



**HAL**  
open science

## Don't judge a book by its cover: The Hcps are not only structural components of the T6SS machinery

Sophie Bleves

### ► To cite this version:

Sophie Bleves. Don't judge a book by its cover: The Hcps are not only structural components of the T6SS machinery. *Virulence*, 2017, 8 (7), pp.1053 - 1054. 10.1080/21505594.2017.1295908. hal-01766077

**HAL Id: hal-01766077**

**<https://amu.hal.science/hal-01766077>**

Submitted on 13 Apr 2018

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

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

1 **Don't judge a book by its cover:**  
2 **the Hcps are not only structural components of the T6SS machinery**

3  
4 Sophie Bleves

5 Aix-Marseille Univ, CNRS, LISM UMR7255, IMM (Institut de Microbiologie de la  
6 Méditerranée), Marseille, France

7 Email: [bleves@imm.cnrs.fr](mailto:bleves@imm.cnrs.fr)

8  
9 **Keywords:** T6SS, Hcp, antibacterial effectors, *Enterobacteriaceae*

10  
11 Although the type VI secretion system (T6SS) was discovered recently (1), it is widespread in  
12 Gram-negative bacteria. This presumably comes from the fitness advantage conferred by the  
13 T6SS (i) in environmental niches against rival bacteria (inter- and intraspecies  
14 competitiveness have been described) and (ii) in the eukaryotic host to commensal bacteria  
15 (2). Moreover in addition to being an anti-eukaryotic weapon (2), the T6SS allows pathogens  
16 to compete with microbiota during host colonization (3). This nanomachine functions as an  
17 inverted contractile phage tail to deliver effectors into bacterial or eukaryotic cells (4). The  
18 contraction of a sheath in the cytoplasm propels an inner tube made of Hcp proteins topped by  
19 a puncturing device consisting of VgrG and PAAR proteins towards target cells. Effectors  
20 associated with this expelled structure are thus translocated into host cells. The first described  
21 T6SS effector was the evolved VgrG1 of *Vibrio cholerae* whose C-terminal extension cross-  
22 links actin (5). Since the discovery of VgrG1, several types of T6SS effectors have been  
23 characterized. They are mainly dedicated to antibacterial activities (2), in some cases to the  
24 battle with eukaryotic hosts (2), or even both (6, 7), which is very original and unusual for  
25 bacterial toxins. Briefly three types of T6SS effectors have been characterized so far: C-  
26 terminal extensions of evolved VgrGs also referred to specialized effectors, specialized  
27 PAAR proteins, and independent toxins also called cargo effectors that target various  
28 components in bacteria (i.e. peptidoglycan, phospholipids, metabolism, nucleic acids) or are  
29 anti-eukaryotic (i.e. bacteria internalization and autophagy). Hcp, VgrG and PAAR proteins  
30 are also implicated in the recruiting and delivery of the cargo effectors (2).

31 Hcp proteins were therefore recognized as structural components of the T6SS machinery that  
32 were also required for effector recruitment until the study of Ma and colleagues in this issue  
33 of *Virulence*. Ma and colleagues reveal a novel function for a new class of Hcps. These Hcps  
34 have been called Hcp-ETs (Haemolysin coregulated protein with C-terminal extension toxins)

35 and act as antibacterial effectors through a specialized C-terminal domain (8). A previous  
36 bioinformatics analysis in various *Salmonella enterica* subspecies (9) identified one Hcp with  
37 a C-terminal extension without conserved domains. This study also mentioned the USP  
38 protein of UPEC (uropathogenic *Escherichia coli*), an Hcp with a putative pyocin extension  
39 (10) and the hypothetical YhhZ protein, an Hcp of *E. coli* (11), which has not yet been further  
40 studied.

41 Here Ma and colleagues performed a systematic search for evolved Hcps in Gram-negative  
42 bacteria and found more than 350 Hcp-ETs encoded by 17 species of *Enterobacteriaceae*.  
43 The Hcp-ETs were classified into five clans according to the conserved domains in their C-  
44 terminal extensions: Hcp-ET1 containing a HNH-DNase domain (a conserved HNH-  
45 endonuclease motif), Hcp-ET2 containing a DUF2235 domain (alpha-beta hydrolase domain)  
46 , Hcp-ET3 containing a pyocin S3 domain, Hcp-ET3-ET4 and orphan ET4 containing a  
47 colicin-DNase domain, and Hcp-ET5 containing a papain-like peptidase domain. Strikingly  
48 these novel toxins are restricted to *Enterobacteriaceae* yet no simple explanation could be  
49 given for this restriction since the C-terminal extensions of Hcp-ETs can be found in other  
50 proteobacteria toxins excluding the hypothesis of a bacterial family specificity. As one might  
51 expect, genes encoding immunity proteins were also found next to Hcp-ET coding genes.  
52 Bacteria produce immunity proteins, which are also known as antitoxins, to prevent fratricide  
53 attack or for self-protection in the case of a toxin that is active in the cytoplasm (2).

54 To gain further insight into the molecular mechanisms of some of these novel T6SS effectors,  
55 the authors performed bacterial competition assays. They showed that the Hcp-ET1 of strain  
56 STEC004 (Shiga toxin-producing *E. coli*) inhibits target cell growth through DNA  
57 degradation and is neutralized by the immunity protein ETI1. They nicely demonstrated that  
58 the Hcp conserved domain (DUF796) in Hcp-ET1 addresses the toxin to the T6SS2  
59 machinery presumably through a heterohexamer formed with two other Hcps of the T6SS2,  
60 namely Hcp2A and Hcp2B. Next, Hcp-ET2 of ETEC (enterotoxigenic *E. coli*) strain PE321  
61 was shown to be evolutionary closed to the Tle1 family of antibacterial T6SS phospholipases  
62 that harbor a GxSxG catalytic motif (12). They found that the Hcp-ET2 cognate immunity  
63 protein, ETI2, directly interacts with Hcp-ET2 and that the DUF796 domain mediates the  
64 targeting to the T6SS machinery. Similarly the authors studied the antibacterial activities of  
65 Hcp-ET3 and of an orphan ET4 in the ETEC strain PE086.

66 Hcp is a key structural component of the T6SS that upon polymerization forms the long rigid  
67 tube that is thrust towards the target cell upon sheath contraction. The data of Ma and  
68 colleagues suggest that in the case of specialized Hcp-ETs, the DUF796 domain can interact

69 with other Hcps to form heterohexameric rings that stack into a tube in the same way that  
70 VgrG heterotrimers stack to form the spike (13). The findings of Ma and colleagues raise  
71 important questions about the localization of the effector extensions within the Hcp tube.  
72 Indeed, the effector domain position should still allow recognition between Hcp subunits and  
73 further assembly. Likewise, the VgrG should accommodate the last Hcp hexamer of the tube  
74 to form the sharp spike. And finally one can ask whether the extension is cleaved or not when  
75 it reaches the target cell?

76

### 77 **Disclosure of Potential Conflicts of Interest**

78 No potential conflicts of interest were disclosed.

79

### 80 **References**

- 81 1. Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF,  
82 Mekalanos JJ. Identification of a conserved bacterial protein secretion system in *Vibrio*  
83 *cholerae* using the *Dictyostelium* host model system. Proc Natl Acad Sci U S A. 2006 Jan  
84 31;103(5):1528-33.
- 85 2. Alcoforado Diniz J, Liu YC, Coulthurst SJ. Molecular weaponry: diverse effectors  
86 delivered by the Type VI secretion system. Cell Microbiol. 2015 Dec;17(12):1742-51. doi:  
87 10.1111/cmi.12532
- 88 3. Sana TG, Flaugnatti N, Lugo KA, Lam LH, Jacobson A, Baylot V, Durand E, Journet L,  
89 Cascales E, Monack DM. *Salmonella* Typhimurium utilizes a T6SS-mediated antibacterial  
90 weapon to establish in the host gut. Proc Natl Acad Sci U S A. 2016 Aug  
91 23;113(34):E5044-51. doi: 10.1073/pnas.1608858113.
- 92 4. Basler M. Type VI secretion system: secretion by a contractile nanomachine. Philos Trans R  
93 Soc Lond B Biol Sci. 2015 Oct 5;370(1679). pii: 20150021. doi: 10.1098/rstb.2015.0021
- 94 5. Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ. Type VI secretion system  
95 translocates a phage tail spike-like protein into target cells where it cross-links actin. Proc  
96 Natl Acad Sci U S A. 2007 Sep 25;104(39):15508-13.
- 97 6. Jiang F, Waterfield NR, Yang J, Yang G, Jin Q. A *Pseudomonas aeruginosa* type VI  
98 secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. Cell Host  
99 Microbe. 2014 May 14;15(5):600-10. doi: 10.1016/j.chom.2014.04.010.
- 100 7. Jiang F, Wang X, Wang B, Chen L, Zhao Z, Waterfield NR, Yang G, Jin Q. The  
101 *Pseudomonas aeruginosa* Type VI Secretion PGAP1-like Effector Induces Host

- 102 Autophagy by Activating Endoplasmic Reticulum Stress. *Cell Rep.* 2016 Aug  
103 9;16(6):1502-9. doi: 10.1016/j.celrep.2016.07.012.
- 104 8. Ma J, Pan Z, Huang J, Sun M, Lu C, Yao H. The Hcp proteins fused with diverse extended-  
105 toxin domains represent a novel pattern of antibacterial effectors in type VI secretion  
106 systems. *Virulence.* 2017 Jan 6:0. doi: 10.1080/21505594.2017.1279374.
- 107 9. Blondel CJ, Jiménez JC, Contreras I, Santiviago CA. Comparative genomic analysis  
108 uncovers 3 novel loci encoding type six secretion systems differentially distributed in  
109 *Salmonella* serotypes. *BMC Genomics.* 2009 Aug 4;10:354. doi: 10.1186/1471-2164-10-  
110 354.
- 111 10. Parret AH, De Mot R. *Escherichia coli*'s uropathogenic-specific protein: a bacteriocin  
112 promoting infectivity? *Microbiology.* 2002 Jun;148(Pt 6):1604-6.
- 113 11. Domka J1, Lee J, Bansal T, Wood TK. Temporal gene-expression in *Escherichia coli* K-  
114 12 biofilms. *Environ Microbiol.* 2007 Feb;9(2):332-46.
- 115 12. Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai SN,  
116 Mougous JD. Diverse type VI secretion phospholipases are functionally plastic  
117 antibacterial effectors. *Nature.* 2013 Apr 25;496(7446):508-12. doi: 10.1038/nature12074.
- 118 13. Hachani A, Lossi NS, Hamilton A, Jones C, Bleves S, Albesa-Jové D, Filloux A. Type VI  
119 secretion system in *Pseudomonas aeruginosa*: secretion and multimerization of VgrG  
120 proteins. *J Biol Chem.* 2011 Apr 8;286(14):12317-27. doi: 10.1074/jbc.M110.193045  
121