies have mostly focused on communication via soluble quorum signalling molecules and not on contact-dependent mechanisms[86,87]. Contact-dependent mechanisms have the advantage, in tight quarters, of also providing information about the number and the identities of immediately adjacent cells. Notably, in 'true' multicellular organisms, direct cell-to-cell signalling is found alongside the production of soluble signalling molecules[88], which highlights the importance of both contact-dependent and contact-independent mechanisms.

It is possible that T6SS effector proteins might have a role in signalling between isogenic cells (FIG. 2c). This could be due to residual activity of the effector, or the E–I complex itself might function as a signalling molecule. The use of toxic effector proteins as signalling molecules is appealing in this respect, as intended recipient organisms successfully interpret the signal, whereas non-intended recipients experience antagonistic effects. Also, the activities of cell wall-remodelling effector proteins are consistent with known mechanisms of interbacterial signalling; for example, in *Mycobacterium tuberculosis* and *Micrococcus luteus*, cell wall-remodelling enzymes have been observed to trigger changes that lead to recovery from the viable but non-culturable (VBNC) growth state[89–92]. Although Gram-positive organisms can be resuscitated by the production of these enzymes by neighbouring cells, the Gram-negative outer membrane inhibits this mechanism. However, T6SS-dependent delivery of resuscitation enzymes to the periplasm could overcome this and enable active cells to 'wake' their neighbours.

Enforcement of social behaviours. Cooperative activities are susceptible to the evolution of non-cooperating organisms, which take advantage of public goods without contributing to the metabolic cost of their production[93,94]. One mechanism that has been proposed to overcome this tendency is enforcement by imposing a cost to individuals that do not participate in cooperation. Intoxication by T6SS effectors could be one means by which enforcement is accomplished (FIG. 2d). The known co-regulation

Toxin–antitoxin systems (TA systems). Systems that consist of a toxic element and an unstable cognate antitoxin that mediate toxicity in the producing cell such that depletion of the antitoxin results in death or senescence of the organism.

Viable but non-culturable (VBNC). Describes a growth state in which a cell is not actively dividing but in which viability is maintained.

Cooperation
The investment of resources by one individual into a process that benefits other individuals. Cooperation is not necessarily mutually beneficial.

of effectors and immunity proteins with social activities would prevent a target organism from failing to cooperate as it would also fail to produce immunity determinants. In *P. aeruginosa* and *P. protegens*, it has been observed that E–I loci are within the Gac–Rsm regulon, which also includes a number of social behaviours, such as the production of exopolysaccharides[10,33,95–97].

Community structure. Intoxication by effector proteins, although deleterious, might also contribute to the three-dimensional architecture of bacterial communities (FIG. 2e). Indeed, a gross defect in biofilm formation has been observed for T6SS mutants in several organisms[98–100]. In isogenic aggregates, cells undergo differentiation. Some of this differentiation, such as senescence or death with subsequent lysis, mirrors the results of attack by antagonistic molecules[101]. The Tse2 effector of *P. aeruginosa* is bacteriostatic to other *P. aeruginosa* cells and could induce senescence. Interestingly, a role for Tse2 in interspecies competition has not been found, and, as it is present in all sequenced *P. aeruginosa* strains, Tse2 is unlikely to be a mediator of intrastrain competition[10]. Another cell fate, lysis — which releases DNA, an important structural component of extracellular matrices — could also be induced by cell wall-degrading or phospholipase effector proteins[15,27,36,102]. As all cells in an isogenic population would be expected to possess both immunity and effector genes, a mechanism for differential susceptibilities to exchanged effectors must be invoked. Heterogeneity in gene expression is frequently observed in cellular aggregates, owing to microenvironmental differences that are found within complex communities[103]. In this manner, positional cues might induce the expression of differential immunity, enabling T6SS-dependent intoxication to occur.

Phage defence. It is known that TA systems can be used to induce suicide in phage-infected bacteria, which protects nearby cells from infection[82]. The importance of this defence mechanism is highlighted by the evolution of phage proteins that inhibit the pathway[104]. T6SS effector proteins could also be used to remove adjacent infected organisms (FIG. 2f). Analogously to TA-mediated phage defence, an infected organism in which the cellular machinery has become hijacked to produce phage particles might then become susceptible to effector intoxication as its pre-existing immunity proteins become depleted. The use of T6S for this purpose may be particularly advantageous, as T6S-assisted suicide does not depend on machinery within the infected cell; thus, this strategy is possibly less susceptible to inhibition by the phage.

Relevant settings for T6S in nature

The studies that are described in the preceding sections provide intriguing insights into the potential roles of T6SSs in bacterial communities. It will be of interest in future *in vivo* or *in situ* investigations to confirm or dispute the predictions that were made on the basis of *in vitro* studies. Below, we identify environmental habitats and *in vivo* systems in which T6S-dependent interactions are likely to occur. These offer a starting point for probing the role of T6S in natural bacterial communities and for exploring the consequences of manipulating T6-mediated interactions.

T6S in polymicrobial infection. Many organisms that encode T6SSs are human pathogens. T6S was initially thought to be involved in virulence by directly targeting eukaryotic cells, but more recent work indicates that targeting eukaryotic cells is a rare property of T6SSs (reviewed in REF. 11). Accordingly, the role of T6S in virulence is more likely to be an indirect one that stems from its role in interbacterial interactions. One way in which T6S could contribute to virulence would be by enabling pathogens to more effectively compete with other host-associated bacteria (FIG. 3). Precedence for the importance of interbacterial antagonism in bacterial pathogenesis comes from the study of bacteriocins; for example, mice that were colonized by the bacteriocin-producing probiotic bacterium *Lactobacillus salivarius* are protected from infection by *Listeria monocytogenes*[105]. Bacteriocin-mediated interactions were also found to predict the outcome of competition between strains of *Streptococcus pneumoniae* in the mouse nasal pharynx[106].

Antagonistic interactions that are mediated by the T6SS could be important for bacterial pathogenesis in a variety of contexts (FIG. 3). First, in order to establish an infection, pathogens must be able to overcome the colonization barrier that is created by the native microflora. This is especially important for enteric pathogens that compete against established populations in the intestines[107]. Although T6S is not known to target Gram-positive organisms, which constitute much of the intestinal microbiota, the numerous enteric pathogens that have T6SSs, including *Salmonella enterica*, *C. rodentium, Aeromonas hydrophila* and enteroaggregative *E. coli*, nonetheless suggests that there is an adaptive role for the system in the gut[50,108–111]. One role may be to selectively target commensal Proteobacteria, such as beneficial *E. coli* strains, that occupy mutually favourable niches. The system may additionally target Bacteroidetes, which is a highly abundant phylum in the gut; however, the activity of the system against non-proteobacterial Gram-negative bacteria is yet to be determined.

When the commensal colonization barrier is compromised — for example, by the disruption of an epithelial surface — the host can become susceptible to infection by many bacteria. In this context, T6S could give pathogens a competitive advantage over other potential colonizers; for example, organisms that have bacterial-targeting T6SSs, including *A. baumanii*, *P. aeruginosa, S. marcescens* and *P. mirabilis*, are common inhabitants of wounds[10,45,48,52,112–114]. After stable colonization, it is conceivable that T6S further enables pathogens to defend their niche by resisting invasion by incoming competitors of the same or of other species (FIG. 3). For example, in the lungs of patients with cystic fibrosis (CF), where clonal populations of *P. aeruginosa* can persist for years, the overall diversity of the species that colonize this habitat decreases as patients age[115–118]. Accordingly, T6S

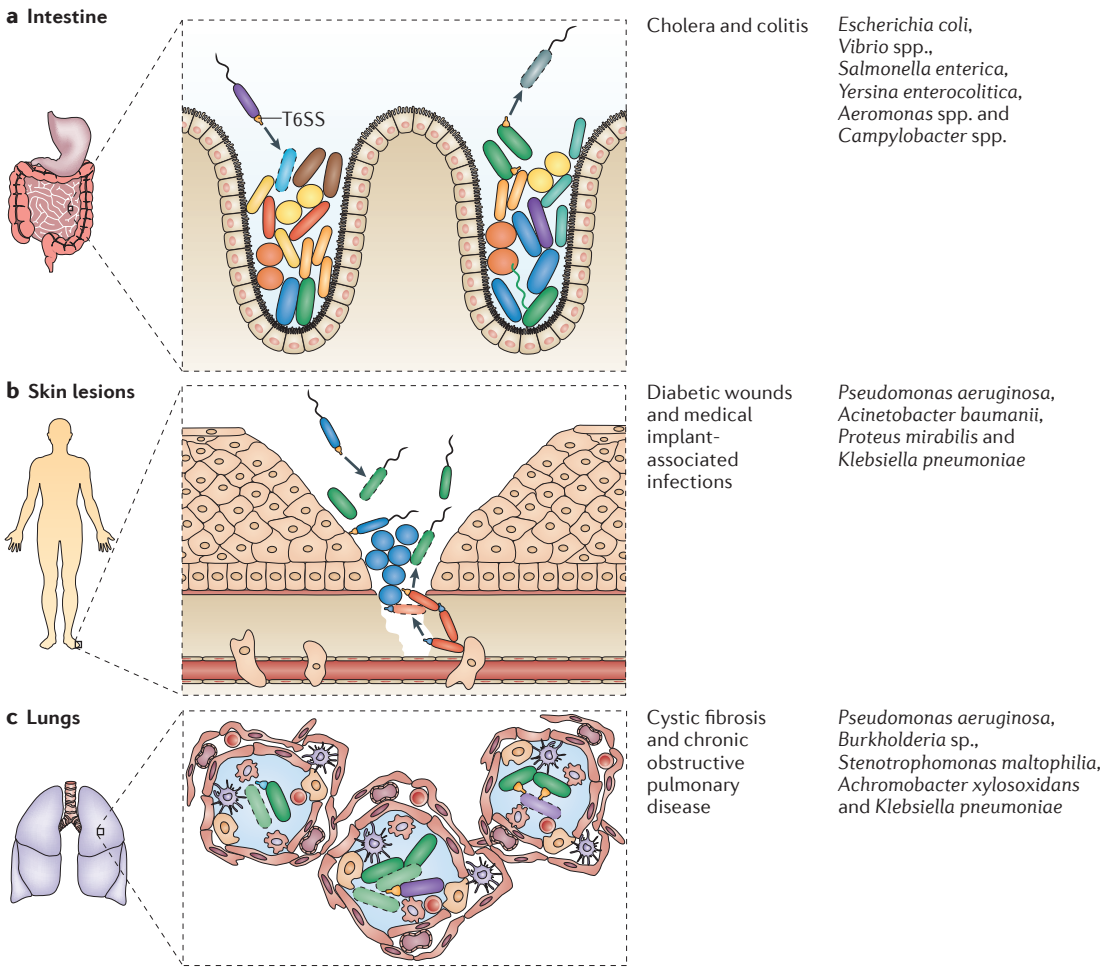


Figure 3 | **Interbacterial T6S and infection.** Potential roles for type VI secretion (T6S)-mediated interactions in disease. Both antagonistic and alternative functions for T6S are depicted. The type VI secretion system (T6SS) is shown as an additional structure on cells; it is shown in orange when mediating an antagonistic function and in blue when its activity has an alternative function. Cells that are being attacked by the T6SSs of other cells are depicted with a dashed outline. **a** | In the intestines, T6S might be used by invading pathogens (intestine close-up, left) or by commensals blocking invading pathogens (intestine close-up, right). **b** | In skin wounds, T6S could be important for competition during colonization (wound close-up, blue versus green cells), could enable established populations to protect their niche from susceptible invaders (wound close-up, red versus green cells) or could facilitate signalling within populations (wound close-up, red cell targeting another red cell). **c** | In chronic lung infections, roles for T6S could include preventing the invasion of an established population by susceptible species (lung close-up, right), facilitating the invasion of a susceptible established population (lung close-up, centre), or contributing to aggregate structure by mediating the lysis of a subpopulation of cells (lung close-up, left).

is one mechanism by which *P. aeruginosa* populations could prevent invasion by other organisms. Consistent with a role for T6SS in CF infections, clinical isolates of *P. aeruginosa* from CF infections frequently have highly active antibacterial T6SSs, and serum from patients with CF has been shown to react to Hcp1 (REFS 97,119).

Chronic infections, such as those in CF lungs or diabetic wounds, are also a habitat that may support functions of T6S beyond bacterial antagonism. Bacterial populations in chronic infections often exist within structured aggregates[120,121]. As described above, the microhabitats in such structures could lead to differential gene expression that facilitates T6-based signalling or cellular differentiation.

T6S in environmental populations. Many organisms that encode T6SSs are not human pathogens. In addition, the human pathogens that possess T6SSs are typically not specialists; rather, they are opportunistic pathogens that also inhabit environmental niches outside the hosts they infect[11]. Therefore, T6S probably has an important role in bacterial competition outside disease settings. Given that T6S requires intimate contact between cells, it is most likely to be important in habitats where bacteria predominately form microcolonies or aggregates. The phyllosphere and the rhizosphere are both habitats that are colonized by diverse bacterial species that are often found clustered together[122,123]. The plant pathogen and leaf surface colonizer *P. syringae* DC3000 tomato has two

Phyllosphere
A habitat that consists of the above-ground surfaces of plants, particularly the leaves.

Rhizosphere
A habitat that consists of plant roots and the surrounding soil that is influenced by their secretions.

T6SSs, one of which has been shown to be important for bacterial antagonism *in vitro*[51,124]. T6SS gene clusters are also common among species from the plant-associated genera *Erwinia*, *Pantoea* and *Pectobacterium*, and the expression of *hcp* and *vrgG* genes in *Pectobacterium atrosepticum* is induced by potato stem extract[125,126]. One study intriguingly suggests that T6S could be important for bacterial interactions in bulk soil that is not associated with plant roots. A T6SS mutant of *P. fluorescens* Pf0–1 grew less well than the wild type in soil that contained indigenous bacteria, but achieved similar growth yields to the wild type in sterilized soil[127].

Conclusions

Many of the recent advances in our understanding of the function of T6S have been made as a result of the identification and characterization of its secreted effectors. Most effectors that have been identified so far show antibacterial activity towards susceptible recipients, which highlights the importance of T6S in mediating interbacterial interactions. The broad range of the targets of T6SS effectors further strengthens the argument for the general importance of the antagonistic function of T6SSs; however, a more nuanced examination of the potential physiological roles for T6S indicates that they extend beyond antagonism to include other effects on bacterial populations.

The potential for T6S to influence the composition of microbial communities suggests tantalizing avenues for its application. The need for new antimicrobials, particularly those that have the ability to target chronic, recalcitrant infections, has never been more apparent. The ability of T6SSs to directly deliver potent antimicrobials to Gram-negative pathogens makes the system an attractive candidate for the engineering of novel antimicrobial mechanisms into probiotic organisms. Such an antibacterial approach would benefit from the capacity of T6S to function in a biofilm, which is a growth state that is notoriously difficult to treat owing to its increased resistance to traditional antimicrobials[14,128]. Depending on the contribution of a given T6SS to the *in vivo* fitness of a bacterial pathogen, an alternative strategy could be to develop inhibitors of its activity. These inhibitors would combat infection by decreasing the ability of pathogens to compete against resident microflora. Finally, the ability of T6S to contribute to bacterial fitness could be harnessed by augmenting the effector repertoires of environmentally beneficial organisms, such as plant growth-promoting bacteria or bioremediation species, to facilitate their ability to compete with indigenous organisms.

1. Roesch, L. F. *et al.* Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J.* **1**, 283–290 (2007).
2. Kostic, A. D., Howitt, M. R. & Garrett, W. S. Exploring host–microbiota interactions in animal models and humans. *Genes Dev.* **27**, 701–718 (2013).
3. Overmann, J. The phototrophic consortium "Chlorochromatium aggregatum" — a model for bacterial heterologous multicellularity. *Adv. Exp. Med. Biol.* **675**, 15–29 (2010).
4. Baker, B. J. & Banfield, J. F. Microbial communities in acid mine drainage. *FEMS Microbiol. Ecol.* **44**, 139–152 (2003).
5. Dekas, A. E., Poretsky, R. S. & Orphan, V. J. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science* **326**, 422–426 (2009).
6. Boetius, A. *et al.* A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**, 623–626 (2000).
7. Strom, S. L. Microbial ecology of ocean biogeochemistry: a community perspective. *Science* **320**, 1043–1045 (2008).
8. Belda-Ferre, P. *et al.* The oral metagenome in health and disease. *ISME J.* **6**, 46–56 (2012).
9. Nielsen, P. H., Saunders, A. M., Hansen, A. A., Larsen, P. & Nielsen, J. L. Microbial communities involved in enhanced biological phosphorus removal from wastewater — a model system in environmental biotechnology. *Curr. Opin. Biotechnol.* **23**, 452–459 (2012).
10. Hood, R. D. *et al.* A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* **7**, 25–37 (2010).
This study provides the first evidence that the T6SS can deliver effector proteins between bacterial cells.
11. Schwarz, S., Hood, R. D. & Mougous, J. D. What is type VI secretion doing in all those bugs? *Trends Microbiol.* **18**, 531–537 (2010).
12. Boyer, F., Fichant, G., Berthod, J., Vandenbrouck, Y. & Attree, I. Dissecting the bacterial type VI secretion system by a genome wide *in silico* analysis: what can be learned from available microbial genomic resources? *BMC Genomics* **10**, 104 (2009).
This paper provides an exhaustive analysis of the distribution and gene content of T6S clusters.
13. Shalom, G., Shaw, J. G. & Thomas, M. S. *In vivo* expression technology identifies a type VI secretion system locus in *Burkholderia pseudomallei* that is induced upon invasion of macrophages. *Microbiology* **153**, 2689–2699 (2007).
14. Schwarz, S. *et al. Burkholderia* type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathog.* **6**, e1001068 (2010).
15. Russell, A. B. *et al.* Type VI secretion delivers bacteriolytic effectors to target cells. *Nature* **475**, 343–347 (2011).
This paper identifies peptidoglycan within the bacterial cell wall as a target of interbacterial T6SS effector proteins.
16. English, G. *et al.* New secreted toxins and immunity proteins encoded within the Type VI secretion system gene cluster of *Serratia marcescens*. *Mol. Microbiol.* **86**, 921–936 (2012).
17. Dong, T. G., Ho, B. T., Yoder-Himes, D. R. & Mekalanos, J. J. Identification of T6SS-dependent effector and immunity proteins by Tn-seq in *Vibrio cholerae*. *Proc. Natl Acad. Sci. USA* **110**, 2623–2628 (2013).
18. Brooks, T. M., Unterweger, D., Bachmann, V., Kostiuk, B. & Pukatzki, S. Lytic activity of the *Vibrio cholerae* type VI secretion toxin VgrG-3 is inhibited by the antitoxin TsaB. *J. Biol. Chem.* **288**, 7618–7625 (2013).
19. Russell, A. B. *et al.* A widespread bacterial type VI secretion effector superfamily identified using a heuristic approach. *Cell Host Microbe* **11**, 538–549 (2012).
20. Benz, J., Sendlmeier, C., Barends, T. R. & Meinhart, A. Structural insights into the effector-immunity system Tse1/Tsi1 from *Pseudomonas aeruginosa*. *PLoS ONE* **7**, e40453 (2012).
21. Zhang, H. *et al.* Structure of the type VI effector–immunity complex (Tae4–Tai4) provides novel insights into the inhibition mechanism of the effector by its immunity protein. *J. Biol. Chem.* **288**, 5928–5939 (2013).
22. Vollmer, W., Pilsl, H., Hantke, K., Holtje, J. V. & Braun, V. Pesticin displays muramidase activity. *J. Bacteriol.* **179**, 1580–1583 (1997).
23. Browder, H. P., Zygmunt, W. A., Young, J. R. & Tavormina, P. A. Lysostaphin: enzymatic mode of action. *Biochem. Biophys. Res. Commun.* **19**, 383–389 (1965).
24. El Ghachi, M. *et al.* Colicin M exerts its bacteriolytic effect via enzymatic degradation of undecaprenyl phosphate-linked peptidoglycan precursors. *J. Biol. Chem.* **281**, 22761–22772 (2006).
25. Kong, K. F., Schneper, L. & Mathee, K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS* **118**, 1–36 (2010).
26. Reynolds, P. E. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**, 943–950 (1989).
27. Chou, S. *et al.* Structure of a peptidoglycan amidase effector targeted to Gram-negative bacteria by the type VI secretion system. *Cell Rep.* **1**, 656–664 (2012).
28. Ding, J., Wang, W., Feng, H., Zhang, Y. & Wang, D. C. Structural insights into the *Pseudomonas aeruginosa* type VI virulence effector Tse1 bacteriolysis and self-protection mechanisms. *J. Biol. Chem.* **287**, 26911–26920 (2012).
29. Zhang, H., Gao, Z.-Q., Su, X.-D. & Dong, Y.-H. Crystal structure of type VI effector Tse1 from *Pseudomonas aeruginosa*. *FEBS Lett.* **586**, 3193–3199 (2012).
30. LeRoux, M. *et al.* Quantitative single-cell characterization of bacterial interactions reveals type VI secretion is a double-edged sword. *Proc. Natl Acad. Sci. USA* **109**, 19804–19809 (2012).
This was the first study to show that inactivation of T6S can provide resistance to T6S-based attack by neighbouring cells.
31. Srikannathasan, V. *et al.* Structural basis for type VI secreted peptidoglycan DL-endopeptidase function, specificity and neutralization in *Serratia marcescens*. *Acta Crystallogr. D Biol. Crystallogr.* **69**, 2468–2482 (2013).
32. Fischetti, V. A. Bacteriophage lysins as effective antibacterials. *Curr. Opin. Microbiol.* **11**, 393–400 (2008).
33. Whitney, J. C. *et al.* Identification, structure and function of a novel type VI secretion peptidoglycan glycoside hydrolase effector–immunity pair. *J. Biol. Chem.* **288**, 26616–26624 (2013).
34. Pukatzki, S., Ma, A. T., Revel, A. T., Sturtevant, D. & Mekalanos, J. J. Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proc. Natl Acad. Sci. USA* **104**, 15508–15513 (2007).
35. Suarez, G. *et al.* A type VI secretion system effector protein, VgrG1, from *Aeromonas hydrophila* that induces host cell toxicity by ADP ribosylation of actin. *J. Bacteriol.* **192**, 155–168 (2010).
36. Russell, A. B. *et al.* Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors. *Nature* **496**, 508–512 (2013).
37. Wilderman, P. J., Vasil, A. I., Johnson, Z. & Vasil, M. L. Genetic and biochemical analyses of a eukaryotic-like phospholipase D of *Pseudomonas aeruginosa* suggest horizontal acquisition and a role for persistence in a chronic pulmonary infection model. *Mol. Microbiol.* **39**, 291–303 (2001).

38. Cascales, E. *et al.* Colicin biology. *Microbiol. Mol. Biol. Rev.* **71**, 158–229 (2007).
This is a comprehensive review of colicins, which are the best characterized of the bacteriocins.
39. Zheng, J., Ho, B. & Mekalanos, J. J. Genetic analysis of anti-amoebae and anti-bacterial activities of the type VI secretion system in *Vibrio cholerae*. *PLoS ONE* **6**, e23876 (2011).
40. Miyata, S. T., Kitaoka, M., Brooks, T. M., McAuley, S. B. & Pukatzki, S. *Vibrio cholerae* requires the type VI secretion system virulence factor VasX to kill *Dictyostelium discoideum*. *Infect. Immun.* **79**, 2941–2949 (2011).
41. Miyata, S.T., Unterweger, D., Rudko, S.P. & Pukatzki, S. Dual expression profile of type VI secretion system immunity genes protects pandemic *Vibrio cholerae*. *PLoS Pathog.* **9**, e1003752 (2013).
42. Geli, V., Baty, D., Pattus, F. & Lazdunski, C. Topology and function of the integral membrane protein conferring immunity to colicin A. *Mol. Microbiol.* **3**, 679–687 (1989).
43. Poole, S. J. *et al.* Identification of functional toxin/immunity genes linked to contact-dependent growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. *PLoS Genet.* **7**, e1002217 (2011).
44. Koskiniemi, S. *et al.* Rhs proteins from diverse bacteria mediate intercellular competition. *Proc. Natl Acad. Sci. USA* **110**, 7032–7037 (2013).
45. Wenren, L. M., Sullivan, N. L., Cardarelli, L., Septer, A. N. & Gibbs, K. A. Two independent pathways for self-recognition in *Proteus mirabilis* are linked by type VI-dependent export. *mBio* **4**, e00374–00313 (2013).
This is a follow-up study to reference 67.
46. Fritsch, M. J. *et al.* Proteomic identification of novel secreted anti-bacterial toxins of the *Serratia marcescens* Type VI secretion system. *Mol. Cell Proteom.* **12**, 2735–2749 (2013).
47. MacIntyre, D. L., Miyata, S. T., Kitaoka, M. & Pukatzki, S. The *Vibrio cholerae* type VI secretion system displays antimicrobial properties. *Proc. Natl Acad. Sci. USA* **107**, 19520–19524 (2010).
48. Murdoch, S. L. *et al.* The opportunistic pathogen *Serratia marcescens* utilizes type VI secretion to target bacterial competitors. *J. Bacteriol.* **193**, 6057–6069 (2011).
49. Salomon, D., Gonzalez, H., Updegraff, B. L. & Orth, K. *Vibrio parahaemolyticus* type VI secretion system 1 is activated in marine conditions to target bacteria, and is differentially regulated from system 2. *PLoS ONE* **8**, e61086 (2013).
50. Gueguen, E. & Cascales, E. Promoter swapping unveils the role of the *Citrobacter rodentium* CTS1 type VI secretion system in interbacterial competition. *Appl. Environ. Microbiol.* **79**, 32–38 (2013).
51. Haapalainen, M. *et al.* Hcp2, a secreted protein of the phytopathogen *Pseudomonas syringae* pv. tomato DC3000, is required for fitness for competition against bacteria and yeasts. *J. Bacteriol.* **194**, 4810–4822 (2012).
52. Carruthers, M. D., Nicholson, P. A., Tracy, E. N. & Munson, R. S. Jr. *Acinetobacter baumannii* utilizes a type VI secretion system for bacterial competition. *PLoS ONE* **8**, e59388 (2013).
53. Dong, C. *et al.* Structural insights into the inhibition of type VI effector Tae3 by its immunity protein Tai3. *Biochem. J.* **454**, 59–68 (2013).
54. Cornforth, D. M. & Foster, K. R. Competition sensing: the social side of bacterial stress responses. *Nature Rev. Microbiol.* **11**, 285–293 (2013).
This review discusses an evolutionary rationale for the observed regulatory patterns that are exhibited in the expression of antagonistic bacterial factors.
55. Basler, M. & Mekalanos, J. J. Type 6 secretion dynamics within and between bacterial cells. *Science* **337**, 815 (2012).
56. Lesic, B., Starkey, M., He, J., Hazan, R. & Rahme, L. G. Quorum sensing differentially regulates *Pseudomonas aeruginosa* type VI secretion locus I and homologous loci II and III, which are required for pathogenesis. *Microbiology* **155**, 2845–2855 (2009).
57. Ishikawa, T., Rompikuntal, P. K., Lindmark, B., Milton, D. L. & Wai, S. N. Quorum sensing regulation of the two hcp alleles in *Vibrio cholerae* O1 strains. *PLoS ONE* **4**, e6734 (2009).
58. Wu, C. F., Lin, J. S., Shaw, G. C. & Lai, E. M. Acid-induced type VI secretion system is regulated by ExoR-ChvG/ChvI signaling cascade in *Agrobacterium tumefaciens*. *PLoS Pathog.* **8**, e1002938 (2012).
59. Robinson, J. B. *et al.* Evaluation of a *Yersinia pestis* mutant impaired in a thermoregulated type VI-like secretion system in flea, macrophage and murine models. *Microb. Pathog.* **47**, 243–251 (2009).
60. Brunet, Y. R., Bernard, C. S., Gavioli, M., Lloubes, R. & Cascales, E. An epigenetic switch involving overlapping fur and DNA methylation optimizes expression of a type VI secretion gene cluster. *PLoS Genet.* **7**, e1002205 (2011).
61. Ishikawa, T. *et al.* Pathoadaptive conditional regulation of the type VI secretion system in *Vibrio cholerae* O1 strains. *Infect. Immun.* **80**, 575–584 (2012).
62. Termine, E. & Michel, G. P. Transcriptome and secretome analyses of the adaptive response of *Pseudomonas aeruginosa* to suboptimal growth temperature. *Int. Microbiol.* **12**, 7–12 (2009).
63. Tilman, D. Resource competition and community structure. *Monogr. Popul. Biol.* **17**, 1–296 (1982).
64. Hawlena, H., Bashey, F., Mendes-Soares, H. & Lively, C. M. Spiteful interactions in a natural population of the bacterium *Xenorhabdus bovienii*. *Am. Nat.* **175**, 374–381 (2010).
65. Vos, M. & Velicer, G. J. Social conflict in centimeter- and global-scale populations of the bacterium *Myxococcus xanthus*. *Curr. Biol.* **19**, 1763–1767 (2009).
66. Chantratita, N. *et al.* Genetic diversity and microevolution of *Burkholderia pseudomallei* in the environment. *PLoS Negl Trop. Dis.* **2**, e182 (2008).
67. Gibbs, K. A., Urbanowski, M. L. & Greenberg, E. P. Genetic determinants of self identity and social recognition in bacteria. *Science* **321**, 256–259 (2008).
This study identified a single genetic locus that is linked to self-recognition in *P. mirabilis*, which is an important bacterial model for social behaviour.
68. Riley, M. A. & Wertz, J. E. Bacteriocins: evolution, ecology, and application. *Annu. Rev. Microbiol.* **56**, 117–137 (2002).
69. Eldar, A. Social conflict drives the evolutionary divergence of quorum sensing. *Proc. Natl Acad. Sci. USA* **108**, 13635–13640 (2011).
70. Strassmann, J. E., Gilbert, O. M. & Queller, D. C. Kin discrimination and cooperation in microbes. *Annu. Rev. Microbiol.* **65**, 349–367 (2011).
71. Ruhe, Z. C., Low, D. A. & Hayes, C. S. Bacterial contact-dependent growth inhibition. *Trends Microbiol.* **21**, 230–237 (2013).
72. Unterweger, D. *et al.* Constitutive type VI secretion system expression gives *Vibrio cholerae* intra- and interspecific competitive advantages. *PLoS ONE* **7**, e48320 (2012).
73. Zhang, W. *et al.* Modulation of a thermoregulated type VI secretion system by AHL-dependent quorum sensing in *Yersinia pseudotuberculosis*. *Arch. Microbiol.* **193**, 351–363 (2011).
74. Bernard, C. S., Brunet, Y. R., Gueguen, E. & Cascales, E. Nooks and crannies in type VI secretion regulation. *J. Bacteriol.* **192**, 3850–3860 (2010).
75. Nadell, C. D., Foster, K. R. & Xavier, J. B. Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol.* **6**, e1000716 (2010).
76. Engelberg-Kulka, H. & Glaser, G. Addiction modules and programmed cell death and antideath in bacterial cultures. *Annu. Rev. Microbiol.* **53**, 43–70 (1999).
77. Yamaguchi, Y., Park, J. H. & Inouye, M. Toxin–antitoxin systems in bacteria and archaea. *Annu. Rev. Genet.* **45**, 61–79 (2011).
78. Li, M. *et al.* Structural basis for type VI secretion effector recognition by a cognate immunity protein. *PLoS Pathog.* **8**, e1002613 (2012).
79. Van Melderen, L. & Saavedra De Bast, M. Bacterial toxin–antitoxin systems: more than selfish entities? *PLoS Genet.* **5**, e1000437 (2009).
80. Yamaguchi, Y. & Inouye, M. Regulation of growth and death in *Escherichia coli* by toxin–antitoxin systems. *Nature Rev. Microbiol.* **9**, 779–790 (2011).
81. Frampton, R., Aggio, R. B., Villas-Boas, S. G., Arcus, V. L. & Cook, G. M. Toxin–antitoxin systems of *Mycobacterium smegmatis* are essential for cell survival. *J. Biol. Chem.* **287**, 5340–5356 (2012).
82. Hazan, R. & Engelberg-Kulka, H. *Escherichia coli* mazEF-mediated cell death as a defense mechanism that inhibits the spread of phage P1. *Mol. Genet. Genom.* **272**, 227–234 (2004).
83. Gerdes, K. & Maisonneuve, E. Bacterial persistence and toxin–antitoxin loci. *Annu. Rev. Microbiol.* **66**, 103–123 (2012).
84. Maisonneuve, E., Castro-Camargo, M. & Gerdes, K. (p)ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity. *Cell* **154**, 1140–1150 (2013).
85. Stoodley, P., Sauer, K., Davies, D. G. & Costerton, J. W. Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* **56**, 187–209 (2002).
86. Parsek, M. R. & Greenberg, E. P. Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol.* **13**, 27–33 (2005).
87. Blango, M. G. & Mulvey, M. A. Bacterial landlines: contact-dependent signaling in bacterial populations. *Curr. Opin. Microbiol.* **12**, 177–181 (2009).
88. Fagotto, F. & Gumbiner, B. M. Cell contact-dependent signaling. *Dev. Biol.* **180**, 445–454 (1996).
89. Downing, K. J. *et al.* Mutants of *Mycobacterium tuberculosis* lacking three of the five rpf-like genes are defective for growth *in vivo* and for resuscitation *in vitro*. *Infect. Immun.* **73**, 3038–3043 (2005).
90. Mukamolova, G. V. *et al.* Muralytic activity of *Micrococcus luteus* Rpf and its relationship to physiological activity in promoting bacterial growth and resuscitation. *Mol. Microbiol.* **59**, 84–98 (2006).
91. Mukamolova, G. V. *et al.* The rpf gene of *Micrococcus luteus* encodes an essential secreted growth factor. *Mol. Microbiol.* **46**, 611–621 (2002).
92. Ravagnani, A., Finan, C. L. & Young, M. A novel firmicute protein family related to the actinobacterial resuscitation-promoting factors by non-orthologous domain displacement. *BMC Genomics* **6**, 39 (2005).
93. West, S. A., Griffin, A. S. & Gardner, A. Evolutionary explanations for cooperation. *Curr. Biol.* **17**, R661–R672 (2007).
This paper provides a comprehensive discussion of the possible mechanisms for the evolution of cooperative behaviour.
94. Diggle, S. P., Griffin, A. S., Campbell, G. S. & West, S. A. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**, 411–414 (2007).
95. Lapouge, K., Schubert, M., Allain, F. H. & Haas, D. Gac/Rsm signal transduction pathway of γ-proteobacteria: from RNA recognition to regulation of social behaviour. *Mol. Microbiol.* **67**, 241–253 (2008).
96. Hassan, K. A. *et al.* Inactivation of the GacA response regulator in *Pseudomonas fluorescens* Pf-5 has far-reaching transcriptomic consequences. *Environ. Microbiol.* **12**, 899–915 (2010).
97. Mougous, J. D. *et al.* A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* **312**, 1526–1530 (2006).
98. Enos-Berlage, J. L., Guvener, Z. T., Keenan, C. E. & McCarter, L. L. Genetic determinants of biofilm development of opaque and translucent *Vibrio parahaemolyticus*. *Mol. Microbiol.* **55**, 1160–1182 (2005).
99. Aschtgen, M. S., Bernard, C. S., De Bentzmann, S., Lloubes, R. & Cascales, E. SciN is an outer membrane lipoprotein required for type VI secretion in enteroaggregative *Escherichia coli*. *J. Bacteriol.* **190**, 7523–7531 (2008).
100. Sha, J. *et al.* Evaluation of the roles played by Hcp and VgrG type 6 secretion system effectors in *Aeromonas hydrophila* SSU pathogenesis. *Microbiology* **159**, 1120–1135 (2013).
101. Webb, J. S., Givskov, M. & Kjelleberg, S. Bacterial biofilms: prokaryotic adventures in multicellularity. *Curr. Opin. Microbiol.* **6**, 578–585 (2003).
102. Whitchurch, C. B., Tolker-Nielsen, T., Ragas, P. C. & Mattick, J. S. Extracellular DNA required for bacterial biofilm formation. *Science* **295**, 1487 (2002).
103. Stewart, P. S. & Franklin, M. J. Physiological heterogeneity in biofilms. *Nature Rev. Microbiol.* **6**, 199–210 (2008).
104. Otsuka, Y. & Yonesaki, T. Dmd of bacteriophage T4 functions as an antitoxin against *Escherichia coli* LsoA and RnlA toxins. *Mol. Microbiol.* **83**, 669–681 (2012).
105. Corr, S. C. *et al.* Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. *Proc. Natl Acad. Sci. USA* **104**, 7617–7621 (2007).
106. Dawid, S., Roche, A. M. & Weiser, J. N. The blp bacteriocins of *Streptococcus pneumoniae* mediate intraspecies competition both *in vitro* and *in vivo*. *Infect. Immun.* **75**, 443–451 (2007).
107. Sekirov, I., Russell, S. L., Antunes, L. C. & Finlay, B. B. Gut microbiota in health and disease. *Physiol. Rev.* **90**, 859–904 (2010).
108. Blondel, C. J., Jimenez, J. C., Contreras, I. & Santiviago, C. A. Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella* serotypes. *BMC Genomics* **10**, 354 (2009).

109. Suarez, G. *et al.* Molecular characterization of a functional type VI secretion system from a clinical isolate of *Aeromonas hydrophila*. *Microb. Pathog.* **44**, 344–361 (2008).
110. Brunet, Y. R., Espinosa, L., Harchouni, S., Mignot, T. & Cascales, E. Imaging type VI secretion-mediated bacterial killing. *Cell Rep.* **3**, 36–41 (2013).
111. Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220–230 (2012).
112. Percival, S. L., Emanuel, C., Cutting, K. F. & Williams, D. W. Microbiology of the skin and the role of biofilms in infection. *Int. Wound J.* **9**, 14–32 (2012).
113. O'Hara, C. M., Brenner, F. W. & Miller, J. M. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin. Microbiol. Rev.* **13**, 534–546 (2000).
114. Hejazi, A. & Falkiner, F. R. *Serratia marcescens*. *J. Med. Microbiol.* **46**, 903–912 (1997).
115. Goddard, A. F. *et al.* Direct sampling of cystic fibrosis lungs indicates that DNA-based analyses of upper-airway specimens can misrepresent lung microbiota. *Proc. Natl Acad. Sci. USA* **109**, 13769–13774 (2012).
116. Cox, M. J. *et al.* Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. *PLoS ONE* **5**, e11044 (2010).
117. Zhao, J. *et al.* Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc. Natl Acad. Sci. USA* **109**, 5809–5814 (2012).
118. Davies, J. C. *Pseudomonas aeruginosa* in cystic fibrosis: pathogenesis and persistence. *Paediatr. Respir. Rev.* **3**, 128–134 (2002).
119. Moscoso, J. A., Mikkelsen, H., Heeb, S., Williams, P. & Filloux, A. The *Pseudomonas aeruginosa* sensor RetS switches type III and type VI secretion via c-di-GMP signalling. *Environ. Microbiol.* **13**, 3128–3138 (2011).
120. Folkesson, A. *et al.* Adaptation of *Pseudomonas aeruginosa* to the cystic fibrosis airway: an evolutionary perspective. *Nature Rev. Microbiol.* **10**, 841–851 (2012).
121. Ehrlich, G. D., Hu, F. Z., Shen, K., Stoodley, P. & Post, J. C. Bacterial plurality as a general mechanism driving persistence in chronic infections. *Clin Orthop Relat Res.* **437**, 20–24 (2005).
122. Vorholt, J. A. Microbial life in the phyllosphere. *Nature Rev. Microbiol.* **10**, 828–840 (2012).
123. Whipps, J. M. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* **52**, 487–511 (2001).
124. Sarris, P. F., Skandalis, N., Kokkinidis, M. & Panopoulos, N. J. *In silico* analysis reveals multiple putative type VI secretion systems and effector proteins in *Pseudomonas syringae* pathovars. *Mol. Plant Pathol.* **11**, 795–804 (2010).
125. De Maayer, P. *et al.* Comparative genomics of the Type VI secretion systems of *Pantoea* and *Erwinia* species reveals the presence of putative effector islands that may be translocated by the VgrG and Hcp proteins. *BMC Genomics* **12**, 576 (2011).
126. Mattinen, L. *et al.* Microarray profiling of host-extract-induced genes and characterization of the type VI secretion cluster in the potato pathogen *Pectobacterium atrosepticum*. *Microbiology* **154**, 2387–2396 (2008).
127. Varivarn, K., Champa, L. A., Silby, M. W. & Robleto, E. A. Colonization strategies of *Pseudomonas fluorescens* Pf0-1: activation of soil-specific genes important for diverse and specific environments. *BMC Microbiol.* **13**, 92 (2013).
128. Parsek, M. R. & Singh, P. K. Bacterial biofilms: an emerging link to disease pathogenesis. *Annu. Rev. Microbiol.* **57**, 677–701 (2003).
129. Leiman, P. G. *et al.* Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proc. Natl Acad. Sci. USA* **106**, 4154–4159 (2009).
130. Kanamaru, S. Structural similarity of tailed phages and pathogenic bacterial secretion systems. *Proc. Natl Acad. Sci. USA* **106**, 4067–4068 (2009).
131. Basler, M., Pilhofer, M., Henderson, G. P., Jensen, G. J. & Mekalanos, J. J. Type VI secretion requires a dynamic contractile phage tail-like structure. *Nature* **483**, 182–186 (2012).
This paper reports the visualization of a contractile filamentous structure that is composed of TssB and TssC.
132. Silverman, J. M., Brunet, Y. R., Cascales, E. & Mougous, J. D. Structure and regulation of the type VI secretion system. *Annu. Rev. Microbiol.* **66**, 453–472 (2012).
133. Cascales, E. & Cambillau, C. Structural biology of type VI secretion systems. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 1102–1111 (2012).
134. Hachani, A. *et al.* Type VI secretion system in *Pseudomonas aeruginosa*: secretion and multimerization of VgrG proteins. *J. Biol. Chem.* **286**, 12317–12327 (2011).
135. Shneider, M. M. *et al.* PAAR-repeat proteins sharpen and diversify the type VI secretion system spike. *Nature* **500**, 350–353 (2013).
136. Silverman, J. M. *et al.* Haemolysin co-regulated protein is an exported receptor and chaperone of type VI secretion substrates. *Mol. Cell* **51**, 584–593 (2013).
137. Pukatzki, S. *et al.* Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc. Natl Acad. Sci. USA* **103**, 1528–1533 (2006).
138. Bladergroen, M. R., Badelt, K. & Spaink, H. P. Infection-blocking genes of a symbiotic *Rhizobium leguminosarum* strain that are involved in temperature-dependent protein secretion. *Mol. Plant Microbe Interact.* **16**, 53–64 (2003).
139. Rao, P. S., Yamada, Y., Tan, Y. P. & Leung, K. Y. Use of proteomics to identify novel virulence determinants that are required for *Edwardsiella tarda* pathogenesis. *Mol. Microbiol.* **53**, 573–586 (2004).
This study reports for the first time that the export of Hcp depends on genes within a conserved cluster.
140. Zheng, J. & Leung, K. Y. Dissection of a type VI secretion system in *Edwardsiella tarda*. *Mol. Microbiol.* **66**, 1192–1206 (2007).
141. Aoki, S. K. *et al.* A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. *Nature* **468**, 439–442 (2010).
142. Nikolakakis, K. *et al.* The toxin/immunity network of *Burkholderia pseudomallei* contact-dependent growth inhibition (CDI) systems. *Mol. Microbiol.* **84**, 516–529 (2012).
143. Anderson, M. S., Garcia, E. C. & Cotter, P. A. The *Burkholderia* bcpAIOB genes define unique classes of two-partner secretion and contact dependent growth inhibition systems. *PLoS Genet.* **8**, e1002877 (2012).
144. Aoki, S. K. *et al.* Contact-dependent growth inhibition requires the essential outer membrane protein BamA (YaeT) as the receptor and the inner membrane transport protein AcrB. *Mol. Microbiol.* **70**, 323–340 (2008).
145. Aoki, S. K. *et al.* Contact-dependent inhibition of growth in *Escherichia coli*. *Science* **309**, 1245–1248 (2005).
This paper reports the discovery of CDI in *E. coli*.
146. Webb, J. S. *et al.* Delivery of CdiA nuclease toxins into target cells during contact-dependent growth inhibition. *PLoS ONE* **8**, e57609 (2013).

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Competing interests statement
The authors declare no competing interests.