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# The type VI secretion system: translocation of effectors and effector-domains

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A number of prominent Gram-negative bacteria use the type VI secretion system (T6SS) to transport proteins across the bacterial envelope. Rapid progress is being made in elucidating the structural components of the T6SS apparatus, and a few effectors have been reported to pass through it. However, this is not the complete story: a family of T6SS proteins, the VgrGs, share structural features with the cell-puncturing device of the T4 bacteriophage, and may be used in a similar fashion by bacteria to puncture host cell membranes and insert the T6SS apparatus into the host cytosol. Interestingly, a number of VgrGs contain C-terminal extensions with effector-domains. Thus, the T6SS may translocate soluble effectors, as well as VgrG effector-domains.

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## Introduction

Gram-negative proteobacteria are known to possess type VI secretion system (T6SS) gene clusters, and this category of bacteria maintains pathogenic or symbiotic interactions with eukaryotic organisms [1]. These bacteria are primarily human, animal, or plant pathogens, but several of the nitrogen-fixing, nonpathogenic rhizobia are also known to deploy functional T6SSs [2]. Many of the pathogens that possess a T6SS pose a serious threat to human health and include several category A or B biowarfare agents such as *Vibrio cholerae*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, *Salmonella typhimurium*, pathogenic *Escherichia coli*, as well as opportunistic and emerging pathogens such as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, *Aeromonas hydrophila*, and *Edwardsiella tarda* [1,3–6,7•,8•,9,10,11•,12,13••,14,15,16••,17].

The T6SS loci (Figure 1), which commonly contain 15–25 genes, were originally designated IAHP (IcmF-associated homologous protein) clusters, after the discovery that one of the genes is highly homologous to *icmF* of the *Legionella pneumophila* type IV secretion system (T4SS) [1,18]. A number of T6SS genes were isolated by their virtue of being essential for secretion or virulence [4,7•,9,11•,12,14,15,16••,19–23]. T6SS gene products that are not secreted are believed to be either structural components of the secretion apparatus, or assist protein translocation in another capacity, like providing the energy to push substrates through the channel of the secretion apparatus. For example, many T6SS clusters contain *clpB* homologs, a class of ATPases that forms a hexameric channel through which it transports proteins in an ATP hydrolysis-dependent mechanism [24]. The ClpB homolog in *P. aeruginosa*, ClpV1, may serve as the energy source for the T6SS apparatus [12], and probably plays a similar role in other bacteria with an active T6SS. A number of putative chaperones that may facilitate the transport of substrates through the T6SS conduit have also been reported [4,7•,16••].

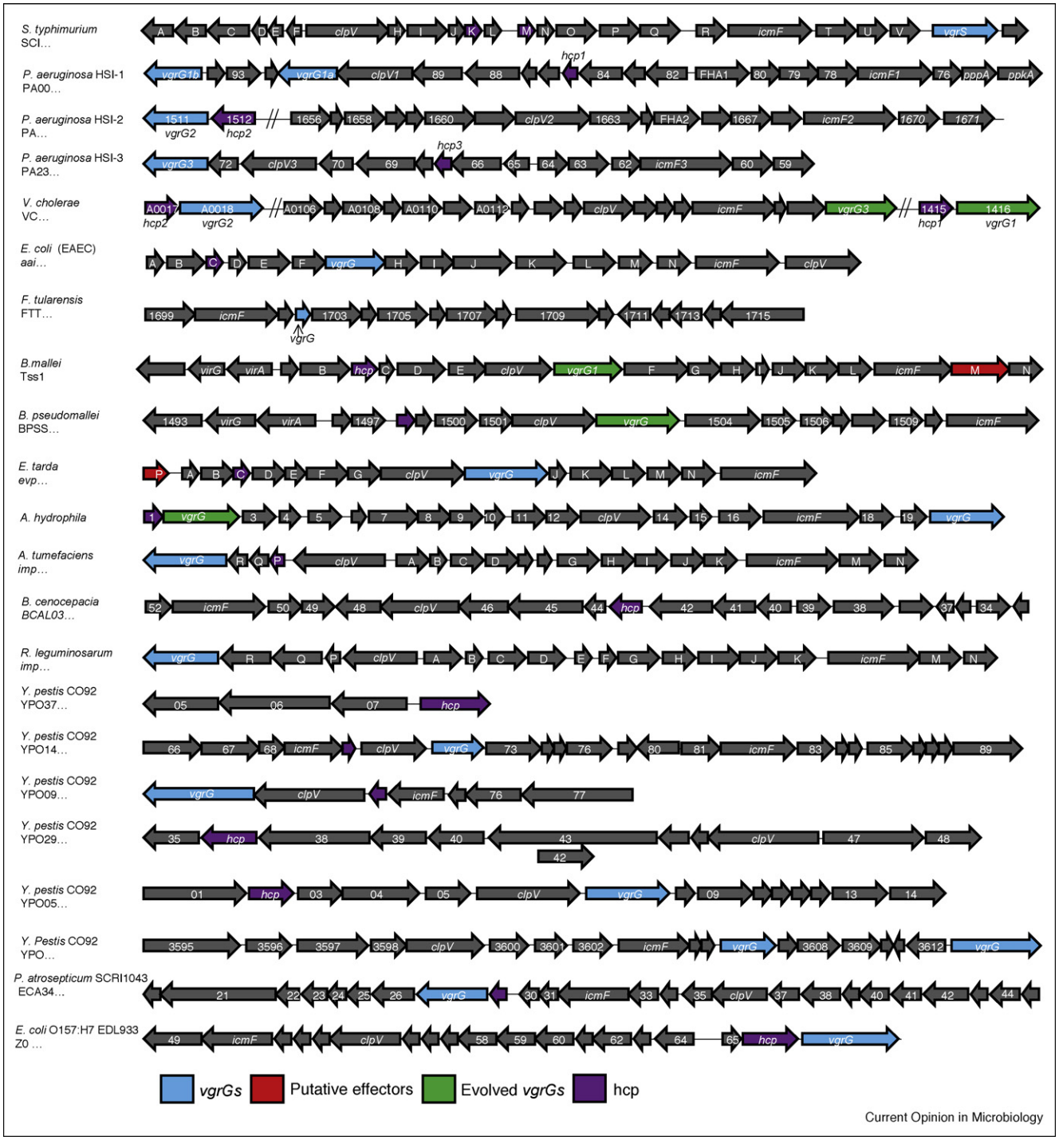
The majority of T6SS components studied so far are not secreted, but are necessary for the secretion of hemolysin coregulated protein (Hcp) and the valine–glycine repeat protein G (VgrG) [1,3–6,7•,8•,9,10,11•,12,13••,14,15,16••,17]. Hcp is secreted by all bacteria with a functional T6SS, and has become a reliable indicator of T6SS function [7•,8•,11•,12,15,19], even though the gene encoding Hcp is not always found in T6SS clusters (Figure 1). The crystal structure of Hcp revealed the formation of hexameric rings [12], which readily polymerize in solution to form tubes with an internal 40-Å diameter that are up to ~100-nm long [25••]. It is conceivable that the Hcp tube forms the conduit of the T6SS through which proteins are transported out of the bacterial cell and into the extracellular space or the cytosol of infected host cells. Thus, it appears that Hcp is a secreted structural T6SS component.

## Secretion of soluble factors

A number of T6SS candidate effector proteins have been recently reported (Table 1). Mutations in these genes

**Glossary**
**EAEC:** enteroaggregative *E. coli*
**IAHP:** IcmF-associated homologous protein
**T4SS:** type IV secretion system
**T6SS:** type VI secretion system
**VgrG:** valine–glycine-repeat protein G

**Figure 1**




The genes of type VI secretion (T6SS). Conserved gene clusters in bacteria with operational T6SSs. Location of genes encoding putative effectors (red arrows), *vgrGs* (blue arrows), *hcp* (purple arrows) and evolved *vgrGs* with C-terminal extensions (green arrows) are highlighted. Bacterial strains and corresponding T6SS gene numbers are listed in the left column.

affect only their own transport and not the assembly of the machine (as indicated by the presence of Hcp and VgrG in the supernatant at wild-type levels). The first evidence that T6SSs secrete soluble effectors came from studies with the plant pathogen *Rhizobium leguminosarum*, which uses its T6SS to transport the small ribose-binding protein RbsB [23]. RbsB homologs are present in many Gram-negative bacteria that harbor T6SSs including

**Table 1**

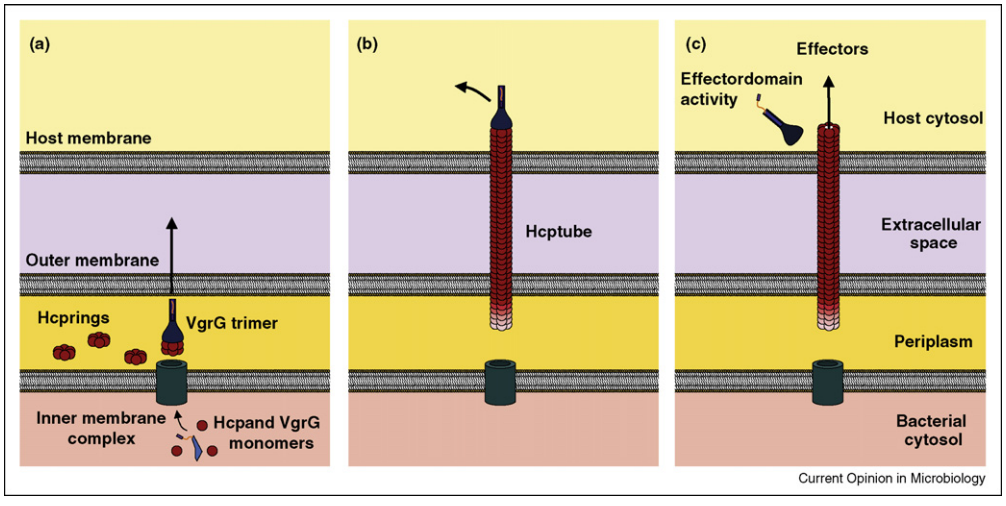
**Putative type VI effectors.**

| Effector | Organism | Length (amino acids) | Weight (kDa) | Canonical hydrophobic signal peptide (predicted by SignalP 3.0) | Conserved domains | Homologs | Reference |
|---|---|---|---|---|---|---|---|
| EvpP | *Edwardsiella tarda* | 185 | 20.3 | No | None | Hypothetical protein MED222_19894 of *Vibrio* sp. *MED222* | [16••] |
| RbsB | *Rhizobium leguminosarum* | 284 | 29.7 | Yes | Sugar transport protein; shares structural features with *V. harveyi* LuxP | Ribose ABC transporter in *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Aeromonas hydrophila*, and *Vibrio* spp. | [23] [38] |
| TssM | *Burkholderia mallei* | 659 | 70.1 | No | Ubiquitin hydrolase domain (PFAM 0443) | Homologs found in *B. pseudomallei*, *B. thailandensis*, and *B. oklahomensis* | [7•] |

*Agrobacterium tumefaciens*, *Aeromonas hydrophila*, and some *Vibrio* species. RbsB and its homologs have been implicated in a variety of different biological functions, including nitrogen fixation, ribose uptake, and chemotaxis [23,26]. Its resemblance with the autoinducer-2 (AI-2)-binding protein LuxP from *V. harveyi*, and the ability of RbsB from *Actinobacillus actinomycetemcomitans* to bind AI-2 [23,26], suggests a possible role in quorum-sensing. However, the biological significance of RbsB in T6SS-mediated has yet to be determined.

Another putative T6SS substrate, EvpP, lacks a canonical hydrophobic signal peptide and is secreted by the T6SS of the human and zoonotic pathogen *Edwardsiella tarda* [16••]. Secretion of EvpP depends on other essential T6SS proteins such as EvpC (Hcp) and EvpI (VgrG), and EvpP translocation is essential for virulence toward blue gourami fish. Surprisingly, sequencing revealed that the N-terminal methionine was missing from secreted EvpP, an observation that has been made for type III secretion system substrates like SopE and Tir from enteropathogenic *E. coli* and *Salmonella dublin*, respectively [27,28]. Whether EvpP is translocated into the host cytosol remains to be shown [16••]. Zheng and Leung, however, demonstrated that EvpP is bound to the Hcp-homolog EvpC inside the bacterial cell and in the extracellular space [16••]. EvpP may interact with Hcp as it travels through the Hcp tube, similar to the soluble T4SS effector CagA from *Helicobacter pylori*, which has been seen bound to the T4SS pili in electron micrographs [29].

**Figure 2**




Model for delivering T6SS effectors and effector-domains. **(a)** Hcp and VgrG monomers are exported into the periplasm through a putative multi-protein complex at the inner membrane. Once in the periplasm, the Hcp monomers form hexameric rings that dock beneath a spike-like VgrG trimer. **(b)** Additional Hcp rings assemble beneath the VgrG tip to create an elongating tube that pushes through the outer membrane. Upon contact with a host membrane, this pilus-like structure punctures through the lipid bilayer. **(c)** The VgrG tip exposes its previously concealed effector-domain to the host cytosol, allowing it to interact with host target molecules and affect host cell function. If the VgrG tip detaches from the T6SS apparatus, it would leave an uncapped Hcp tube, which may now act as a conduit for effector protein delivery into the host cell.

Alternatively, EvpP may be pushed out into the extracellular space by an extending Hcp tube together with the VgrG tip (Figure 2).

In the zoonotic pathogen *B. mallei*, the T6SS gene cluster is part of the VirAG virulence regulon [7•]. This gene cluster encodes the putative effector TssM, which is thought to function as a deubiquitinase [7•]. This class of proteins removes ubiquitin-tags from proteins that are targeted for degradation [30]. Although it is intriguing to speculate that T6SSs secrete effectors that interfere with the ubiquitin-degradation pathway of host cells, evidence for T6SS-dependent secretion of TssM remains to be shown [7•].

To summarize, a number of proteins that require the T6SS for secretion have been reported, setting a precedent for a new class of T6SS components that may function as effectors. Their encoding genes are located either inside or in proximity to T6SS clusters. Unbiased future genetic and proteomics approaches will determine if there are effector genes located elsewhere on the chromosome. Once identified, the next step will be to determine whether their gene products are injected into the host cytosol, and if so, how they mediate virulence.

## T6SS components share structural features with the needle complex of T4 bacteriophage

Another class of proteins that are found in culture supernatants of bacteria with T6SSs, besides Hcp, are the VgrG proteins. These proteins share structural features with the cell-puncturing device of T4 bacteriophage (also referred to as the needle complex) [31]. The needle complex is positioned at the distal end of the tail tube and is used to puncture the bacterial envelope to insert the phage tail tube and to inject phage DNA.

The T4 bacteriophage needle complex consists of trimers of two proteins, gp27 and gp5, which associate in a $(gp27)_3$–$(gp5)_3$ complex. The $(gp5)_3$-portion of the complex contains a C-terminal triple stranded β-helix, which provides the needle-shaped structure that punctures biological membranes. The VgrG family of T6SS components shares structural features with the T4 $(gp27)_3$–$(gp5)_3$ needle complex. According to the HHpred threading program, which identifies protein domains based upon hidden Markov model profile–profile alignments [32], the N-terminal portion of VgrGs shares structural features with the gp44 protein of bacteriophage Mu, while the C-terminal portion aligns with the gp5 protein of T4 phage [33•]. Because Mu gp44 is a structural ortholog of T4's gp27, it was concluded that VgrGs closely resemble a $(gp27)_3$–$(gp5)_3$ needle complex with a characteristic β-helix cell-puncturing structure [33•]. Unlike phage, which requires two separate protein assemblies, $(gp27)_3$ and $(gp5)_3$, to produce the needle complex, bacterial VgrGs apparently use a single polypeptide to form $(VgrG)_3$ complexes. That VgrGs do indeed form multimeric complexes has been shown experimentally when immunoprecipitation of VgrG-1 pulled down VgrG-2 and VgrG-3 in *V. cholerae* [33•]. The exact composition of these VgrG complexes, or whether VgrGs also form homotrimeric structures, cannot be concluded from this experiment. In conclusion, on the basis of its homology to the T4 needle complex and the fact that T6SS components are required to detect VgrGs in culture supernatants [33•], it was proposed that VgrGs are part of the surface-exposed portion of the T6SS apparatus [34].

## Some VgrGs carry C-terminal extensions

Interestingly, some VgrGs carry C-terminal extensions (Table 2). In some *E. coli* strains, VgrGs (and sometimes Hcp) are part of the so-called rearrangement hot spots (*Rhs*) — repetitive DNA sequences responsible for genetic duplications [35]. As a result, up to eight *Rhs* elements can be found in the genomes of some *E. coli* strains. Lin *et al.* hypothesized that these duplications resulted from unequal recombination events between different *Rhs* elements on the K-12 chromosome [35]. Besides being able to multiply in a single genome, *Rhs* elements may participate in horizontal gene transfer as mobile genetic elements to generate the heterologous population of C-terminal extensions currently associated with VgrG proteins.

The C-terminal extensions of some VgrGs carry functional domains (Table 2). Because these extensions may function as effector-domains, these VgrGs have been named 'evolved VgrGs' [33•]. Even though most T6SS clusters contain VgrG genes, only a small minority of clusters encodes evolved VgrGs. Two prominent representatives for bacteria with T6SS clusters that encode evolved VgrGs are *V. cholerae* and *B. pseudomallei*. The majority of evolved VgrG genes are not part of T6SS clusters, but are scattered throughout bacterial genomes, often in the same arrangement with *hcp* as found in *Rhs* elements (see above). Interestingly, the genomes of some bacteria with T6SSs, including *S. typhimurium*, enteroaggregative *E. coli*, and *F. tularensis*, do not encode evolved VgrGs (Table 2), but still exhibit a T6SS-dependent virulence phenotype [4,9,11•,20]. Thus, evolved VgrGs are likely to carry out a bonus function needed by some, but not all pathogens with a T6SS.

The C-terminal extensions carry a variety of conserved domains implicated in an array of biological functions, including crosslinking of host actin, degradation of the peptidoglycan layer (found in the periplasm of Gram-negative bacteria), and ADP-ribosylation of host proteins (Table 2). The best-studied example for evolved VgrGs to date is the *V. cholerae* VgrG-1 protein. VgrG-1 carries an actin-crosslinking domain and when expressed in mammalian cells, causes the collapse of the host cytoskeleton

**Table 2**

**List of VgrGs in bacterial species with functional type VI secretion systems (T6SS).**

| Organism | Disease | Total number of VgrGs | Evolved VgrGs | Full length | Extension length | Homology of extension |
|---|---|---|---|---|---|---|
| *Vibrio cholerae* | Cholera and cholera-like illness | 3 | VC1416 | 1163 | 515 | Actin crosslinking domain |
| | | | VCA0123 | 1017 | 385 | Peptidoglycan degradation and chitosanase |
| *Pseudomonas aeruginosa* | Bacterial infections of the lung, skin, and eye | 10 | PA0262 | 1019 | 185 | Unknown |
| *Yersinia pestis* | Bubonic plague | 6 | YPO2725 | 921 | 250 | Pro-Glu-Thr-Thr-Ala repeats |
| *Francisella tularensis* | Tularemia | 1 | None | – | – | – |
| *Burkholderia cenocepacia* | Cystic fibrosis i nfections | 10 | BCAS0667 | 999 | 270 | Class 3 lipase |
| | | | BCAL1359 | 1233 | 345 | Unknown |
| *Burkholderia mallei* | Glanders (respiratory infections in equines) | 7 | BMAA0737 | 1007 | 215 | Unknown |
| *Burkholderia pseudomallei* | Melioidosis | 13 | BPSS0105 | 899 | 200 | Pro-Thr repeats |
| | | | BPSS1503 | 1007 | 215 | Unknown |
| | | | BPSS2046 | 1111 | 211 | Unknown |
| *Salmonella typhimurium* | Salmonellosis | 1 | None | – | – | – |
| Enteroaggregative *Escherichia coli* | Gastroenteritis | 3 | None | – | – | – |
| *Aeromonas hydrophila* | Gastroenteritis | 3 | AHA_119 | 927 | 275 | ADP-ribosyltransferase |
| *Pectobacterium atrosepticum* | Blackleg in potatoes (plant pathogen) | 5 | None | – | – | – |
| *Edwardsiella tarda* | Hemorrhagic septicemia (fish pathogen); gastrointestinal and extraintestinal infections (humans) | 1 | None | – | – | – |
| *Agrobacterium tumefaciens* | Crown gall disease (plant pathogen) | 2 | None | – | – | – |
| *Rhizobium leguminosarum* | Nitrogen fixation on pea plants (plant symbiont) | 1 | None | – | – | – |
| *Escherichia coli* O157:H7 | Severe (often bloody) diarrhea, hemolytic uremic syndrome | 3 | None | – | – | – |

[36]. This interaction between VgrG-1 and actin is direct and requires ATP as the energy source [33•].

## T6SS effector delivery

Because the macromolecular structure of the T6SS has not yet been resolved, it is not known how T6SS machines assemble or deliver effectors. Considering that over 20 gene products form the secretion apparatus, a full understanding is not imminent. The experimental evidence published to date supports a model for T6SS effector delivery that, in part, mimics the mechanism of the T4 bacteriophage in delivering substrates into *E. coli* (Figure 2) [31]. According to this model, Hcp and VgrGs form a pilus that is displayed on the bacterial surface. VgrG molecules are assigned a dual function, both as membrane-puncturing tips on the end of an Hcp tube and, in the case of evolved VgrGs, as an effector molecule through the activity of C-terminal effector-domains. The presentation of effector-domains attached to the machine component VgrG suggests a novel mechanism of effector presentation, closely resembling the type V secretion system in which a single effector protein transports itself to the bacterial surface [37]. If this model is correct, the T6SS utilizes parts of the machine itself to interfere with processes in the host cell.

The model proposed here requires a T6SS complex in the bacterial inner membrane to export proteins like Hcp and VgrGs into the periplasm (Figure 2a). Bioinformatic analysis suggests that a number of T6SS gene products localize to the inner membrane [34], and these proteins may be involved in shuttling Hcp and VgrG across the inner membrane. Mougous *et al.* showed that Hcp is found in the periplasm of *P. aeruginosa* [13••], representing at the very least a temporary localization to this compartment. VgrG proteins, once in the periplasm, may assemble into a trimeric complex resembling the T4 bacteriophage cell-puncturing device. After the VgrG-complex is assembled, Hcp rings may dock beneath it and form a tube as more rings are added (Figure 2a). The growing Hcp tube might pass through a pore in the outer membrane, or perhaps use the VgrG tip to puncture the membrane and allow tube extension outwards into the extracellular space (Figure 2b). This model accounts for

the codependency of Hcp and VgrGs for secretion as noted by multiple investigators [16••,33•]. In the absence of Hcp, VgrGs cannot reach the extracellular space on top of an extending Hcp pilus, while in the absence of VgrGs, the extending tube cannot pass through the outer membrane.

When the bacterium comes in close contact with a host cell, the T6SS structures protruding from the bacterial surface puncture the host membrane (i.e. plasma or vesicular membrane) with their VgrG tip. According to the model, the trimeric VgrG tip is pushed through the membrane, and the C-terminal effector-domain, which until this time might have been hidden in the cavity of the trimeric VgrG complex, unfolds and interacts with its respective host targets in the cytosol (Figure 2c). It is also possible that this domain is cleaved off from the VgrG, allowing it to interact with host targets distant from the VgrG puncture site. In addition, the VgrG trimer may also act as a 'cap' on an Hcp conduit, which detaches after puncturing through the host membrane to allow translocation of effector proteins from the bacterium directly into the host cytosol (Figure 2c).

## Conclusions

An increasing number of genes are being identified that are required for T6SS-mediated virulence. The majority of these genes seem to encode structural components of the secretion apparatus. The next great task will be to identify new effector molecules, characterize those presented here, and determine if these molecules indeed pass through a T6SS conduit to reach the host cytosol. In the case of VgrG proteins, the challenge will be to determine if these molecules indeed have dual functions: to insert the T6SS apparatus into the host cytosol and to perform effector functions.

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## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- • of special interest

1. Das S, Chaudhuri K: **Identification of a unique IAHP (IcmF associated homologous proteins) cluster in *Vibrio cholerae* and other proteobacteria through in silico analysis**. *In Silico Biol* 2003, **3**:287-300.

2. Das S, Chakrabortty A, Banerjee R, Roychoudhury S, Chaudhuri K: **Comparison of global transcription responses allows identification of *Vibrio cholerae* genes differentially expressed following infection**. *FEMS Microbiol Lett* 2000, **190**:87-91.

3. Yen YT, Bhattacharya M, Stathopoulos C: **Genome-wide in silico mapping of the secretome in pathogenic *Yersinia pestis* KIM**. *FEMS Microbiol Lett* 2008, **279**:56-63.

4. de Bruin OM, Ludu JS, Nano FE: **The *Francisella* pathogenicity island protein IglA localizes to the bacterial cytoplasm and is needed for intracellular growth**. *BMC Microbiol* 2007, **7**:1.

5. Gray CG, Cowley SC, Cheung KK, Nano FE: **The identification of five genetic loci of *Francisella novicida* associated with intracellular growth**. *FEMS Microbiol Lett* 2002, **215**:53-56.

6. Nano FE, Zhang N, Cowley SC, Klose KE, Cheung KK, Roberts MJ, Ludu JS, Letendre GW, Meierovics AI, Stephens G *et al.*: **A *Francisella tularensis* pathogenicity island required for intramacrophage growth**. *J Bacteriol* 2004, **186**:6430-6436.

7. • Schell MA, Ulrich RL, Ribot WJ, Brueggemann EE, Hines HB, Chen D, Lipscomb L, Kim HS, Mrazek J, Nierman WC *et al.*: **Type VI secretion is a major virulence determinant in *Burkholderia mallei***. *Mol Microbiol* 2007, **64**:1466-1485.
The VirAG two-component regulatory system was known to be necessary for virulence in a hamster model of *B. mallei*. Using whole-genome DNA microarrays, overexpression of VirAG led to the upregulation of several genes, including a cluster corresponding to a T6SS. Mutations of genes found in this cluster demonstrated that the T6SS is required for virulence toward hamsters. A secreted Hcp homolog was also identified when VirAG was overexpressed. This Hcp homolog was indirectly shown to be produced *in vivo* as it is recognized by antisera from an infected human, horse, and mice.

8. • Shalom G, Shaw J, Thomas M: **In vivo expression technology identifies a type VI secretion system locus in *Burkholderia pseudomallei* that is induced upon invasion of macrophages**. *Microbiology* 2007, **153**:2689-2699.
Using *in vivo* expression technology (IVET) the authors demonstrated that genes belonging to a T6SS cluster in *Burkholderia pseudomallei* are upregulated after being taken up by murine macrophages. This suggests that the *B. pseudomallei* T6SS may have a role in intracellular growth.

9. Parsons DA, Heffron F: ***sciS*, an *icmF* homolog in *Salmonella enterica* serovar Typhimurium, limits intracellular replication and decreases virulence**. *Infect Immun* 2005, **73**:4338-4345.

10. Folkesson A, Lofdahl S, Normark S: **The *Salmonella enterica* subspecies I specific centisome 7 genomic island encodes novel protein families present in bacteria living in close contact with eukaryotic cells**. *Res Microbiol* 2002, **153**:537-545.

11. • Dudley EG, Thomson NR, Parkhill J, Morin NP, Nataro JP: **Proteomic and microarray characterization of the AggR regulon identifies a pheU pathogenicity island in enteroaggregative *Escherichia coli***. *Mol Microbiol* 2006, **61**:1267-1282.
AggR was initially identified as a regulator of genes on the virulence plasmid pAA2 of EAEC strain EC042. A proteomics and microarray approach led to the identification of a T6SS gene cluster that is under the regulation of AggR. The genes in this cluster encode homologs of hallmark proteins from other characterized T6SSs. The authors identified AaiC, a protein with no known homologs, which is dependent on other genes in the cluster for its secretion. It is of note that *aaiA* and *aaiC* are found in several other strains of EAEC around the world which further implicates the T6SS as an important factor in pathogenesis.

12. Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL, Joachimiak G, Ordonez CL, Lory S *et al.*: **A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus**. *Science* 2006, **312**:1526-1530.

13. •• Mougous JD, Gifford CA, Ramsdell TL, Mekalanos JJ: **Threonine phosphorylation post-translationally regulates protein secretion in *Pseudomonas aeruginosa***. *Nat Cell Biol* 2007, **9**:797-803.
This paper suggests that the regulation of the T6SS in *P. aeruginosa* PAO1 is influenced by two proteins, PpkA and PppA. PpkA is a kinase which becomes activated and autophosphorylated under certain environmental conditions whereas PppA is a phosphatase which counteracts the actions of PpkA. Both proteins act on a common substrate, Fha1, which contains a forkhead-associated domain. The authors demonstrate that both PpkA and Fha1 are required for Hcp secretion into the supernatant. Using fluorescence microscopy they also indicate that Fha1 is necessary for ClpV1 localization to T6SS foci. Once the environmental signal is removed, PppA can dephosphorylate Fha1 and possibly PpkA as well switching off the T6SS.

14. Aubert DF, Flannagan RS, Valvano MA: **A novel sensor kinase-response regulator hybrid controls biofilm formation and type VI secretion system activity in *Burkholderia cenocepacia***. *Infect Immun* 2008, **76**:1979-1991.

15. Suarez G, Sierra JC, Sha J, Wang S, Erova TE, Fadl AA, Foltz SM, Horneman AJ, Chopra AK: **Molecular characterization of a functional type VI secretion system from a clinical isolate of *Aeromonas hydrophila***. *Microb Pathog* 2008, **44**:344-361.

16. •• Zheng J, Leung KY: **Dissection of a type VI secretion system in *Edwardsiella tarda***. *Mol Microbiol* 2007, **66**:1192-1206.
This paper provides detailed characterization of the EVP cluster (T6SS) in *Edwardsiella tarda* and identifies two previously unreported secreted proteins, EvpI and EvpP which are secreted in a T6-dependent manner. By constructing several gene knockouts, it was demonstrated that EvpP is a putative effector of the *E. tarda* T6SS and it depends on 13 EVP genes for secretion. EvpI (VgrG) and EvpC (Hcp) were shown to physically interact and also exert a mutual dependency for secretion from the cell. Finally, all but one EVP gene is required for virulence in the blue gourami fish model.

17. Rao PS, Yamada Y, Tan YP, Leung KY: **Use of proteomics to identify novel virulence determinants that are required for *Edwardsiella tarda* pathogenesis**. *Mol Microbiol* 2004, **53**:573-586.

18. Segal G, Purcell M, Shuman HA: **Host cell killing and bacterial conjugation require overlapping sets of genes within a 22-kb region of the *Legionella pneumophila* genome**. *Proc Natl Acad Sci U S A* 1998, **95**:1669-1674.

19. Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ: **Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system**. *Proc Natl Acad Sci U S A* 2006, **103**:1528-1533.

20. Ludu JS, de Bruin OM, Duplantis BN, Schmerk CL, Chou AY, Elkins KL, Nano FE: **The *Francisella* pathogenicity island protein PdpD is required for full virulence and associates with homologues of the type VI secretion system**. *J Bacteriol* 2008, **190**:4584-4595.

21. Mattinen L, Nissinen R, Riipi T, Kalkkinen N, Pirhonen M: **Host-extract induced changes in the secretome of the plant pathogenic bacterium *Pectobacterium atrosepticum***. *Proteomics* 2007, **7**:3527-3537.

22. Wu HY, Chung PC, Shih HW, Wen SR, Lai EM: **Secretome analysis uncovers an Hcp-family protein secreted via a type VI secretion system in *Agrobacterium tumefaciens***. *J Bacteriol* 2008, **190**:2841-2850.

23. Bladergroen MR, Badelt K, Spaink HP: **Infection-blocking genes of a symbiotic *Rhizobium leguminosarum* strain that are involved in temperature-dependent protein secretion**. *Mol Plant Microbe Interact* 2003, **16**:53-64.

24. Lee S, Sowa ME, Watanabe YH, Sigler PB, Chiu W, Yoshida M, Tsai FT: **The structure of ClpB: a molecular chaperone that rescues proteins from an aggregated state**. *Cell* 2003, **115**:229-240.

25. •• Ballister ER, Lai AH, Zuckermann RN, Cheng Y, Mougous JD: **In vitro self-assembly of tailorable nanotubes from a simple protein building block**. *Proc Natl Acad Sci U S A* 2008, **105**:3733-3738.
Prior to this paper, it was known that individual Hcp proteins can interact to form homohexamers. Here the authors demonstrate that these hexameric rings are capable of stacking on top of one another forming a nanotube with a diameter of 9 nm. With an internal diameter of 4 nm, it is possible that Hcp forms part of the structural secretion complex, allowing for effector proteins to pass through its centre.

26. Binnie RA, Zhang H, Mowbray S, Hermodson MA: **Functional mapping of the surface of *Escherichia coli* ribose-binding protein: mutations that affect chemotaxis and transport**. *Protein Sci* 1992, **1**:1642-1651.

27. Wood MW, Rosqvist R, Mullan PB, Edwards MH, Galyov EE: **SopE, a secreted protein of *Salmonella dublin*, is translocated into the target eukaryotic cell via a sip-dependent mechanism and promotes bacterial entry**. *Mol Microbiol* 1996, **22**:327-338.

28. Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB: **Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells**. *Cell* 1997, **91**:511-520.

29. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, Konig W *et al.*: ***Helicobacter* exploits integrin for type IV secretion and kinase activation**. *Nature* 2007, **449**:862-866.

30. Love KR, Catic A, Schlieker C, Ploegh HL: **Mechanisms, biology and inhibitors of deubiquitinating enzymes**. *Nat Chem Biol* 2007, **3**:697-705.

31. Kanamaru S, Leiman PG, Kostyuchenko VA, Chipman PR, Mesyanzhinov VV, Arisaka F, Rossmann MG: **Structure of the cell-puncturing device of bacteriophage T4**. *Nature* 2002, **415**:553-557.

32. Soding J, Biegert A, Lupas AN: **The HHpred interactive server for protein homology detection and structure prediction**. *Nucleic Acids Res* 2005, **33**:W244-W248.

33. • 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 U S A* 2007, **104**:15508-15513.
The authors provide evidence that in the T6SS of *V. cholerae*, Hcp is dependent on VgrG-1 and VgrG-2 to be secreted into culture supernatants. Immunoprecipitation data indicate that the VgrG proteins interact with one another to form a larger protein complex. The VgrGs also share homology with the bacteriophage T4 tailspike puncturing device. Thus, this protein complex may form part of the structural secretion apparatus allowing for the transport of other proteins out of the bacterial cell.

34. Cascales E: **The type VI secretion toolkit**. *EMBO Rep* 2008, **9**:735-741.

35. Lin R, Capage M, Hill C: **A repetitive DNA sequence, rhs, is responsible for duplications within the *Escherichia coli* K-12 chromosome**. *J Mol Biol* 1984, **177**:1-18.

36. Sheahan KL, Cordero CL, Satchell KJ: **Identification of a domain within the multifunctional *Vibrio cholerae* RTX toxin that covalently cross-links actin**. *Proc Natl Acad Sci U S A* 2004, **101**:9798-9803.

37. Gerlach RG, Hensel M: **Protein secretion systems and adhesins: the molecular armory of Gram-negative pathogens**. *Int J Med Microbiol* 2007, **297**:401-415.

38. James D, Shao H, Lamont RJ, Demuth DR: **The *Actinobacillus actinomycetemcomitans* ribose binding protein RbsB interacts with cognate and heterologous autoinducer 2 signals**. *Infect Immun* 2006, **74**:4021-4029.