

# What is type VI secretion doing in all those bugs?

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**The identification of bacterial secretion systems capable of translocating substrates into eukaryotic cells via needle-like appendages has opened fruitful and exciting areas of microbial pathogenesis research. The recent discovery of the type VI secretion system (T6SS) was met with early speculation that it too acts as a 'needle' that pathogens aim at host cells. New reports demonstrate that certain T6SSs are potent mediators of inter-bacterial interactions. In light of these findings, we examined earlier data indicating its role in pathogenesis. We conclude that although T6S can, in rare instances, directly influence interactions with higher organisms, the broader physiological significance of the system is likely to provide defense against simple eukaryotic cells and other bacteria in the environment. The crucial role of T6S in bacterial interactions, along with its presence in many organisms relevant to disease, suggests that it might be a key determinant in the progression and outcome of certain human polymicrobial infections.**

## Type VI secretion: from discovery to a preliminary structure–function model

The large gene clusters that are now known to encode type VI secretion systems (T6SSs) were first shown to participate in protein export and proposed to be the mark of a novel secretion system by Spaink and colleagues in 2003 [1]. A report that closely followed was the earliest to demonstrate that secretion of hemolysin coregulated protein (Hcp), a hallmark of all T6SSs subsequently identified, depends on other genes in this cluster [2].

Found in 123 sequenced species of bacteria (as of January 2010), the T6SS might be the most common of the large specialized secretion systems [3]. The T6S gene cluster consists of approximately 15 conserved genes, and many contain a number of functionally relevant accessory elements. Multiple evolutionarily distinct T6SSs are often present in a single genome; for example, the genome of *Burkholderia pseudomallei* encodes six apparent T6SSs, which account for a remarkable 2% of its genome [4,5]. Detailed reviews of the genetic requirements for T6S have been published elsewhere [6–9] and this information will not be discussed at length here. Figure 1 provides a schematic depiction of the T6SS and summarizes key aspects of its function and mechanism.

Much of the speculation regarding the structure and mechanism of T6S is based on similarities between two of

its conserved components, Hcp and valine-glycine repeat protein G (VgrG), to bacteriophage tail proteins. Hcp and VgrG, which are transported to the extracellular milieu in a manner dependent on most of the conserved T6S genes, are structurally similar to bacteriophage tail tube [gene product (gp)19] and spike complex (gp27/gp5) proteins, respectively [10–14]. Based in part on this structural similarity and in part on their lack of obvious toxin or effector activities, these proteins have been postulated to function as extracellular appendages of the secretion apparatus. Additional similarities between core T6S components and bacteriophage have been noted; TssE (COG3518) is a conserved T6S protein that shares sequence homology with the phage baseplate protein gp25 [14] and co-purified TssB–TssC (VipA–VipB; COG3516 and COG3517) proteins were found to oligomerize into a tubule with dimensions similar to that of the bacteriophage tail sheath [15]. The structure and sequence-based homology of T6S and bacteriophage tail proteins has led to the hypothesis that the two systems function analogously. As such, the T6S system is thought to exert its influence on targeted cells by a puncturing mechanism mediated most immediately by VgrG [14]. According to this model, the T6S apparatus is in essence an inverted phage tail on the surface of a bacterium. This model is consistent with the general findings that the effects of T6S require direct cell–cell contact and that VgrG gains access to the cytoplasm of targeted cells.

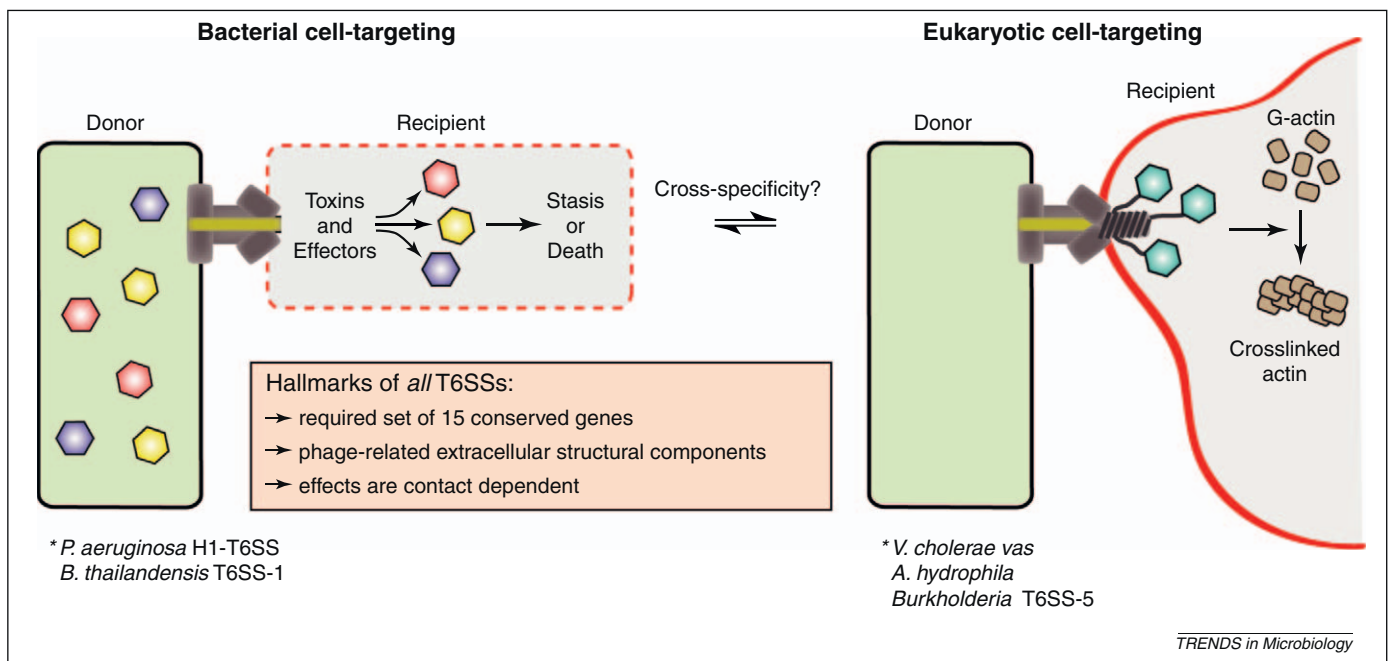
## Reports linking T6S to virulence and host cell interactions

In the absence of additional knowledge, the relatedness of T6S components to bacteriophage tail proteins would lead to speculation that the system might play a role in inter-bacterial interactions. However, the earliest reports on this system linked it to host interactions and virulence and thereby set the stage for subsequent studies that further probed this capacity. These studies have yielded important fundamental insights into the system and have produced unequivocal evidence that, to widely varying degrees, T6S can play a role in pathogenesis. This subject has been thoroughly reviewed [7,16–18] and will receive only partial coverage in this article.

Perhaps the most dramatic virulence defects reported to date for T6S mutants derive from studies of T6SS-5 in *Burkholderia mallei* [4], *Burkholderia thailandensis* [3] and *B. pseudomallei* [5,19]. The system appears to be absolutely essential for virulence in mammalian hosts in this group of closely related organisms. However, the

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**Figure 1.** Schematic depiction of bacterial and host cell-targeting T6SSs. Asterisks indicate the particular system depicted. Additional representative T6SSs of each specificity are listed and are discussed in detail in the text. The *P. aeruginosa* H1-T6SS (left) is postulated to target at least three proteins (hexagons) to other bacterial cells [51]. One of these proteins was shown to be a toxin (Tse2), whereas the function(s) of the remaining two is unknown (Tse1 and Tse3). Although cell contact is required for H1-T6SS-dependent targeting of Tse2, the subcellular compartment (e.g. cytosol, periplasm and outer membrane) to which the Tse proteins are delivered within the recipient cell remains unknown. The *V. cholerae* vas secretion system (right) functions by translocating an effector domain linked to VgrG into the cytosol of eukaryotic recipient cells [26]. This domain promotes cell rounding by catalyzing the formation of G-actin crosslinks, which are not capable of forming F-actin [78].

effects of T6S on virulence and host interactions are less pronounced [20–24] or even negligible in most instances (Table 1 and further discussion below). Of note, we do not consider the *Francisella* pathogenicity island (FPI) gene cluster a T6SS, because of its apparent lack of 10 of the 13 strictly conserved T6S-associated genes [9]. Furthermore, all of the conserved T6S genes lacking in the FPI have been experimentally demonstrated to be required for T6S-dependent phenotypes in at least one instance [16,25].

Despite the large number of reports describing either a virulence phenotype or modification of host interactions in T6S mutants, there are few cases in which a molecular understanding of its involvement is known. A series of studies on the *Vibrio cholerae* virulence associated secretion (*vas*) system elegantly demonstrated that its effects on defense against amoebae, macrophage cell rounding and intestinal inflammation in infant mice are attributable to the actin crosslinking activity of a translocated effector (Figure 1) [21,26–28]. Interestingly, this effector activity has been mapped to the C-terminal domain of an exported VgrG family protein [27]. A T6SS from *Aeromonas hydrophila* might also exert its effects on host cells in this manner. A VgrG family protein translocated into host cells via the T6SS of this bacterium bears a C-terminal domain with ADP-ribosyltransferase activity that targets actin [29]. Although VgrG is a conserved component of all T6SSs and is required for a functional secretion apparatus, bioinformatic analyses have predicted that only a small number of species encode VgrG proteins with fused effector domains. The single ‘stand-alone’ T6S substrate that has been linked to virulence thus far is EvpP from *Edwardsiella tarda*; however, the function of this protein is not known [25,30].

In this report, we re-evaluate the general significance of T6S in bacterial pathogenesis. We conclude that, contrary

to the current thrust of this burgeoning field, it is likely that the majority of T6SSs do not participate directly in critical host cell interactions. Although the function of the vast majority of T6SSs remains unexplored, existing experimental data combined with the organismal distribution of the system argue for its broad significance in bacterial fitness in the environment. As discussed below, recent findings from our laboratory and reinterpretation of earlier studies suggest that a common function of the system could be to mediate interactions between contacting bacterial cells. Thus, the T6SS could be relevant to the outcome of the many polymicrobial human diseases in which organisms that possess this system take part (Table 2). We hope this article will generate interest in investigating alternative functions of the system.

### Data inconsistent with T6S as a host-targeting virulence factor

For most microbiologists, the relevance of their work to disease is paramount. Therefore, researchers studying T6S in a wide range of pathogens have investigated the role of this system in relevant infection models. Considering that negative data are rarely published, a surprisingly large number of reports indicate a lack of involvement of T6S in virulence [20,31–38]. The first section of Table 1 provides a list of these studies and, where available, details of the infection model, phenotypes measured and bacterial mutants used. In addition to direct experimental findings, studies using whole genome approaches to identify virulence factors have generally failed to implicate T6S [39]. The second section of Table 1 contains a partial list of reports that used signature tagged mutagenesis (STM) to study organisms harboring T6S in animal infection models, but did not identify

**Table 1. Findings from organismal infection models inconsistent with a general role for T6SSs in virulence**

Direct experimental observation <sup>a</sup>												
Bacterial species	Gene(s) inactivated	Locus tag(s)	Organismal model	Phenotype(s) measured	Ref							
<i>Vibrio cholerae</i>	<i>hcp-1 hcp-2</i>	VC_1415, VC_A0017	Mouse	LD <sub>50</sub> , intestinal colonization	[33]							
<i>Yersinia pestis</i>	T6S cluster	YPO0499-YPO0516	Flea, mouse	Infectivity (flea), host survival (mouse)	[32]							
<i>Escherichia coli</i> (EAEC) <sup>b</sup>	Not indicated	Not indicated	Mouse	Intestinal colonization	[31]							
<i>E. coli</i> (UPEC)	<i>hcp clpV</i>	c3391-c3392	Mouse	Bladder and kidney colonization	[34]							
	T6S cluster	c3398-c3404										
<i>Pseudomonas syringae</i>	<i>clpV</i>	Psyr_2658	Bean	Epiphytic colonization, foliar disease symptoms	[35]							
<i>Pectobacterium atrosepticum</i>	<i>icmF</i>	ECA3432	Potato	Tuber maceration (rot)	[36]							
<i>Agrobacterium tumefaciens</i>	<i>icmF</i>	Atu4332	Potato	Tuber tumorigenesis	[38]							
	T6S cluster	Atu4330-Atu4343										
<i>Vibrio anguillarum</i>	<i>vtsA-H</i>	GQ118992 <sup>c</sup>	Trout	LD <sub>50</sub>	[37]							
<i>Salmonella</i> Typhimurium	T6S cluster	STM0266-STM0294 <sup>d</sup>	Mouse	Host survival, spleen colonization	[20]							
Signature-tagged mutagenesis <sup>e</sup>												
Bacterial species	STM numbers <sup>f</sup>		Animal model <sup>h</sup>	Functional categories of hits							Ref	
	Mutants	Hits		T3S	M	T	D	R	S	U		
<i>Yersinia enterocolitica</i>	2016	55	9	Mouse (spleen)	+	—	+	+	+	+	+	[42]
<i>Yersinia pseudotuberculosis</i>	603	31	10	Mouse (spleen)	—	+	+	—	+	+	+	[44]
<i>E. coli</i> (EHEC)	1900	79	6	Cow (intestine)	+	+	+	+	+	+	+	[43]
<i>Salmonella</i> Gallinarum	1152	20	5	Chicken (spleen)	+	+	—	—	+	+	+	[46]
<i>S. Typhimurium</i>	1045	119	4	Pig (intestine)	+	+	+	+	+	+	+	[40]
<i>Klebsiella pneumoniae</i>	2200	29	7	Mouse (intestine)	NA	+	+	+	+	+	+	[45]
<i>V. cholerae</i>	1100	23	5	Mouse (intestine)	NA	+	+	+	+	+	+	[41]

Abbreviations: EAEC, enteroaggregative *E. coli*; UPEC, uropathogenic *E. coli*; EHEC, enterohemorrhagic *E. coli*; LD<sub>50</sub>, median lethal dose; T3S, type III secretion; M, metabolism; T, transposers; D, DNA-associated; R, regulation; S, surface structures and motility; U, unknown; NA, organism does not possess T3S.

<sup>a</sup>Studies in which at least one essential T6S gene was inactivated and no statistically significant virulence defect was observed.

<sup>b</sup>Results presented as data not shown and not described in detail.

<sup>c</sup>*V. anguillarum* locus tags not available; GenBank accession number provided instead.

<sup>d</sup>Locus tags based on *S. Typhimurium* strain LT2.

<sup>e</sup>Studies in which T6S was not identified as important for colonization in an animal model of infection.

<sup>f</sup>Mutants indicates the total number of insertions screened and hits refers to the total number of mutants that showed a defect.

<sup>g</sup>Approximate number of expected T6S mutants within transposon library based on genome size, number of insertions obtained, number of T6S clusters and a T6S cluster size of 20 kilobases.

<sup>h</sup>Colonized organ in parentheses.

genes within the system [40–46]. At the same time, these studies almost universally identified T3S genes when this system was present. Because the T6SS was not yet identified when some of these studies were conducted, we included only screens in which all ‘hits’ were provided so that we could manually search for those within T6S clusters. Some of the bacteria analyzed in these screens possess multiple T6SSs; therefore, functional redundancy or compensation among the systems could be one explanation for failing to implicate a given system in pathogenesis. However, this has generally not been shown to occur with T6SSs, and those of a single organism are typically evolutionarily distinct and differentially regulated [3–6,11,19,32,47–49].

A study conducted by Levesque and colleagues provides one example where a T6SS was identified in an STM screen [50]. This study used the chronic rat lung infection model, in which mutant *Pseudomonas aeruginosa* pools are encased within agar beads and delivered directly into the lung by a cannula. Interestingly, mutations within several genes in Hcp secretion island I (HSI-I), which encodes a bacterial cell-targeting T6SS (discussed below) [51], led to decreased fitness of the bacterium in this model. In the agar bead infection model, bacteria are confined within a solid support, similar to conditions that are most permissive of HSI-I-encoded T6SS (H1-T6SS) activity [3,51]. In this model, the fitness defect of T6S mutants could stem from direct competition with other bacteria

within the infection pool rather than from sensitivity to host-derived factors or the host environment.

Using a novel non-obese diabetic-*scid* *IL2r<sup>null</sup>* murine model to screen for determinants of *Salmonella enterica* serovar Typhi infection, Libby *et al.* identified several insertions in the *Salmonella* pathogenicity island-6-encoded T6SS [52]. Heffron and coworkers had shown previously that a deletion of a conserved gene (*icmF*) within the orthologous system of *Salmonella* Typhimurium yielded a strain that was hypervirulent in mice [22]. This apparent contradiction could be explained by specific adaptations of the system to the different infectious lifestyles of these organisms, or, if the system is involved in interbacterial interactions, to differences in the requirement for the system in monotypic infections versus those with pooled mutants.

### T6SSs and T6S-like elements are critically involved in interbacterial interactions

Our group has become intrigued by the ability of T6S to target bacterial cells and its subsequent role in interbacterial interactions. Our interest in this area began with the identification of a small group of proteins that are substrates of the *P. aeruginosa* H1-T6SS, Tse (type VI secretion exported) proteins 1–3 [17,51]. One of these, Tse2, is a toxin active against prokaryotic and eukaryotic cells when expressed intracellularly; however, the H1-T6SS targets this toxin exclusively to bacterial cells [51,53]. When grown

**Table 2. T6SS-positive organisms found in polymicrobial human diseases**

Type of infection	Bacterial species
Respiratory	<i>Pseudomonas aeruginosa</i>
	<i>Burkholderia cepacia</i> complex
	<i>Burkholderia pseudomallei</i>
	<i>Ralstonia pickettii</i>
	<i>Acinetobacter baumannii</i>
	<i>Klebsiella pneumoniae</i>
	<i>Proteus mirabilis</i>
	<i>Chromobacterium violaceum</i>
	<i>Bordetella</i> spp. ( <i>parapertussis</i> , <i>bronchiseptica</i> , <i>petrii</i> )
Gastrointestinal	<i>Escherichia coli</i> (EHEC)
	<i>Salmonella enterica</i> serovars (Typhimurium, Typhi)
	<i>Shigella sonnei</i>
	<i>Campylobacter concisus</i>
	<i>Vibrio</i> spp. ( <i>cholerae</i> , <i>parahaemolyticus</i> , <i>vulnificus</i> )
	<i>Aeromonas</i> spp.
	<i>Yersinia enterocolitica</i>
Skin/abscesses	<i>Acinetobacter baumannii</i>
	<i>Proteus mirabilis</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Klebsiella pneumoniae</i>
	<i>Escherichia coli</i>

under conditions that promote close contact with a Tse2-secreting strain, *P. aeruginosa* cells lacking Tse2 immunity are efficiently outcompeted.

Recently, one of the five T6SSs in *B. thailandensis*, T6SS-1 (BTH\_I2954-BTH\_I2968), was found to be involved in interspecies interactions with several other proteobacteria. In growth competition assays, *B. thailandensis* cells lacking T6SS-1 function were sensitized to growth arrest induced by direct cell contact with a specific group of bacteria that includes *Pseudomonas putida*, *Pseudomonas fluorescens* and *Serratia proteamaculans* [3]. Additionally, wild-type *B. thailandensis* was able to persist with *P. putida* in mixed flow cell biofilm assays, whereas *B. thailandensis* lacking T6SS-1 was rapidly displaced. Taken together, these studies provide compelling evidence that T6SSs can be a decisive factor in the interactions between bacterial cells of the same, or differing, species. The observation that cell–cell contact is a requisite for the influence of T6S on both host and bacterial cells suggests that this relatively conserved secretion system could use a common mechanism for targeting diverse cell types.

Other groups have also provided links, albeit less direct, between T6S and interbacterial interactions. In *Proteus mirabilis*, a VgrG homolog was shown to be important in the social behavior of self versus non-self recognition [54]. This self-identity pathway, encoded by the identification of self (*ids*) genes, is responsible for the boundary formation observed between different *P. mirabilis* strains. The *ids* gene cluster also includes an Hcp homolog, which was not implicated in boundary formation. Additionally, several studies have linked T6S to biofilm formation, another process considered to reflect social behavior [55–61].

Three laboratories independently found that T6S mutants in *Yersinia pestis* [32], *Pectobacterium atrosepticum* [62] and *Acinetobacter baylyi* [63] displayed abnor-

malities in growth regulation *in vitro*. In each case, the authors noted that strains lacking a functional T6SS grew more robustly than the wild type. In *Y. pestis* and *P. atrosepticum*, increased *in vitro* proliferation of the T6S mutants was correlated with increased fitness in relevant *in vivo* infection models: survival in macrophages and potato tuber maceration, respectively [32,62]. These findings show that T6SSs can be important for regulating cell density in a manner entirely independent of the host, and that this dysregulation can influence disease progression. Therefore, careful consideration needs to be given to the interpretation of studies that report T6S-dependent repression of virulence [1,22,64]. These findings could be explained by intraspecies-targeting T6SSs that regulate proliferation, rather than by a direct effect of T6S on the host cell.

### A genomic view suggests a broad role for T6S in the environment

Looking beyond experimental data, which provides insight into only a handful of bacterial species, a genomic view of T6S further suggests that the system is in many cases more important for bacterial fitness in the environment than in pathogenesis. Simply observing that a small fraction of the >100 T6SS<sup>+</sup> organisms are pathogens or symbionts, or are otherwise associated with eukaryotic cells, does little to make a case for their general role in bacterial interactions. Regardless of their pathogenic potential, most proteobacteria are likely to interface and interact with an array of simple eukaryotic organisms in the environment. Moreover, two T6SSs have been shown to provide protection against such organisms. In fact, one of these, the *V. cholerae* *vas* system, was found to be essential for resisting predation by amoebae [28], but not for causing disease in an infant mouse model [33]. Broad conclusions from genomic data are therefore not feasible, and a closer look is required.

One way to use genomics to learn about the likely roles of T6SSs is to compare the repertoire of these systems in the genomes of bacteria that are closely related, yet specialize in the occupation of different niches. *B. pseudomallei*, a highly virulent pathogen with a large environmental reservoir, possesses six evolutionarily distinct T6SSs. A unique set of five of these is shared with its close relatives *B. thailandensis*, a soil saprophyte of relatively low virulence, and *B. mallei*, an obligate zoonotic pathogen [3–5]. Interestingly, three of the *B. mallei* T6SS gene clusters appear to be degraded and probably inactivated by mutations in essential core genes of the systems [3,4]. Because *B. mallei* recently derived from *B. pseudomallei*, this suggests that the function of these systems became dispensable following the transition from an environmental free-living lifestyle to that of a strict host-associated pathogen [65]. This is emphasized by the recent finding that the most deteriorated *B. mallei* system, T6SS-1, which retains only four of the 13 conserved *tss* genes, is involved in interspecies bacterial interactions in *B. thailandensis* [3]. Unlike *B. mallei*, each T6SS of *B. pseudomallei* and *B. thailandensis* has a full complement of conserved *tss* genes [35]. These observations suggest that the majority of the *Burkholderia* T6SSs are not major participants in the interactions of



these bacteria with eukaryotic cells. Bolstering this interpretation is the finding that a *B. thailandensis* strain lacking the function of four of its five T6SSs (T6SS-5 intact) is as virulent as the wild type [3].

The representation of T6S in species of the *Bordetella* genus also argues that the predominant role of the system is in the environment. *Bordetella petrii*, the only known environmentally adapted species in the genus [66], possesses two T6SSs [9]. This organism is most often isolated from the environment, lacks the toxins of pathogenic bordetellae, and has not been etiologically associated with a disease state [66,67]. *Bordetella bronchiseptica* and *Bordetella parapertussis*, members of the *B. bronchiseptica* cluster, possess only one of the systems found in *B. petrii*, and the most host-restricted and virulent member of this subspecies, *Bordetella pertussis*, lacks T6S altogether [67,68].

Bacteriocins are a diverse group of molecules that can be passively or actively released by most bacteria [69]. Although the function of these proteins remains contentious from an evolutionary perspective, it is clear that many can function as narrow host range antimicrobials [69,70]. Intriguingly, several T6SSs have associated elements with homology to bacteriocins. A VgrG protein with a C-terminal domain that resembles a bacteriocin of the S-type pyocin subfamily is located within the SPI-21 T6S gene cluster of *S. enterica* subspecies *arizonae* (IIIa) [71]. Additionally, a pyocin immunity protein is found flanking *vgrG* and three other immunity genes are located elsewhere in SPI-21. In uropathogenic *Escherichia coli*, a gene encoding an apparent fusion of Hcp to an S-type pyocin has also been found [71–73]. This spatial association linking bacteriocin genes with T6SS gene clusters does not appear to be a unique feature of enteric organisms. A predicted operon encoding a putative outer membrane bacteriocin efflux protein, a colicin V-processing peptidase and a putative bacteriocin secretion protein is located two genes upstream of T6SS-1 of *B. pseudomallei* (BPSL3092-94).

The observations presented above are based exclusively on sequence analyses, and therefore provide only circumstantial support for the hypothesis that T6S is not generally utilized as a canonical virulence factor by bacteria. However, the cited examples indicate a negative correlation between the pathogenic potential and the abundance of T6SSs within certain groups of bacteria. The prevalence of T6SSs in environmentally adapted bacteria, which are likely to encounter a high diversity of competing microorganisms, could reflect specialization of systems for particular cell types. In light of experimental data demonstrating a major role for certain T6SSs in bacterial competition [3,51], the noted associations between bacteriocin-related elements and certain T6SSs imply that a significant fraction of T6SSs participate in interbacterial interactions [71,73].

### Concluding remarks and future directions

Without a detailed mechanism for T6S-dependent effects on recipient cells, it remains impossible to predict whether a system that can target eukaryotic cells can also target bacterial cells, or *vice versa*. There is only a limited amount of information to suggest that cellular specificity might be

### Box 1. Outstanding questions

- What are the physiological role(s) and adaptive significance of T6S-mediated interbacterial interactions in the environment?
- What roles do bacterial cell-targeting T6SSs play in human infections?
- Are host- and bacterial cell-targeting T6SSs discernible by sequence or gene content?
- Are there T6SSs that can target both eukaryotic and prokaryotic cells?
- To date, few substrates of the T6SS have been identified. Are there other T6S substrates that await identification?
- Components of the T6SS appear to be related to bacteriophage proteins. By analogy, are proteins the only T6S substrates, or could DNA also be exported through the system?
- By what mechanism does the T6S apparatus target substrates and effector domains to recipient cells?

hardwired into the secretion apparatus; the bacteria-targeting T6SSs of *P. aeruginosa* and *B. thailandensis* have not been shown to affect eukaryotic cells, nor has the eukaryotic cell-targeting T6SS-5 of *B. thailandensis* been implicated in interbacterial interactions [3,51]. Studies have demonstrated that the T6SSs of *V. cholerae* and *A. hydrophila* act on host actin [27,74]. It could be that the substrate specificity of the T6S effectors of these organisms evolved from an earlier role in interbacterial interactions, in which they might have acted upon the actin homolog MreB in targeted cells [75]. It remains to be experimentally evaluated whether such systems could possess dual specificity.

Clearly, there are many important questions and exciting directions to pursue related to bacteria-targeting T6SSs (Box 1). Perhaps those that should be addressed most immediately are: (i) what are the physiological role(s) and adaptive significance of T6S-mediated interbacterial interactions in the environment, and (ii) what role does T6S play in human infections? Although in this article we highlighted evidence that many T6SSs are unlikely to directly mediate host interactions, this does not negate the impact that these systems – if indeed they participate in interbacterial interactions – could have on the outcomes of many human polymicrobial diseases [76]. In such cases, the relative numbers of organisms representing any given bacterial taxon could depend as greatly on fitness against competing bacteria as against host-derived factors. Even in infections considered monotypic, pathogens must first overcome other pathogens and commensal organisms to reach the site of infection and establish dominance (bacterial interference) [77]. Table 2 provides a summary of organisms that possess T6S and participate in human polymicrobial diseases. Whether focused on the role of T6S in interactions with host cells or other bacteria, continued investigation of this frontier promises to yield insights into an important and underexplored aspect of the physiology of many Gram-negative cells.

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