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

Sophie Bleves

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

Sophie Bleves

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

Email: bleves@imm.cnrs.fr

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

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

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

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

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

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

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

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

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. 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 Jan 31;103(5):1528-33.
2. Alcoforado Diniz J, Liu YC, Coulthurst SJ. Molecular weaponry: diverse effectors delivered by the Type VI secretion system. Cell Microbiol. 2015 Dec;17(12):1742-51. doi: 10.1111/cmi.12532
3. Sana TG, Flaugnatti N, Lugo KA, Lam LH, Jacobson A, Baylot V, Durand E, Journet L, Cascales E, Monack DM. *Salmonella* Typhimurium utilizes a T6SS-mediated antibacterial weapon to establish in the host gut. Proc Natl Acad Sci U S A. 2016 Aug 23;113(34):E5044-51. doi: 10.1073/pnas.1608858113.
4. Basler M. Type VI secretion system: secretion by a contractile nanomachine.Philos Trans R Soc Lond B Biol Sci. 2015 Oct 5;370(1679). pii: 20150021. doi: 10.1098/rstb.2015.0021
5. 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 Sep 25;104(39):15508-13.
6. 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 May 14;15(5):600-10. doi: 10.1016/j.chom.2014.04.010.
7. Jiang F, Wang X, Wang B, Chen L, Zhao Z, Waterfield NR, Yang G, Jin Q.T he *Pseudomonas aeruginosa* Type VI Secretion PGAP1-like Effector Induces Host

Autophagy by Activating Endoplasmic Reticulum Stress. Cell Rep. 2016 Aug 9;16(6):1502-9. doi: 10.1016/j.celrep.2016.07.012.

8. Ma J, Pan Z, Huang J, Sun M, Lu C, Yao H. The Hcp proteins fused with diverse extended-toxin domains represent a novel pattern of antibacterial effectors in type VI secretion systems. Virulence. 2017 Jan 6:0. doi: 10.1080/21505594.2017.1279374.

9. Blondel CJ, Jiménez JC, Contreras I, Santiviago CA. Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella* serotypes. BMC Genomics. 2009 Aug 4;10:354. doi: 10.1186/1471-2164-10-354.

10. Parret AH, De Mot R. *Escherichia coli*'s uropathogenic-specific protein: a bacteriocin promoting infectivity? Microbiology. 2002 Jun;148(Pt 6):1604-6.

11. Domka J1, Lee J, Bansal T, Wood TK. Temporal gene-expression in *Escherichia coli* K-12 biofilms. Environ Microbiol. 2007 Feb;9(2):332-46.

12. Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai SN, Mougous JD. Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors. Nature. 2013 Apr 25;496(7446):508-12. doi: 10.1038/nature12074.

13. Hachani A, Lossi NS, Hamilton A, Jones C, Bleves S, Albesa-Jové D, Filloux A. Type VI secretion system in *Pseudomonas aeruginosa*: secretion and multimerization of VgrG proteins. J Biol Chem. 2011 Apr 8;286(14):12317-27. doi: 10.1074/jbc.M110.193045