MICROBIOLOGY

MICROBIOLOGY SOCIETY PRIZE LECTURE

Coulthurst, *Microbiology* 2019;165:503–515

DOI 10.1099/mic.0.000789

The Type VI secretion system: a versatile bacterial weapon

Sarah Coulthurst*

Abstract

The Type VI secretion system (T6SS) is a protein nanomachine that is widespread in Gram-negative bacteria and is used to translocate effector proteins directly into neighbouring cells. It represents a versatile bacterial weapon that can deliver effectors into distinct classes of target cells, playing key roles in inter-bacterial competition and bacterial interactions with eukaryotic cells. This versatility is underpinned by the ability of the T6SS to deliver a vast array of effector proteins, with many distinct activities and modes of interaction with the secretion machinery. Recent work has highlighted the importance and diversity of interactions mediated by T6SSs within polymicrobial communities, and offers new molecular insights into effector delivery and action in target cells.

INTRODUCTION

The Type VI secretion system (T6SS) is a protein nanomachine deployed by many Gram-negative bacteria to translocate effector proteins directly into target cells. Following its recognition as the sixth major protein secretion system in Gram-negative bacteria [1, 2], the T6SS was initially believed to function as a classical virulence factor, namely to deliver effector proteins that destroy or manipulate the cells of eukaryotic host organisms ('anti-eukaryotic T6SS'). However it has subsequently become clear that the primary function of the T6SS is as a device for inter-bacterial competion. In other words, bacteria use the T6SS to deliver toxic antibacterial effectors into rival bacterial cells ('anti-bacterial T6SS'). More recently, the range of known uses of the T6SS has expanded further, including action against microbial fungi and scavenging of scarce metal ions. In parallel, recent work has revealed the importance of interactions mediated by antibacterial T6SSs in varied polymicrobial communities, and offers new molecular insights into effector delivery and action in target cells.

It has been estimated that >25 % of proteobacteria encode at least one T6SS in their genome [3]. These T6SSs, whilst sharing the same 13 or 14 core components (TssA-M, PAAR), show considerable variation in terms of gene content and function and can be split into six sub-families (1, 2, 3, 4a, 4b, 5) [4, 5]. Moreover, two further, evolutionarily divergent, T6SS-like systems have been reported. One is found on the *Francisella* pathogenicity island and designated T6SS^{ii}, the other is found in the phylum *Bacteroidetes* and designated T6SS^{iii}, whilst the widespread and well-characterized proteobacterial T6SS can be termed T6SS^{i}. T6SS^{ii} and T6SS^{iii} have distinct components but a common overall mode of action when compared to the canonical T6SS^{i} [6, 7]. A given bacterial species may contain between one and six different T6SSs, with the complement of T6SSs present frequently varying between individual strains. In some bacteria, one T6SS is used for two distinct roles: for example, the T6SS in *Vibrio cholerae* is both antibacterial and anti-eukaryotic [8], whilst in other cases different T6SSs fulfil distinct roles: for example, T6SS-5 and T6SS-1 in *Burkholderia thailandensis* have exclusively anti-host and antibacterial activity, respectively [9]. In general, different bacterial species and strains use and tailor their T6SS(s) for specific roles according to the niche and strategy of the organism. In addition to variation in number and type of T6SS *per se*, there is also considerable diversity in effector portfolio. Furthermore, regulation of T6SS gene expression is highly organism-specific and matched with the biological role the system is required to fulfil. For example, the anti-host T6SS of *Burkholderia mallei* is regulated by the global virulence regulator VirAG, whilst the 'defensive' antibacterial H1-T6SS of *Pseudomonas aeruginosa* is post-transcriptionally activated by cell damage-derived signals via the RetS/Gac/Rsm pathway [10, 11]. The aim of this review is to showcase the diversity and breadth of functions mediated by the T6SS, and to highlight the widespread importance of this system in many contexts.

Received 29 January 2019; Accepted 22 February 2019; Published 20 March 2019

Author affiliation: Division of Molecular Microbiology, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK.

***Correspondence:** Sarah Coulthurst, s.j.coulthurst@dundee.ac.uk

Keywords: Bacterial protein secretion; Type VI secretion system; bacterial effector proteins; polymicrobial communities.

Abbreviations: ACD, actin crosslinking domain; EAEC, Enteroaggregative *E. coli*; OMV, outer membrane vesicle; PQS, *Pseudomonas* quinolone signal; QS, quorum sensing; T6SS, Type VI secretion system.

MECHANISM OF EFFECTOR DELIVERY BY THE T6SS

The T6SS is a large and dynamic 'nanomachine' that uses a contraction mechanism to propel an extracellular puncturing structure out of the secreting cell. The force of this propulsion can further drive the puncturing structure, which is decorated with effector proteins, into an immediately adjacent cell, thus achieving contact-dependent translocation of effector proteins into target cells (summarized in Fig. 1).

Assembly and firing of the T6SS machinery

As reviewed in detail elsewhere [12–15], the T6SS is an envelope-spanning apparatus assembled from 14 core components (TssA-M, PAAR) and comprising several distinct sub-assemblies: membrane complex, cytoplasmic baseplate, cytoplasmic contractile sheath and expelled puncturing structure. The puncturing structure consists of a tube of stacked hexameric rings of the Hcp (TssD) protein, topped by a spike comprising a VgrG (TssI) trimer with a PAAR domain-containing protein tip. Assembly begins with the formation of the bell-shaped membrane complex, comprising ten or twelve copies each of the outer membrane protein TssJ and the inner membrane proteins TssLM [15, 16]. The cytoplasmic face of the membrane complex then docks the baseplate, which consists of six $TssEF_2G(K_3)_2$ 'wedges' around a central $VgrG_3$PAAR unit. TssK acts as a connector linking the baseplate to the membrane complex, by interacting with TssFG and the cytoplasmic domains of TssLM [15, 17, 18]. The Hcp tube can then assemble onto the base of VgrG, extending into the cytoplasm. Simultaneously, a helical sheath structure comprising TssBC subunits polymerizes around the Hcp tube in an extended, high-energy 'primed' conformation [19–21]. Rapid (<2 ms) contraction of the TssBC sheath drives the Hcp-VgrG-PAAR structure through the baseplate and membrane complex, out of the secreting cell. Contraction of the TssBC sheath, which can span the width of the producing cell, provides sufficient power and reach for the expelled Hcp-VgrG-PAAR structure to also breach an appropriately located recipient cell [21–23]. Following contraction, the contracted sheath is specifically depolymerized by the ATPase TssH whilst the effectors are somehow released inside the target cell. A role for the final core T6SS component, TssA, in capping the distal end of the Hcp-TssBC structure and coordinating assembly of the inner tube and the sheath has recently been described [24, 25].

The contraction-based mechanism of the T6SS is related to the injection mechanism of contractile bacteriophages, with close structural similarities between the Hcp-VgrG-PAAR structure and the phage tail tube and tail spike, between the TssBC sheath and the tail sheath, and between the TssEFG and $gp25gp6_2gp7$ wedge units [12, 17]. However, in contrast to the phage system, the T6SS can be reused for multiple firing events by the same cell. Indeed dynamic cycles of T6SS assembly–contraction–disassembly have been observed using fluorescence microscopy imaging of individual T6SSs in a number of organisms, including *V. cholerae*, enteroaggregative *Escherichia coli* (EAEC), *P. aeruginosa* and *Serratia marcescens* [16, 26–28].

Accessory components

In addition to the core components above, T6SSs are diversified and further tailored for their function by the possession of accessory structural or regulatory components which are present in some, but not all, T6SSs. In some systems an additional membrane complex component, SciZ/TagL, provides a peptidoglycan-binding functionality normally present in TssL, whilst, conversely, T6SSs may also co-opt peptidoglycan hydrolase enzymes to assist in the formation of the membrane complex through the cell wall [29–31]. In EAEC, a membrane-associated TssA-family protein, TagA, interacts with TssA in order to 'catch', stop and stabilize the extending TssBC sheath when it reaches the opposite side of the cell [32]. Although some other T6SSs possess a second TssA-family protein which is likely to function similarly, many do not, leaving open the question of how else this function might be achieved. Another protein, TagJ, may help to recruit TssH to the contracted sheath in some systems [33].

Many T6SSs also contain conserved post-translational regulatory components. In *P. aeruginosa* and *S. marcescens*, a membrane-bound protein threonine kinase, PpkA, phosphorylates the T6SS-associated Fha protein, overcoming inhibition mediated by a negative regulator, TagF, thereby allowing assembly of an active T6SS. An antagonistic phosphatase, PppA, prom30otes spatial relocation of the T6SS machinery between firing events. Interestingly, the two systems are used to increase the efficiency of T6SS antibacterial activity in distinct ways. In *P. aeruginosa*, the TagQRST complex detects incoming T6SS-mediated attacks from neighbouring cells, activating PpkA and thus causing the H1-T6SS to assemble and fire back towards the attacker, making it a 'defensive' system. In *S. marcescens*, the upstream regulator of PpkA is a distinct protein, RtkS, and the signal for activation is independent of incoming attack or cell–cell contact, resulting in an 'offensive' system which can attack passive or aggressive neighbours (13, 28; and references therein). Other variations also exist, including PpkA-mediated phosphorylation of TssL in *A. tumefaciens* [34].

Modes of effector delivery

An important aspect of the versatility of the T6SS is its ability to deliver a large variety of different types of effector proteins. To achieve this, effectors can associate with the expelled Hcp-VgrG-PAAR structure through multiple distinct mechanisms in order to be translocated between cells (Fig. 1). Cargo effectors non-covalently interact with specific Hcp, VgrG or PAAR proteins, whilst 'specialized' effectors comprise modular proteins in which additional effector domains are covalently fused to the C-terminus of Hcp, VgrG or PAAR proteins [13, 35]. Hcp-dependent cargo effectors are relatively small, bind within the lumen of the Hcp hexamer and are recognized and stabilized by this

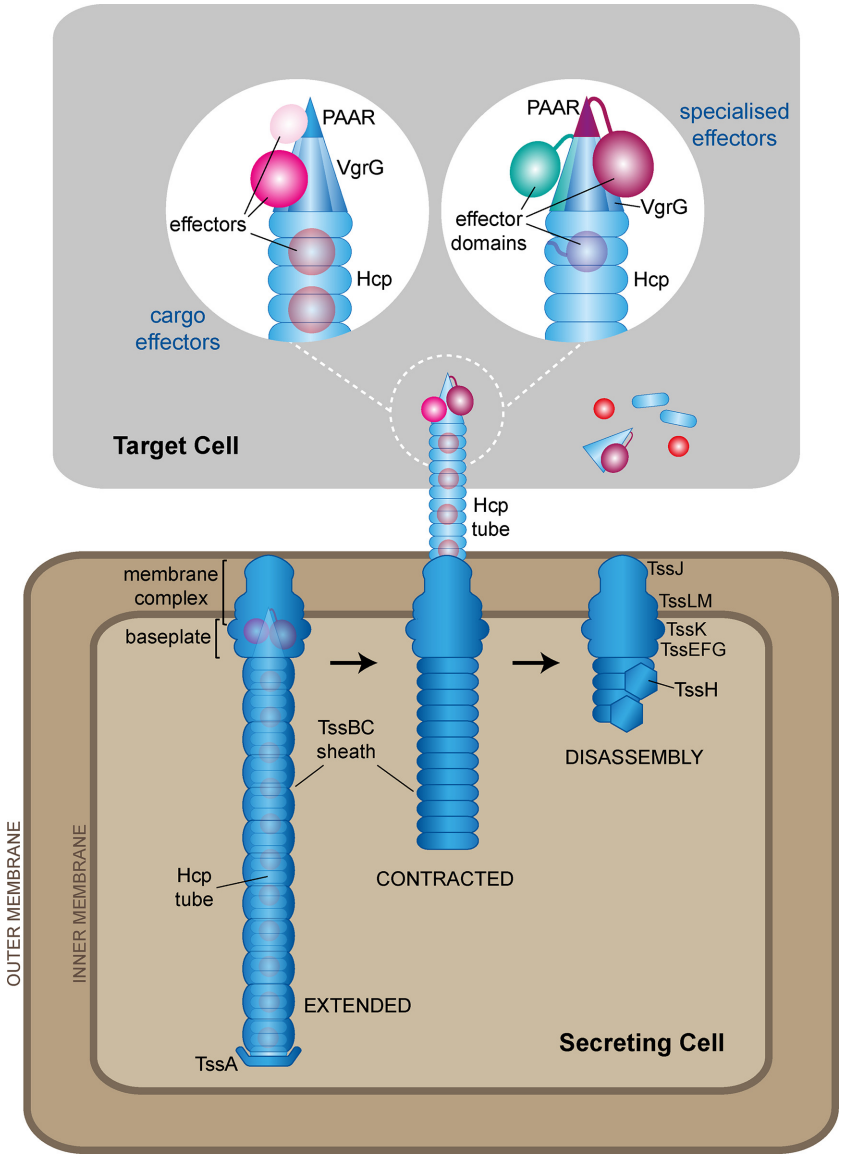

Fig. 1. Effector delivery by the Type VI secretion system of a Gram-negative bacterium. Schematic illustration of the current model for the contraction-based 'firing' mechanism of the T6SS. The T6SS assembles with the contractile TssBC sheath in an 'extended' conformation. Contraction of this sheath, which is anchored to a cytoplasmic baseplate docked on an envelope-spanning membrane complex, drives the Hcp-VgrG-PAAR puncturing structure out of the cell. An adjacent target cell can also be breached by this tube-spike structure. Following contraction, TssH depolymerizes the contracted sheath and the T6SS disassembles at least partially, ready for a new round of firing. The insets illustrate the different ways in which effectors can interact with the expelled Hcp-VgrG-PAAR puncturing structure in order to be translocated out of the secreting cell and into a target cell. 'Cargo' effectors non-covalently interact with Hcp, VgrG or PAAR proteins, whilst 'specialized' effectors consist of an effector domain covalently fused to a VgrG or Hcp protein or a protein containing a PAAR repeat-containing domain. For further details, see text.

interaction, as first described for the antibacterial effectors Tse2, Tse1 (Tae1^{PA}) and Tse3 (Tge1^{PA}) of *P. aeruginosa* [36]. VgrG-dependent cargo effectors interact with specific VgrG proteins to sit on the outside of the spike and include many phospholipase effectors, such as Tle1^{EC} from EAEC [37]. Examples of PAAR-interacting cargo effectors have also recently been reported, including TseT in *P. aeruginosa* [38]. Many different examples of specialized VgrG effectors (also termed 'evolved VgrGs') have been described, including VgrG-1 of *V. cholerae*, which possesses a C-terminal actin crosslinking domain (ACD) [39]. PAAR domain-containing specialized effectors are also widespread and diverse, including many nuclease toxins. These can be based simply on a PAAR domain followed by an effector domain, but also include a group of large polymorphic toxins known as Rhs proteins. In the latter, a conserved central Rhs repeat

domain is predicted to form a shell-like structure around a C-terminal effector domain, with different Rhs proteins possessing a multitude of distinct C-terminal domains and associated immunity proteins [40–43]. It also appears that effector domains may on occasion be fused at the N-terminal end of PAAR proteins, further emphasizing their modularity [44]. Finally, a family of specialized Hcp effectors with a number of distinct C-terminal effector domains, present in members of the *Enterobacteriaceae*, has recently been described [45]. Thus all three components of the expelled puncturing structure can be used for both cargo and specialized effector delivery modes. A given T6SS is typically associated with multiple VgrG, PAAR and/or Hcp homologues, with or without specialized effector domains, allowing for delivery of many effectors. Specific combinations of these homologues define functional tube-spike units and determine the effectors translocated by that firing event [46]. However, the frequency and significance of the formation of VgrG heterotrimers, or of 'mixed' Hcp tubes, is currently unclear.

In some cases, effector recruitment and therefore delivery by the T6SS requires a further 'chaperone' or 'adaptor' protein. Several unrelated but widespread families of such chaperones have been described to date. EagR/EagT (DUF1795) proteins bind the N-terminal PAAR-containing domains of Rhs and Tse6-like effectors, stabilizing transmembrane regions which may ultimately permit the effectors to cross the recipient cell inner membrane and allowing the effector to be loaded onto the cognate VgrG [46, 47]. Tap-1/TecL (DUF4213) family proteins allow the interaction and loading of VgrG-dependent cargo effectors, including TseL and Tde1, onto the cognate VgrG. These are modular adaptors, where the C-terminal half of the protein varies with the associated effector, providing a mechanism for new effectors to be horizontally acquired and interact with existing VgrG homologues via recombination within the Tap-1 gene [48, 49]. A chaperone (TecT) facilitating interaction of a PAAR-dependent cargo effector with the cognate PAAR protein has also been reported, with PAAR competing with a co-chaperone for access to the chaperone [38]. None of these families of chaperones appear to be secreted with their effectors, rather their role is to protect and load specific effectors onto the machinery prior to secretion. Consistent with the different routes of effector secretion, there is no universal secretion signal for substrates of the T6SS. No such signal is required for specialized effectors, whilst cargo effectors require specific 3D interactions with their cognate VgrG/PAAR/Hcp protein, assisted in many cases by specific chaperones. One secretion motif (MIX) has been identified in a subset of effectors widespread in *Vibrionaceae* [50]; this motif is likely to define an adaptor domain interacting with one of the core components.

ANTIBACTERIAL EFFECTORS

T6SSs represent widespread and potent weapons for killing or inhibiting rival bacterial cells, both within and between species. This is achieved by the delivery of broad-spectrum antibacterial effectors (Fig. 2), with a given system typically able to deliver one or more representatives of several different effector families.

Classes and modes of action of T6SS-delivered antibacterial effectors

A large number of T6SS effectors that target the peptidoglycan cell wall of recipient bacteria have been identified. These peptidoglycan hydrolases can be divided into at least five families of peptidoglycan amidases (Tae1–4, TaeX), which cleave specific bonds within the peptide cross-bridges of the cell wall, and four families of peptidoglycan glycoside hydrolases (Tge1–3, VgrG-3), which cleave the backbone glycan chains [51–54]. The inner membrane is also a common target of T6SS effectors. Five families of lipase/phospholipase effector (Tle1–4, includes effectors with phospholipase A_1 or A_2 activity, and Tle5, phospholipase D) have been described [37, 55]. Additionally two effectors, VasX and Tse4, have been reported to form pores or channels in the membrane [56, 57]. All these effectors targeting the cell wall or membrane act in/from the periplasm, being generally non-toxic if expressed in the cytoplasm and in some cases activated by periplasmic insertion of disulphide bonds [58]. This suggests that the major destination for incoming effectors is likely to be the periplasm of target cells, which may also inform on why the T6SS does not appear to act against Gram-positive cells lacking this compartment. However, T6SSs also deliver effectors which act in the bacterial cytoplasm. A number of T6SS-dependent nuclease effectors have been described (e.g. DNases Tde1 and RhsAB) with many more, particularly PAAR-containing specialized effectors, predicted to possess DNase, RNase or deaminase activity according to bioinformatic analyses [43, 48, 59, 60]. T6SS effectors can also act by degrading an essential cytoplasmic cofactor, as revealed by the identification of two families of $NAD(P)^+$ hydrolase effectors (Tse6/Tne1 and Tne2) [61, 62]. Recently a T6SS-delivered ADP-ribosyltransferase toxin, Tre1, which modifies FtsZ by the addition of ADP-ribose and thus inhibits cell division, has been described. Such toxins are often used by bacteria against eukaryotic cells, but this work suggests they may also be used by several inter-bacterial toxin delivery systems including the T6SS [63]. Cytoplasmic-acting effectors may reach the cytoplasm in several ways: by a minority of T6SS delivery events reaching the cytoplasm directly, by effectors incorporating transmembrane domains to allow their own traversal of the inner membrane, and/or by target cell protein-mediated import similar to Cdi toxins; to date, some evidence has been presented for each possibility [47, 62, 64, 65]. Whilst numerous and varied T6SS-dependent antibacterial effectors have already been reported, it seems clear that the portfolio of effectors and of effector modes of action will continue to grow, given that many effectors identified to date still have no known or readily predictable function and that new effectors will be revealed by experimental and bioinformatic analysis of increasing numbers of bacterial strains and species.

Self-protection by specific immunity proteins

Any bacterial cell possessing an antibacterial T6SS must possess a means to prevent self-intoxication by its own effectors (cytoplasmic-acting effectors, prior to secretion) and intoxication by effectors delivered into it by its neighbouring sibling cells (all effectors, incoming). This is achieved through specific immunity proteins, which are encoded adjacent to the gene for the cognate effector protein. Immunity proteins reside in the cellular compartment of action of the effector and normally bind tightly to the effector to physically prevent toxicity (Fig. 2) [40]. For example, immunity proteins against peptidoglycan hydrolase effectors are soluble or lipid-anchored periplasmic proteins which specifically bind to their cognate effectors and block the active site [54, 66, 67]. Interestingly, the Tri1 immunity protein, which protects against the Tre1 ADP-ribosyltransferase effector, has a novel dual function. In addition to a typical, specific active site occlusion mechanism, it also has an enzymatic ADP-ribosylhydrolase activity which removes the modification added by the effector and confers broad resistance to related toxins [63].

Evolution and acquisition of effector–immunity pairs

T6SS-mediated inter-bacterial competition occurs between and within bacterial species, mediated by considerable variation in effector–immunity portfolio, even between strains of the same species. In addition to diversity in the number and type of effector, there is also variation within effector families resulting in related but specific and mutually incompatible effector–immunity pairs. In general, effector–immunity pairs appear to be horizontally acquired in an inter-bacterial 'arms race'. In the case of specialized effectors, there is some evidence, particularly for Rhs proteins, that homologous recombination events allow the facile exchange of one C-terminal effector domain plus cognate downstream immunity gene for another pair, resulting in highly variable loci which can mediate competition between strains [68]. For cargo effectors, whilst simple effector–immunity pairs can be acquired in isolation, it appears that these are often acquired together with linked chaperone or VgrG proteins which are predicted to allow their delivery in the recipient background [4, 49, 69]. So-called 'orphan' immunity proteins, which do not confer resistance to effectors currently possessed by the organism, are frequently encoded downstream of a related 'active' effector–immunity pair. These may be retained from formerly active effector–immunity pairs where the effector has been lost, or represent newly acquired genes, in both cases probably able to confer protection against effectors delivered by other strains [40, 70, 71]. Many T6SS effectors appear to contain modular toxin domains which can be used for inter-bacterial competition in several contexts, such as cargo or specialized T6SS effectors, effectors delivered by contact-dependent inhibition (Cdi) or Type VII (ESAT/Esx) secretion systems, or toxin domains of colicins. For example, Tne2 domains are found in putative T6SS cargo effectors, in Rhs and smaller PAAR specialized T6SS effectors, and in proteins containing LXG and WXG motifs associated with T7SSs [61], whilst CdiA toxin domains are frequently shared with T6SS-associated Rhs proteins and can also be found in putative T6SS cargo effectors [72, 73].

One reason why antibacterial T6SSs are so effective is likely to be because multiple distinct effectors can be delivered at the same time. One one hand, this means that a rival cell cannot simply protect itself by becoming spontaneously resistant to one effector or acquiring one immunity protein. Perhaps more importantly, simultaneous delivery of effectors targeting the cell wall, cell membrane and cellular DNA, for example or attacking one target with several different enzymatic activities, is likely to lead to more efficient killing than any effector alone. Indeed, synergy between several different combinations of effectors has been demonstrated in *P. aeruginosa* [56]. This study also suggested that multiple effectors may protect against variations in environmental conditions that might reduce the efficacy of an individual toxin.

ROLES OF ANTIBACTERIAL T6SSS IN BACTERIAL COMMUNITIES

The killing activity conferred by antibacterial T6SSs can be extremely potent during *in vitro* co-culture experiments, with T6SS-wielding cells often able to virtually eliminate similar numbers of susceptible competitor cells within a few hours. The obvious next question, then, is how that activity is used and relevant in 'real-life' microbial communities and niches. Once T6SS-dependent antibacterial activity had been identified [74], it became clear that reports of virulence defects in T6SS mutants should be carefully evaluated since decreased fitness of a T6SS mutant could be due to loss of ability to compete against the resident microbiota or co-infecting pathogens, rather than the T6SS having a direct effect on host cells. Some years on, there is now evidence that antibacterial T6SSs can markedly influence the composition of host-associated communities and affect the outcome for the host. Antibacterial T6SSs can also play roles in the social behaviour of bacteria, for example recognition of self, and facilitate horizontal gene transfer by releasing DNA from prey cells.

Polybacterial host-associated communities

A number of studies have demonstrated a role for antibacterial T6SSs in overcoming colonization resistance of the gut microbiota towards pathogens. The T6SS of *Salmonella typhimurium* was found to be required for successful establishment of infection in the gut if the resident microbial community was intact, whilst the T6SS of *Shigella sonnei* increased its ability to outcompete commensal *E. coli* and persist in a mouse model [75, 76]. In *V. cholerae*, T6SS-mediated antibacterial activity against the host commensal microbiota was shown to increase intestinal colonization and activate host innate immunity genes in an infant mouse model, and to contribute to colonization of the middle small intestine in an infant rabbit model [77, 78]. Interestingly, in

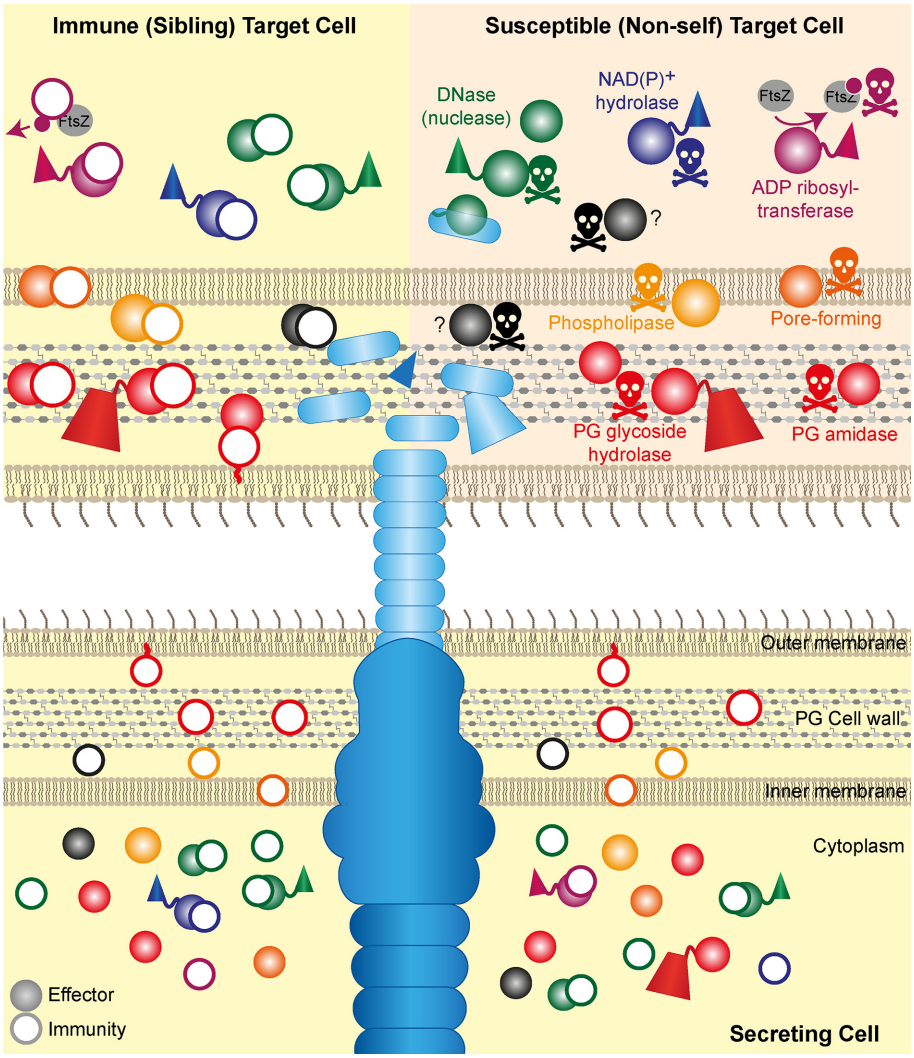

Fig. 2. Antibacterial effectors and cognate immunity proteins. Schematic illustration of modes of action of T6SS-delivered antibacterial effector proteins and their neutralization by self-protecting immunity proteins. The T6SS can deliver a variety of toxic effector proteins into a target cell, which may act on different cellular targets including the peptidoglycan cell wall, cellular nucleic acids and the inner membrane. Immunity proteins specific for each individual effector are localized at the site of action of their cognate effector and typically neutralize the toxin by direct binding and physical inhibition. Immunity proteins protect the secreting cell from its own toxins prior to secretion, if they act in the cytoplasm and are not shielded by another structure such as an Rhs repeat domain (this possibility is not depicted here). Immunity proteins also protect genetically identical cells from the action of all the effectors delivered by their neighbouring sibling. Note that only one PAAR-containing protein and one VgrG trimer can be delivered in a single firing event, although several are included here for illustrative purposes. PG, peptidoglycan.

a *Drosophila* infection model, T6SS-mediated killing of a subpopulation of commensal bacteria was somehow actively required to trigger host destruction by *V. cholerae* [79]. In contrast, in a zebrafish model, removal of a pre-colonized symbiotic species from the gut in a manner dependent on the action of the *V. cholerae* T6SS was not due to antibacterial activity, but rather to a direct impact of the VgrG-1 ACD on host intestinal movements, emphasizing the potential complexities of T6SS-dependent *in vivo* interactions [80]. Members of the *Bacteroidales* are major constituents of the human gut microbiota and commonly possess up to three distinct architectures of T6SS^{iii}, with T6SS loci frequently transferred among co-resident species in the gut [81]. T6SS-mediated competition has been observed between strains of *B. fragilis in vivo*, and appears to play a key role in establishing a stable community of compatible *Bacteriodes* strains in a given individual [82–84]. Antibacterial T6SS activity has also been implicated in generating colonization resistance. A symbiotic *Bacteroides fragilis* strain was shown to utilize its T6SS and the effector Bte2 to exclude a pathogen, a toxigenic strain of *B. fragilis*, *in vivo*, protecting the host from colitis [85]. It will be interesting to

see whether *Bacteroides* also utilize the T6SS against invading proteobacterial gut pathogens *in vivo*. In the bee gut, competition mediated by antibacterial T6SSs, and in particular highly diverse and readily exchangeable Rhs effector domains, appears to be an important driver of fitness and evolution within the microbiota [86].

Complex polymicrobial communities are also found associated with plant hosts, in particular within the rhizosphere. Antibacterial T6SSs are frequently found in both pathogenic and symbiotic or beneficial plant-associated bacteria [87]. This suggests that antibacterial T6SSs are involved in establishing and protecting beneficial plant-associated communities and in invasion of these communities by pathogens. In support of this concept, T6SS-dependent antibacterial activity in the plant-protecting rhizosphere bacterium *Pseudomonas putida* was shown to reduce colonization and necrosis caused by the phytopathogen *Xanthomonas campestris* when co-infiltrated into *Nicotiana* leaves [88]. In contrast, the antibacterial T6SS of the plant pathogen *A. tumefaciens* is effective against *P. aeruginosa* within a plant but not on lab media [48].

A very specific symbiotic relationship is that between the squid *Euprymna scolopes* and *Vibrio fischeri*, in which the bacteria colonize individual crypts in the squid light organ and ultimately bioluminesce. It appears that an antibacterial T6SS plays a key role in selecting and spatially separating strains of *V. fischeri* colonizing the light organ. Individual crypts cannot be colonized by two or more incompatible strains, where incompatibility is conferred by a difference in T6SS and effector–immunity complement [89]. In general, host-associated bacterial communities typically exist in a biofilm state, where aggregates of cells are adherent to a surface within a polymeric extracellular matrix. This scenario is likely to be conducive to T6SS-dependent interactions, and indeed T6SS genes are frequently co-regulated with biofilm genes and the T6SS has been shown to allow persistence of *Burkholderia* in a mixed biofilm [9]. On the other hand, it has been suggested that extracellular polysaccharide may, in some cases, provide a physical barrier that reduces the effectiveness of T6SS attacks [90].

Social behaviour and acquisition of genetic material

Antibacterial T6SSs can represent a means by which one individual strain or genotype can distinguish self from non-self (i.e. from closely related strains). In some cases, this manifests as the formation of a boundary between two populations. In *Proteus mirabilis*, the formation of Dienes lines, macroscopic boundaries between swarms of two non-identical strains, is dependent on T6SS-mediated killing of non-siblings via strain-specific effector–immunity pairs [91, 92]. Similarly, in *Myxococcus xanthus*, delivery of a T6SS nuclease toxin was shown to be required for the inability of colonies of different strains to merge [93]. The T6SS has also been proposed to be a means of policing quorum sensing (QS) 'cheats', spontaneous QS mutants that benefit from the production of common goods (e.g. extracellular enzymes) by a population, whilst no longer producing the goods themselves. In *Burkholderia thailandensis*, QS activation of effector-immunity gene expression was shown to result in QS mutant cheats being eliminated by QS-proficient cells intoxicating them with effectors to which they are no longer immune [94]. Even within a genetically uniform population, antibacterial T6SSs may be able to promote phenotypic homogeneity by allowing fitter cells to eliminate starving or otherwise less-fit cells from the population. Somewhat similar to elimination of QS cheats, starving cells of *M. xanthus* have reduced levels of T6SS and TsxI immunity proteins, allowing them to be killed by delivery of the TsxE effector by healthy neighbouring cells [95]. Mathematical modelling has provided support for the idea that T6SS-mediated interbacterial competition can lead to spatial separation of non-identical bacterial populations, and suggests that this separation can favour the evolution of cooperation within the segregated population [96].

A distinct ecological role for antibacterial T6SSs is in facilitating DNA uptake, and thus horizontal gene transfer, in naturally competent bacteria. T6SS-mediated lysis of non-self cells, whether closely or more distantly related, results in release of DNA from the targeted cell which can then be taken up by the T6SS-wielding attacker. This role was first identified in *V. cholerae*, where T6SS gene expression is co-regulated with that of the competence machinery [97]. Further studies in this organism indicated that T6SS effector–immunity genes themselves can be transferred in this way, and thus an attacker can acquire new weapons from its prey [98]. Similarly, T6SS-mediated horizontal gene acquisition has been demonstrated in *Acinetobacter* [99, 100]. Transfer of genetic material from prey *E. coli* to *Acinetobacter* was frequent enough to allow observation of functional transformation in real time, which may help to explain why *Acinetobacter baumannii* is able to acquire antibiotic resistance so rapidly in the clinic [99].

ANTI-HOST T6SS EFFECTORS

In addition to widespread and versatile utilization of antibacterial T6SSs, bacteria can also use T6SSs to directly target eukaryotic cells, including those of host organisms. There have been many reports of virulence, host response and host cell interaction phenotypes dependent on a functional T6SS, in a range of bacterial pathogens [101]. However given the caveat that such phenotypes may be an indirect result of the action of an antibacterial T6SS, for example against the host microbiota, it is pertinent to consider only those cases where T6SS effectors responsible for direct action against host cells have been identified. During the first decade of T6SS research, these were few in number: VgrG proteins with C-terminal actin crosslinking, actin ADP ribosylase and host membrane fusion domains; another VgrG protein with a tubulin-binding domain that modulates microtubule-mediated bacterial internalization; and two cargo phospholipase D effectors that also facilitate internalization by binding Akt and activating the PI3K pathway; all reviewed by Hachani and co-workers [101].

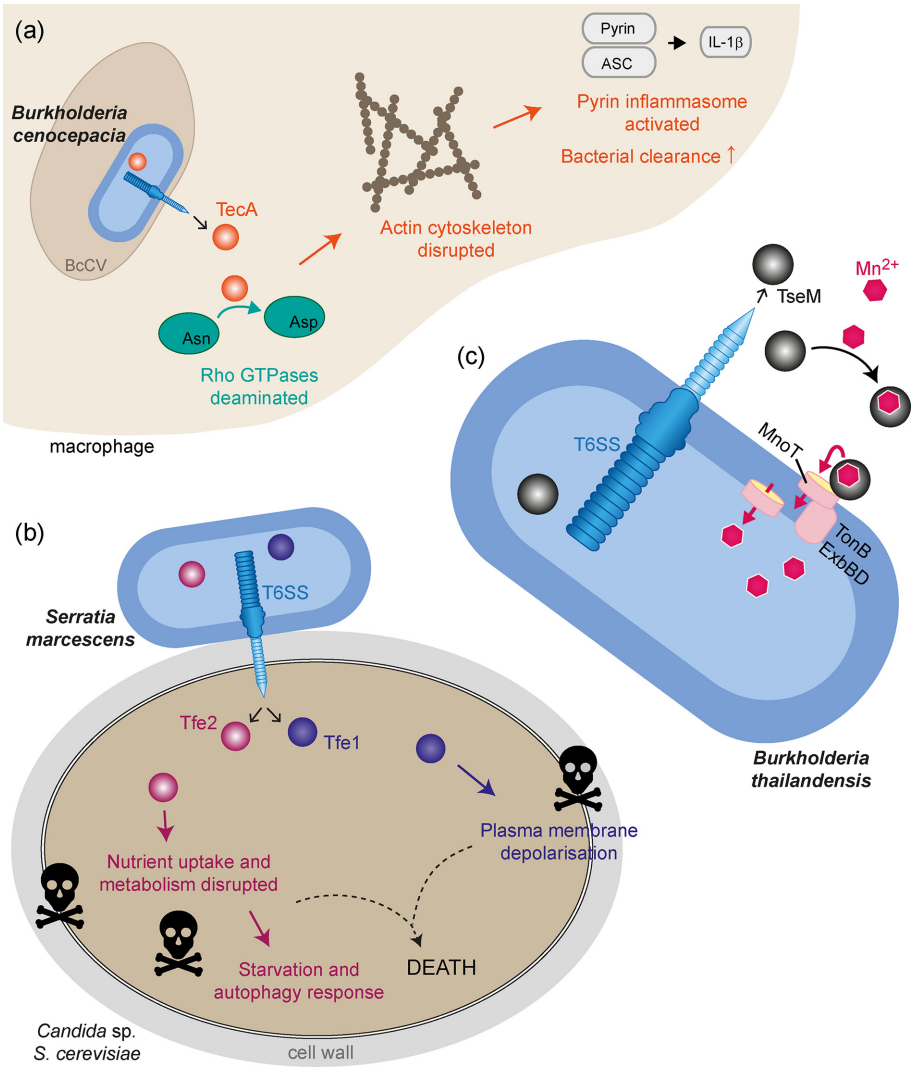

Fig. 3. Examples of Type VI secreted effectors with roles distinct from antibacterial toxins. Schematic illustration of current models for the action of the anti-host effector TecA (a), anti-fungal effectors Tfe1 and Tfe2 (b) and metallophore effector TseM (c). (a) *Burkholderia cenocepacia* delivers TecA from within the *B. cenocepacia*-containing vacuole (BcCV), causing deamidation of specific asparagine residues in Rho family GTPases RhoA and Rac1, leading to disruption of the actin cytoskeleton, activation of the pyrin inflammasome and pyroptosis and increasing bacterial clearance [106]. (b) *S. marcescens* delivers Tfe1 and Tfe2 into fungal cells, including *Candida albicans* and *Saccharomyces cerevisiae*. The action of Tfe1 leads to depolarization of the fungal plasma membrane without formation of large aspecific pores. The action of Tfe2 disrupts inter-related pathways involved in sulfate assimilation, plasma membrane nutrient transport and amino acid metabolism, leading to a starvation response including induction of autophagy. Intoxication by Tfe1 and Tfe2 can eventually cause cell death [114]. (c) *B. thailandensis* uses its T6SS-4 to translocate TseM to the extracellular milieu, where it binds Mn^{2+}. TseM loaded with Mn^{2+} interacts with the outer membrane TonB-dependent receptor MnoT, which is associated with a TonB-ExbD-ExbB complex, transferring Mn^{2+} from TseM to MnoT and allowing its active import across the outer membrane. Either the SitABCD or MntH transporters may then be utilized to import Mn^{2+} across the inner membrane [119].

These phospholipase effectors, PldA and PldB of *P. aeruginosa*, are also antibacterial effectors (Tle5 family) which exert toxicity from the periplasm of target cells lacking the cognate immunity proteins. This highlights another aspect of the versatility of the T6SS: not only is the same system sometimes able to deliver dedicated anti-eukaryotic and antibacterial effectors (e.g. the ACD-containing VgrG-1 and peptidoglycan hydrolase-containing VgrG-3 proteins delivered by the *V. cholerae* T6SS), but some effectors are able to act trans-kingdom, against both eukaryotic and prokaryotic cells [51, 102].

More recently, a number of other anti-host effectors has been reported. The T6SS of *Francisella tularensis* is required for virulence and intracellular proliferation, via phagosomal escape into the cytoplasm. Four effectors, PdpCD and OpiAB, delivered by this system and contributing to intra-macrophage growth, have been identified. PdpC is the

major determinant of phagosomal escape, whilst OpiA can assist in this process through its PI(3)-kinase activity [103–105]. One of the first T6SS effectors identified, EvpP of *Edwardsiella tarda*, was recently shown to prevent activation of the NLRP3 inflammasome by inhibiting the Ca^{2+}-dependent MAPK-Jnk pathway, whilst TecA of *B. cenocepacia* was shown to cause activation of the Pyrin inflammasome via its Rho GTPase deaminase activity and the resulting cytoskeleton disruption (Fig. 3) [106, 107]. Actin rearrangement is also induced by a CNF1-like toxin delivered by *Vibrio parahaemolyticus*, whilst a Tle4-family phospholipase of *P. aeruginosa* causes disruption of the endoplasmic reticulum [108, 109]. In addition, a catalase effector from EHEC, KatN, was proposed to act against reactive oxygen species within host cells [110].

THE T6SS AS A WEAPON AGAINST EUKARYOTIC MICROBIAL COMPETITORS

It is clear from the studies described in the previous section that the bacterial T6SS is able to deploy effector proteins against eukaryotic cells, and it is also generally appreciated that many polymicrobial communities, including those relevant clinically, contain both bacteria and fungi [111]. Therefore it is perhaps not surprising that the versatility of the T6SS extends to its use against fungal cells. Early indications that this could be the case came from observations of T6SS-dependent inhibition of the yeast *Cryptococcus carnescens* by the phytopathogen *Pseudomonas syringae* and an increase in T6SS gene expression in a biocontrol strain of *Pseudomonas fluorescens* when colonizing plant roots in the presence of the fungal phytopathogen *Gaeumannomyces graminis* [112, 113]. Recently, the T6SS of *S. marcescens*, previously believed to be exclusively an antibacterial T6SS, was shown to possess anti-fungal activity against *S. cerevisiae* and *Candida* spp., and it was by studying this system that the first T6SS-delivered anti-fungal effector proteins were identified. These effectors, Tfe1 and Tfe2, have distinct actions against target fungal cells, ultimately leading to fungal cell death. Tfe1-mediated intoxication leads to plasma membrane depolarization without the formation of aspecific pores, whilst Tfe2 intoxication disrupts nutrient uptake and amino acid metabolism and leads to the induction of autophagy, probably as a starvation reponse (Fig. 3) [114]. Tfe1 and Tfe2 represent new classes of effector proteins, with no obvious similarity to other effectors or proteins of known function, and their precise mode of action remains to be elucidated. It is likely that T6SS-dependent anti-fungal activity is widespread. Not only can homologues of Tfe1 and Tfe2 be detected in other T6SS-wielding bacteria, but typical T6SS effector identification criteria are likely to miss anti-fungal effectors. These criteria include (1) association with T6SS genes, (2) antibacterial activity and adjacently encoded immunity protein, and/or (3) the presence of known toxin or effector domains, all of which would have excluded Tfe1 and Tfe2. This highlights the importance of unbiased approaches such as secretomics for the identification of new effectors. The discovery of T6SS-dependent anti-fungal activity suggests that the contribution of the T6SS to shaping polymicrobial communities is broader and more important than previously appreciated. Extending this idea further, there is also evidence that the T6SS can also act against other single-celled eukaryotes, namely amoebae. Indeed, the T6SS was first identified through the requirement for an intact T6SS for *V. cholerae* to resist predation by *Dictyostelium discoideum* [2]. Further work revealed that the pore-forming effector VasX and the actin crosslinking effector VgrG-1 are required for virulence against *Dictyostelium*, in addition to having antibacterial activity or anti-host activity, respectively [115, 116]. Thus the *V. cholerae* T6SS can be utilized against bacterial competitors in the host or environment, against amoebal predators in the environment,and against the host directly, representing a truly versatile and multi-purpose weapon.

A CONTACT-INDEPENDENT ROLE FOR T6SS IN METAL UPTAKE

A further role for the T6SS, this time not requiring effector delivery into target cells, has also been described. In this case, the T6SS is responsible for secretion of effectors to the extracellular milieu which allow the bacteria to take up particular metal ions. In *Yersinia pseudotuberculosis*, T6SS-4 secretes a Zn^{2+}-binding protein, YezP, under conditions of oxidative stress. Secreted YezP is proposed to scavenge Zn^{2+} ions and thus contribute to survival in the presence of reactive oxygen species produced by the host [117]. Similarly, *B. thailandensis* T6SS-4 secretes a related Zn^{2+}-binding effector, TseZ, which allows Zn^{2+} uptake under conditions of oxidative stress via interaction of extracellular TseZ with the TonB-dependent outer membrane haem receptor HmuR [118]. The *B. thailandensis* T6SS-4 is also responsible for the secretion of another metallophore effector, TseM. TseM is required for manganese uptake under conditions of oxidative stress and interacts with MnoT, another TonB-dependent outer membrane receptor, to achieve transport of Mn^{2+} into the cell (Fig. 3) [119]. Importantly, it was demonstrated that possession of TseZ/HmuR or TseM/MnoT, and T6SS-4, by *B. thailandensis* provides a competitive advantage against other bacteria during co-culture in conditions where the respective metal ion is limiting but cells are not in contact. Thus T6SS-dependent secretion of metallophores to the media allows the producing organism to effectively scavenge scarce metal ions from the environment and thus outcompete bacterial competitors without directly harming them. Both metal uptake systems were also required for full virulence in a *Galleria* model, indicating an important role in metal acquisition within a host environment [118, 119]. It is not yet clear whether T6SS-4 in *B. thailandensis* and *Y. pseudotuberculosis*, and related systems in other organisms, are also used to deliver toxic effector proteins into target cells, or whether they are used exclusively for metallophore secretion; certainly their transcriptional regulation, responsive to oxidative stress and metal limitation, appears to be tailored for a metal-scavenging function.

A distinct role for a T6SS effector in metal uptake has been reported for the *P. aeruginosa* H3-T6SS-dependent effector TseF. Extracellular TseF binds PQS-Fe^{2+} complexes incorporated within outer membrane vesicles (OMVs). (PQS, Pseudomonas quinolone signal, is a quorum-sensing signalling molecule with iron-chelating properties.) TseF also interacts with the outer membrane siderophore receptor FptA and porin OprF, and is proposed to mediate $Fe2^{+}$ uptake by delivering OMV-associated PQS-Fe^{2+} to these receptors for transport into the cell [120]. It is currently unclear why TseF or the metallophores above would be secreted by the T6SS, a system normally considered to be designed for effector translocation into cells. Nevertheless, these effectors once again highlight the breadth and versatility of T6SS function, as well as raising the possibility that other proteins that function extracellularly may utilize the T6SS for their secretion.

CONCLUDING REMARKS

It is now clear that the bacterial T6SS is used for an impressively broad range of functions, all linked by the purpose of increasing the competitive fitness of the secreting cell. While anti-host T6SSs allow the bacterium to compete with host defence mechanisms, antibacterial and anti-fungal T6SSs allow the bacterium to compete with rival microbes, both closely and distantly related. Furthermore, those T6SSs and effectors used for contact-independent metal scavenging allow the secreting bacterium to compete with both host defences and co-resident microbes. This functional versatility is permitted by an ever-growing repertoire of diverse effector proteins, and the ability to hook them up to the delivery device in many different ways. It is likely that the portfolio of known effector proteins will continue to increase rapidly, particularly given the evolutionary pressures of inter-bacterial competition and the recent realization that effectors may also have non-toxic extracellular roles. The extent of T6SS-related contributions to myriad bacterial interactions has been further expanded by the validated inclusion of T6SS^{ii} (*Francisella*) and T6SS^{iii} (*Bacteriodetes*) alongside the canonical T6SS^{i} systems, with an even more distantly related T6SS-like system ('T6SS^{iv}') recently described [121]. Importantly, recent work has not only revealed new molecular- and atomic-level details of the mechanism of this intriguing machinery, but it has also begun to demonstrate the importance of T6SS-mediated cell–cell interactions in a variety of 'real-life', or at least relevant model, communities. It is likely that the future will reveal many more examples of communities whose composition, dynamics and properties will be shaped by T6SSs of several flavours. Finally, it is exciting to note that T6SS could provide several potential opportunities for the development of new antimicrobial strategies. It might be possible to inhibit the T6SS itself to reduce the fitness of a T6SS-wielding pathogen, although the broad distribution of the system in other bacteria including commensals would require consideration of specificity. Another avenue, although with similar issues when considering complex communities, could be the generation of engineered 'biocontrol' strains to target pathogens of concern. Finally, by studying T6SS effectors and the impact that they have on target cells, we may learn more about the basic physiology of host, bacterial or fungal cells and how to effectively inhibit them. From both a fundamental and translational point of view, exciting times lie ahead regarding this versatile, widespread and effective bacterial nanoweapon.

Funding information
S. J. C. is supported by the Wellcome Trust (Senior Fellowship in Basic Biomedical Science, 104556/Z/14/Z).

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M *et al.* A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* 2006;312:1526–1530.
2. Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D *et al.* Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc Natl Acad Sci* 2006;103:1528–1533.
3. Bingle LE, Bailey CM, Pallen MJ. Type VI secretion: a beginner's guide. *Curr Opin Microbiol* 2008;11:3–8.
4. Barret M, Egan F, Fargier E, Morrissey JP, O'Gara F. Genomic analysis of the Type VI secretion systems in *Pseudomonas* spp.: novel clusters and putative effectors uncovered. *Microbiology* 2011;157:1726–1739.
5. 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* 2009;10:104.
6. Clemens DL, Ge P, Lee BY, Horwitz MA, Zhou ZH. Atomic structure of T6SS reveals interlaced array essential to function. *Cell* 2015;160:940–951.
7. Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ *et al.* A Type VI secretion-related pathway in *Bacteroidetes* mediates interbacterial antagonism. *Cell Host Microbe* 2014;16:227–236.
8. MacIntyre DL, Miyata ST, Kitaoka M, Pukatzki S. The *Vibrio cholerae* Type VI secretion system displays antimicrobial properties. *Proc Natl Acad Sci* 2010;107:19520–19524.
9. Schwarz S, West TE, Boyer F, Chiang WC, Carl MA *et al.* *Burkholderia* Type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. *PLoS Pathog* 2010;6:e1001068.
10. Leroux M, Kirkpatrick RL, Montauti EI, Tran BQ, Peterson SB *et al.* Kin cell lysis is a danger signal that activates antibacterial pathways of *Pseudomonas aeruginosa*. *elife* 2015;4.
11. Schell MA, Ulrich RL, Ribot WJ, Brueggemann EE, Hines HB *et al.* Type VI secretion is a major virulence determinant in *Burkholderia mallei*. *Mol Microbiol* 2007;64:1466–1485.
12. Brackmann M, Nazarov S, Wang J, Basler M. Using force to punch holes: mechanics of contractile nanomachines. *Trends Cell Biol* 2017;27:623–632.
13. Cianfanelli FR, Monlezun L, Coulthurst SJ. Aim, load, fire: the Type VI secretion system, a bacterial nanoweapon. *Trends Microbiol* 2016;24:51–62.
14. Clemens DL, Lee BY, Horwitz MA. The *Francisella* Type VI secretion system. *Front Cell Infect Microbiol* 2018;8:121.
15. Nguyen VS, Douzi B, Durand E, Roussel A, Cascales E *et al.* Towards a complete structural deciphering of Type VI secretion system. *Curr Opin Struct Biol* 2018;49:77–84.

16. Durand E, Nguyen VS, Zoued A, Logger L, Péhau-Arnaudet G *et al.* Biogenesis and structure of a Type VI secretion membrane core complex. *Nature* 2015;523:555–560.
17. Cherrak Y, Rapisarda C, Pellarin R, Bouvier G, Bardiaux B *et al.* Biogenesis and structure of a Type VI secretion baseplate. *Nat Microbiol* 2018;3:1404–1416.
18. Nazarov S, Schneider JP, Brackmann M, Goldie KN, Stahlberg H *et al.* Cryo-EM reconstruction of Type VI secretion system baseplate and sheath distal end. *Embo J* 2018;37:e97103.
19. Kudryashev M, Wang RY-R, Brackmann M, Scherer S, Maier T *et al.* Structure of the Type VI secretion system contractile sheath. *Cell* 2015;160:952–962.
20. Renault MG, Zamarreno Beas J, Douzi B, Chabalier M, Zoued A *et al.* The gp27-like hub of VgrG serves as adaptor to promote Hcp tube assembly. *J Mol Biol* 2018;430:3143–3156.
21. Wang J, Brackmann M, Castaño-Díez D, Kudryashev M, Goldie KN *et al.* Cryo-EM structure of the extended type VI secretion system sheath-tube complex. *Nat Microbiol* 2017;2:1507–1512.
22. Basler M. Type VI secretion system: secretion by a contractile nanomachine. *Philos Trans R Soc Lond B Biol Sci* 2015;370: 20150021.
23. Vettiger A, Winter J, Lin L, Basler M. The Type VI secretion system sheath assembles at the end distal from the membrane anchor. *Nat Commun* 2017;8:16088.
24. Dix SR, Owen HJ, Sun R, Ahmad A, Shastri S *et al.* Structural insights into the function of Type VI secretion system TssA subunits. *Nat Commun* 2018;9:4765.
25. Zoued A, Durand E, Brunet YR, Spinelli S, Douzi B *et al.* Priming and polymerization of a bacterial contractile tail structure. *Nature* 2016;531:59–63.
26. Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ. Type VI secretion requires a dynamic contractile phage tail-like structure. *Nature* 2012;483:182–186.
27. Basler M, Ho BT, Mekalanos JJ. Tit-for-tat: Type VI secretion system counterattack during bacterial cell-cell interactions. *Cell* 2013;152:884–894.
28. Ostrowski A, Cianfanelli FR, Porter M, Mariano G, Peltier J *et al.* Killing with proficiency: integrated post-translational regulation of an offensive Type VI secretion system. *PLoS Pathog* 2018;14: e1007230.
29. Aschtgen MS, Gavioli M, Dessen A, Lloubès R, Cascales E. The SciZ protein anchors the enteroaggregative *Escherichia coli* Type VI secretion system to the cell wall. *Mol Microbiol* 2010;75:886–899.
30. Santin YG, Cascales E. Domestication of a housekeeping transglycosylase for assembly of a Type VI secretion system. *EMBO Rep* 2017;18:138–149.
31. Weber BS, Hennon SW, Wright MS, Scott NE, de Berardinis V *et al.* Genetic dissection of the Type VI secretion system in *Acinetobacter* and identification of a novel peptidoglycan hydrolase, TagX, required for Its biogenesis. *mBio* 2016;7:e01253–16.
32. Santin YG, Doan T, Lebrun R, Espinosa L, Journet L *et al.* In vivo TssA proximity labelling during Type VI secretion biogenesis reveals TagA as a protein that stops and holds the sheath. *Nat Microbiol* 2018;3:1304–1313.
33. Förster A, Planamente S, Manoli E, Lossi NS, Freemont PS *et al.* Coevolution of the ATPase ClpV, the sheath proteins TssB and TssC, and the accessory protein TagJ/HsiE1 distinguishes Type VI secretion classes. *J Biol Chem* 2014;289:33032–33043.
34. Lin JS, Wu HH, Hsu PH, Ma LS, Pang YY *et al.* Fha interaction with phosphothreonine of TssL activates Type VI secretion in *Agrobacterium tumefaciens*. *PLoS Pathog* 2014;10:e1003991.
35. Durand E, Cambillau C, Cascales E, Journet L. VgrG, Tae, Tle, and beyond: the versatile arsenal of Type VI secretion effectors. *Trends Microbiol* 2014;22:498–507.
36. Silverman JM, Agnello DM, Zheng H, Andrews BT, Li M *et al.* Haemolysin coregulated protein is an exported receptor and chaperone of Type VI secretion substrates. *Mol Cell* 2013;51: 584–593.
37. Flaugnatti N, Le TT, Canaan S, Aschtgen MS, Nguyen VS *et al.* A phospholipase A1 antibacterial Type VI secretion effector interacts directly with the C-terminal domain of the VgrG spike protein for delivery. *Mol Microbiol* 2016;99:1099–1118.
38. Burkinshaw BJ, Liang X, Wong M, Le ANH, Lam L *et al.* A Type VI secretion system effector delivery mechanism dependent on PAAR and a chaperone-co-chaperone complex. *Nat Microbiol* 2018;3:632–640.
39. Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ. Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proc Natl Acad Sci* 2007;104:15508–15513.
40. Alcoforado Diniz J, Coulthurst SJ. Intraspecies competition in *Serratia marcescens* Is mediated by Type VI-Secreted Rhs effectors and a conserved effector-associated accessory protein. *J Bacteriol* 2015;197:2350–2360.
41. Hachani A, Allsopp LP, Oduko Y, Filloux A. The VgrG proteins are "à la carte" delivery systems for bacterial Type VI effectors. *J Biol Chem* 2014;289:17872–17884.
42. Whitney JC, Beck CM, Goo YA, Russell AB, Harding BN *et al.* Genetically distinct pathways guide effector export through the Type VI secretion system. *Mol Microbiol* 2014;92:529–542.
43. Zhang D, de Souza RF, Anantharaman V, Iyer LM, Aravind L. Polymorphic toxin systems: Comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. *Biol Direct* 2012;7:18.
44. Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ *et al.* PAAR-repeat proteins sharpen and diversify the Type VI secretion system spike. *Nature* 2013;500:350–353.
45. Ma J, Pan Z, Huang J, Sun M, Lu C *et al.* The Hcp proteins fused with diverse extended-toxin domains represent a novel pattern of antibacterial effectors in Type VI secretion systems. *Virulence* 2017;8:1189–1202.
46. Cianfanelli FR, Alcoforado Diniz J, Guo M, de Cesare V, Trost M *et al.* VgrG and PAAR proteins define distinct versions of a functional Type VI secretion system. *PLoS Pathog* 2016;12:e1005735.
47. Quentin D, Ahmad S, Shanthamoorthy P, Mougous JD, Whitney JC *et al.* Mechanism of loading and translocation of Type VI secretion system effector Tse6. *Nat Microbiol* 2018;3:1142–1152.
48. Ma LS, Hachani A, Lin JS, Filloux A, Lai EM. *Agrobacterium tumefaciens* deploys a superfamily of Type VI secretion DNase effectors as weapons for interbacterial competition in planta. *Cell Host Microbe* 2014;16:94–104.
49. Unterweger D, Kostiuk B, Ötjengerdes R, Wilton A, Diaz-Satizabal L *et al.* Chimeric adaptor proteins translocate diverse Type VI secretion system effectors in *Vibrio cholerae*. *Embo J* 2015;34:2198–2210.
50. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA *et al.* Marker for Type VI secretion system effectors. *Proc Natl Acad Sci* 2014;111:9271–9276.
51. Brooks TM, 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* 2013;288:7618–7625.
52. Ma J, Sun M, Pan Z, Lu C, Yao H. Diverse toxic effectors are harbored by vgrG islands for interbacterial antagonism in Type VI secretion system. *Biochim Biophys Acta Gen Subj* 2018;1862: 1635–1643.
53. Russell AB, Singh P, Brittnacher M, Bui NK, Hood RD *et al.* A widespread bacterial Type VI secretion effector superfamily identified using a heuristic approach. *Cell Host Microbe* 2012;11: 538–549.
54. Whitney JC, Chou S, Russell AB, Biboy J, Gardiner TE *et al.* Identification, structure, and function of a novel Type VI

secretion peptidoglycan glycoside hydrolase effector-immunity pair. *J Biol Chem* 2013;288:26616–26624.

55. **Russell AB, Leroux M, Hathazi K, Agnello DM, Ishikawa T *et al.*** Diverse Type VI secretion phospholipases are functionally plastic antibacterial effectors. *Nature* 2013;496:508–512.
56. **Lacourse KD, Peterson SB, Kulasekara HD, Radey MC, Kim J *et al.*** Conditional toxicity and synergy drive diversity among antibacterial effectors. *Nat Microbiol* 2018;3:440–446.
57. **Miyata ST, Unterweger D, Rudko SP, Pukatzki S.** Dual expression profile of Type VI secretion system immunity genes protects pandemic Vibrio cholerae. *PLoS Pathog* 2013;9:e1003752.
58. **Mariano G, Monlezun L, Coulthurst SJ.** Dual role for DsbA in attacking and targeted bacterial cells during Type VI secretion system-mediated competition. *Cell Rep* 2018;22:774–785.
59. **Koskiniemi S, Lamoureux JG, Nikolakakis KC, T'kint de Roodenbeke C, Kaplan MD *et al.*** Rhs proteins from diverse bacteria mediate intercellular competition. *Proc Natl Acad Sci* 2013; 110:7032–7037.
60. **Ma J, Sun M, Dong W, Pan Z, Lu C *et al.*** PAAR-Rhs proteins harbor various C-terminal toxins to diversify the antibacterial pathways of Type VI secretion systems. *Environ Microbiol* 2017;19: 345–360.
61. **Tang JY, Bullen NP, Ahmad S, Whitney JC.** Diverse NADase effector families mediate interbacterial antagonism via the Type VI secretion system. *J Biol Chem* 2018;293:1504–1514.
62. **Whitney JC, Quentin D, Sawai S, Leroux M, Harding BN *et al.*** An interbacterial NAD(P)$^{(+)}$ glycohydrolase toxin requires elongation factor Tu for delivery to target cells. *Cell* 2015;163:607–619.
63. **Ting SY, Bosch DE, Mangiameli SM, Radey MC, Huang S *et al.*** Bifunctional immunity proteins protect bacteria against FtsZ-Targeting ADP-ribosylating toxins. *Cell* 2018;175:1380–1392.
64. **Vettiger A, Basler M.** Type VI secretion system substrates are transferred and reused among sister cells. *Cell* 2016;167:99–110.
65. **Willett JL, Gucinski GC, Fatherree JP, Low DA, Hayes CS.** Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways. *Proc Natl Acad Sci* 2015; 112:11341–11346.
66. **Russell AB, Hood RD, Bui NK, Leroux M, Vollmer W *et al.*** Type VI secretion delivers bacteriolytic effectors to target cells. *Nature* 2011;475:343–347.
67. **Srikannathasan V, English G, Bui NK, Trunk K, O'Rourke PE *et al.*** Structural basis for Type VI secreted peptidoglycan DL-endopeptidase function, specificity and neutralization in *Serratia marcescens*. *Acta Crystallogr D Biol Crystallogr* 2013;69:2468–2482.
68. **Koskiniemi S, Garza-Sánchez F, Sandegren L, Webb JS, Braaten BA *et al.*** Selection of orphan Rhs toxin expression in evolved *Salmonella enterica* serovar typhimurium. *PLoS Genet* 2014;10:e1004255.
69. **Fitzsimons TC, Lewis JM, Wright A, Kleifeld O, Schittenhelm RB *et al.*** Identification of novel a*cinetobacter baumannii* Type VI secretion system antibacterial effector and immunity pairs. *Infect Immun* 2018;86.
70. **Alcoforado Diniz J, Liu YC, Coulthurst SJ.** Molecular weaponry: diverse effectors delivered by the Type VI secretion system. *Cell Microbiol* 2015;17:1742–1751.
71. **Kirchberger PC, Unterweger D, Provenzano D, Pukatzki S, Boucher Y.** Sequential displacement of Type VI secretion system effector genes leads to evolution of diverse immunity gene arrays in *Vibrio cholerae*. *Sci Rep* 2017;7:45133.
72. **Batot G, Michalska K, Ekberg G, Irimpan EM, Joachimiak G *et al.*** The CDI toxin of *Yersinia kristensenii* is a novel bacterial member of the RNase A superfamily. *Nucleic Acids Res* 2017;45: 5013–5025.
73. **Poole SJ, Diner EJ, Aoki SK, Braaten BA, T'kint de Roodenbeke C *et al.*** Identification of functional toxin/immunity genes linked to contact-dependent growth inhibition (CDI) and rearrangement hotspot (Rhs) systems. *PLoS Genet* 2011;7:e1002217.
74. **Hood RD, Singh P, Hsu F, Güvener T, Carl MA *et al.*** A Type VI secretion system *of Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* 2010;7:25–37.
75. **Anderson MC, Vonaesch P, Saffarian A, Marteyn BS, Sansonetti PJ.** *Shigella sonnei* encodes a functional T6SS used for interbacterial competition and niche occupancy. *Cell Host Microbe* 2017; 21:769–776.
76. **Sana TG, Flaugnatti N, Lugo KA, Lam LH, Jacobson A *et al.*** *Salmonella* typhimurium utilizes a T6SS-mediated antibacterial weapon to establish in the host gut. *Proc Natl Acad Sci* 2016; 113:E5044–E5051.
77. **Fu Y, Ho BT, Mekalanos JJ.** Tracking *Vibrio cholerae* cell-cell interactions during infection reveals bacterial population dynamics within intestinal microenvironments. *Cell Host Microbe* 2018;23:274–281.
78. **Zhao W, Caro F, Robins W, Mekalanos JJ.** Antagonism toward the intestinal microbiota and its effect on *Vibrio cholerae* virulence. *Science* 2018;359:210–213.
79. **Fast D, Kostiuk B, Foley E, Pukatzki S.** Commensal pathogen competition impacts host viability. *Proc Natl Acad Sci* 2018;115: 7099–7104.
80. **Logan SL, Thomas J, Yan J, Baker RP, Shields DS *et al.*** The *Vibrio cholerae* Type VI secretion system can modulate host intestinal mechanics to displace gut bacterial symbionts. *Proc Natl Acad Sci* 2018;115:E3779–E3787.
81. **Coyne MJ, Roelofs KG, Comstock LE.** Type VI secretion systems of human gut *Bacteroidales* segregate into three genetic architectures, two of which are contained on mobile genetic elements. *BMC Genomics* 2016;17:58.
82. **Chatzidaki-Livanis M, Geva-Zatorsky N, Comstock LE.** *Bacteroides fragilis* Type VI secretion systems use novel effector and immunity proteins to antagonize human gut *Bacteroidales* species. *Proc Natl Acad Sci* 2016;113:3627–3632.
83. **Verster AJ, Ross BD, Radey MC, Bao Y, Goodman AL *et al.*** The Landscape of Type VI secretion across human gut microbiomes reveals its role in community composition. *Cell Host Microbe* 2017;22:411–419.
84. **Wexler AG, Bao Y, Whitney JC, Bobay LM, Xavier JB *et al.*** Human symbionts inject and neutralize antibacterial toxins to persist in the gut. *Proc Natl Acad Sci* 2016;113:3639–3644.
85. **Hecht AL, Casterline BW, Earley ZM, Goo YA, Goodlett DR *et al.*** Strain competition restricts colonization of an enteric pathogen and prevents colitis. *EMBO Rep* 2016;17:1281–1291.
86. **Steele MI, Kwong WK, Whiteley M, Moran NA.** Diversification of Type VI secretion system toxins reveals ancient antagonism among bee gut microbes. *mBio* 2017;8.
87. **Bernal P, Llamas MA, Filloux A.** Type VI secretion systems in plant-associated bacteria. *Environ Microbiol* 2018;20:1–15.
88. **Bernal P, Allsopp LP, Filloux A, Llamas MA.** The *Pseudomonas putida* T6SS is a plant warden against phytopathogens. *Isme J* 2017;11:972–987.
89. **Speare L, Cecere AG, Guckes KR, Smith S, Wollenberg MS *et al.*** Bacterial symbionts use a Type VI secretion system to eliminate competitors in their natural host. *Proc Natl Acad Sci* 2018;115: E8528–E8537.
90. **Toska J, Ho BT, Mekalanos JJ.** Exopolysaccharide protects *Vibrio cholerae* from exogenous attacks by the type 6 secretion system. *Proc Natl Acad Sci* 2018;115:7997–8002.
91. **Alteri CJ, Himpsl SD, Pickens SR, Lindner JR, Zora JS *et al.*** Multicellular bacteria deploy the Type VI secretion system to preemptively strike neighboring cells. *PLoS Pathog* 2013;9: e1003608.
92. **Wenren LM, Sullivan NL, Cardarelli L, Septer AN, Gibbs KA.** Two independent pathways for self-recognition in *Proteus*

mirabilis are linked by Type VI-dependent export. *mBio* 2013;4: e00374–13.

93. Gong Y, Zhang Z, Liu Y, Zhou XW, Anwar MN *et al.* A nuclease-toxin and immunity system for kin discrimination in *Myxococcus xanthus*. *Environ Microbiol* 2018;20:2552–2567.
94. Majerczyk C, Schneider E, Greenberg EP. Quorum sensing control of Type VI secretion factors restricts the proliferation of quorum-sensing mutants. *eLife* 2016;5.
95. Troselj V, Treuner-Lange A, Søgaard-Andersen L, Wall D. Physiological heterogeneity triggers sibling conflict mediated by the Type VI secretion system in an aggregative multicellular bacterium. *mBio* 2018;9.
96. McNally L, Bernardy E, Thomas J, Kalziqi A, Pentz J *et al.* Killing by Type VI secretion drives genetic phase separation and correlates with increased cooperation. *Nat Commun* 2017;8: 14371.
97. Borgeaud S, Metzger LC, Scrignari T, Blokesch M. The Type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer. *Science* 2015;347:63–67.
98. Thomas J, Watve SS, Ratcliff WC, Hammer BK. Horizontal gene transfer of functional Type VI killing genes by natural transformation. *mBio* 2017;8.
99. Cooper RM, Tsimring L, Hasty J. Inter-species population dynamics enhance microbial horizontal gene transfer and spread of antibiotic resistance. *elife* 2017;6.
100. Ringel PD, Hu D, Basler M. The role of Type VI secretion system effectors in target Cell lysis and subsequent horizontal gene transfer. *Cell Rep* 2017;21:3927–3940.
101. Hachani A, Wood TE, Filloux A. Type VI secretion and anti-host effectors. *Curr Opin Microbiol* 2016;29:81–93.
102. Jiang F, Waterfield NR, Yang J, Yang G, Jin Q. A *Pseudomonas aeruginosa* Type VI secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. *Cell Host Microbe* 2014;15: 600–610.
103. Brodmann M, Dreier RF, Broz P, Basler M. *Francisella* requires dynamic Type VI secretion system and ClpB to deliver effectors for phagosomal escape. *Nat Commun* 2017;8:15853.
104. Eshraghi A, Kim J, Walls AC, Ledvina HE, Miller CN *et al.* Secreted effectors encoded within and outside of the *Francisella* pathogenicity island promote intramacrophage growth. *Cell Host Microbe* 2016;20:573–583.
105. Ledvina HE, Kelly KA, Eshraghi A, Plemel RL, Peterson SB *et al.* A Phosphatidylinositol 3-kinase effector alters phagosomal maturation to promote intracellular growth of *Francisella*. *Cell Host Microbe* 2018;24:285–295.
106. Aubert DF, Xu H, Yang J, Shi X, Gao W *et al.* A *Burkholderia* Type VI effector deamidates Rho GTPases to activate the pyrin inflammasome and trigger inflammation. *Cell Host Microbe* 2016;19:664–674.
107. Chen H, Yang D, Han F, Tan J, Zhang L *et al.* The Bacterial T6SS effector EvpP prevents NLRP3 inflammasome activation by inhibiting the Ca(2+)-dependent MAPK-Jnk pathway. *Cell Host Microbe* 2017;21:47–58.
108. Jiang F, Wang X, Wang B, Chen L, Zhao Z *et al.* The *Pseudomonas aeruginosa* Type VI secretion PGAP1-like effector induces host autophagy by activating endoplasmic reticulum stress. *Cell Rep* 2016;16:1502–1509.
109. Ray A, Schwartz N, de Souza Santos M, Zhang J, Orth K *et al.* Type VI secretion system MIX-effectors carry both antibacterial and anti-eukaryotic activities. *EMBO Rep* 2017;18:1978–1990.
110. Wan B, Zhang Q, Ni J, Li S, Wen D *et al.* Type VI secretion system contributes to Enterohemorrhagic *Escherichia coli* virulence by secreting catalase against host reactive oxygen species (ROS). *PLoS Pathog* 2017;13:e1006246.
111. Peleg AY, Hogan DA, Mylonakis E. Medically important bacterial-fungal interactions. *Nat Rev Microbiol* 2010;8:340–349.
112. Haapalainen M, Mosorin H, Dorati F, Wu RF, Roine E *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* 2012;194:4810–4822.
113. Marchi M, Boutin M, Gazengel K, Rispe C, Gauthier JP *et al.* Genomic analysis of the biocontrol strain *Pseudomonas fluorescens* Pf29Arp with evidence of T3SS and T6SS gene expression on plant roots. *Environ Microbiol Rep* 2013;5:393–403.
114. Trunk K, Peltier J, Liu YC, Dill BD, Walker L *et al.* The Type VI secretion system deploys antifungal effectors against microbial competitors. *Nat Microbiol* 2018;3:920–931.
115. Miyata ST, Kitaoka M, Brooks TM, McAuley SB, Pukatzki S. *Vibrio cholerae* requires the Type VI secretion system virulence factor VasX to kill *Dictyostelium discoideum*. *Infect Immun* 2011;79: 2941–2949.
116. Zheng J, Ho B, Mekalanos JJ. Genetic analysis of anti-amoebae and anti-bacterial activities of the Type VI secretion system in *Vibrio cholerae*. *PLoS One* 2011;6:e23876.
117. Wang T, Si M, Song Y, Zhu W, Gao F *et al.* Type VI secretion system transports Zn2+ to combat multiple stresses and host immunity. *PLoS Pathog* 2015;11:e1005020.
118. Si M, Wang Y, Zhang B, Zhao C, Kang Y *et al.* The Type VI secretion system engages a redox-regulated Dual-functional heme transporter for zinc acquisition. *Cell Rep* 2017;20:949–959.
119. Si M, Zhao C, Burkinshaw B, Zhang B, Wei D *et al.* Manganese scavenging and oxidative stress response mediated by Type VI secretion system in *Burkholderia thailandensis*. *Proc Natl Acad Sci* 2017;114:E2233–E2242.
120. Lin J, Zhang W, Cheng J, Yang X, Zhu K *et al.* A Pseudomonas T6SS effector recruits PQS-containing outer membrane vesicles for iron acquisition. *Nat Commun* 2017;8:14888.
121. Böck D, Medeiros JM, Tsao HF, Penz T, Weiss GL *et al.* In situ architecture, function, and evolution of a contractile injection system. *Science* 2017;357:713–717.

Edited by: D. J. Kelly