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

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► 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-01766077v1>

Submitted on 13 Apr 2018

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

3
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9 **Keywords:** T6SS, Hcp, antibacterial effectors, *Enterobacteriaceae*

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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

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