1
2
3
4
5 **Adaptor proteins of type VI secretion system effectors**
6
7
8

9 Daniel Unterweger^{1#}, Benjamin Kostiuk¹ & Stefan Pukatzki^{1,2*}
10
11

12 ¹Department of Medical Microbiology and Immunology, University of Alberta,
13 Edmonton, AB T6G 2S2, Canada

14 ²Department of Immunology & Microbiology, University of Colorado School of Medicine,
15 Aurora, CO 80045, USA

16 [#]Current address: Department of Zoology, University of Oxford, Oxford OX1 3PS, United
17 Kingdom
18
19

20 *Corresponding author: Pukatzki, S. (stefan.pukatzki@ucdenver.edu)
21

22 Keywords (2-6): Type VI secretion system, adaptor protein, accessory protein, chaperone,
23 effector translocation, diversity

24 **Abstract** (max 50 words, currently 46)

25 Bacteria use the type VI secretion system (T6SS) to kill neighboring cells. One key feature of the
26 T6SS is the secretion of diverse effectors. Here, we discuss six publications that describe three
27 superfamilies of T6SS proteins, each dedicated to mediate the secretion of cognate effectors.

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

Text

The type VI secretion system (T6SS) is a contractile puncturing device, enabling bacteria to kill neighboring prokaryotic and eukaryotic cells in a contact-dependent manner. Contraction of a cytoplasmic outer sheath results in the ejection of an inner tube (comprised of Hcp subunits) that can penetrate into a neighboring cell [1]. Effectors are found within the tube and on top of the tip of the tube. Once translocated, the effectors act in a toxic manner unless inhibited by T6SS immunity proteins produced by the receiving cell. T6SS effectors differ in the mechanism by which they associate with the tip of the T6SS machinery tube, as well as in their biochemical activity. The first T6SS effectors to be described were the so-called valine glycine-rich proteins G (VgrG) that exhibit structural homology to the cell-puncturing device of T4 bacteriophage, establishing the tip of the T6SS. VgrG proteins consist of a core that can be fused to a C-terminal domain with effector activity [2]. In addition, VgrGs contain a region that binds the so-called PAAR-domain (proline-alanine-alanine-arginine domain) of a defined class of effector proteins [1]. To define the mechanisms by which diverse T6SS effectors are translocated, we summarize the recent characterization of proteins with domains of unknown function (DUFs) that have been labeled as accessory [3], adaptor [4], or chaperone proteins [5; 6].

These adaptors are responsible for the secretion of effectors with or without PAAR domains. Liang *et al.* and Unterweger *et al.* independently described a member of the superfamily of proteins with a conserved DUF4123 domain required for the secretion of effectors lacking a PAAR domain in *Vibrio cholerae* [4; 6]. Diniz *et al.*, Whitney *et al.* and Cianfanelli *et al.* characterized representatives of the superfamily of proteins with a conserved DUF1795 domain that are needed for the secretion of PAAR-domain effectors in *Serratia marcescens* and *Pseudomonas aeruginosa* [3; 5; 7]. More recently, Bondage *et al.* identified a third group of

adaptor proteins characterized by the DUF2169 in *Agrobacterium tumefaciens* [8]. Adaptor proteins representing three superfamilies that have been studied experimentally are compiled in Table 1 along with the effectors they translocate and other pertinent information.

Table 1. Experimentally studied adaptor proteins subdivided by DUF superfamily

Superfamily	Adaptor	Effector	Effector activity	Effector-region for VgrG interaction	Species	Reference
DUF1795	EagR1	Rhs1	Unknown	PAAR-domain	<i>S. marcescens</i>	3
	EagR2	Rhs2	DNase	PAAR-domain	<i>S. marcescens</i>	7
	EagT6	Tse6	NAD(P) ⁺ Glycohydrolase	PAAR-domain	<i>P. aeruginosa</i>	5
DUF2169	Atu3641	Tde2	DNase	unknown	<i>A. tumefaciens</i>	8
DUF4123	Tap-1 (TecL)	TseL	Lipase	unknown	<i>V. cholerae</i>	4, 6
	VasW	VasX	Pore formation	unknown	<i>V. cholerae</i>	10
	TecC	TseC	Unknown	unknown	<i>A. hydrophila</i>	6
	Tap-1 (Atu4349)	Tde1	DNase	unknown	<i>A. tumefaciens</i>	8

Biochemical studies of the proteins belonging to the DUF4123 and DUF1795 superfamilies have demonstrated direct interactions with their cognate effectors are required form protein complexes with VgrG proteins [3-6; 8]. The observation that some PAAR domain-containing effectors are unable to bind to VgrG by themselves further supports the notion that DUF1789 proteins are required for the stability and three-dimensional conformation of effectors. Indeed, adaptor

proteins of all three superfamilies were reported to stabilize their cognate effector indicating that these proteins act as chaperones [5; 8; 9]. Genetic and biochemical experiments indicate that DUF4123, DUF1795 and DUF2169 proteins are also required for the secretion of cognate effectors and subsequent killing of targeted prey bacteria [3-8]. Importantly, DUF4123, DUF1795 and DUF2169 proteins are only required for the delivery of their cognate effector, but not for the functionality of the T6SS [3-6; 8; 10]. Thus, adaptor proteins of the three superfamilies differ in this aspect from Hcp proteins, which form the inner T6SS tube and can act as chaperones for small effectors therein [11].

Experiments conducted by Whitney *et al.* suggest that DUF1795 protein EagT6 of *P. aeruginosa* binds to the N-terminal half of PAAR domain-containing effector Tse6 - thereby stabilizing Tse6 [5]. The DUF1795 superfamily protein EagR1 has been described to interact also with the N-terminal domain of its cognate effector, Rhs1, in *S. marcescens* [7]. The crystal structure of EagT6 resembles the shape of a horseshoe and the authors proposed a model in which the chaperone EagT6 shields hydrophobic transmembrane helices of Tse6 during the translocation from one bacterium to another [5]. However, the DUF1795 superfamily proteins of *S. marcescens* do not appear to accompany their secreted effectors as they are not detected in supernatants analyzed by mass spectrometry [7]. Western-blot analysis of culture supernatants also failed to detect the DUF4123 protein Tap-1 (TecL) or DUF2169 protein Atu3641 in culture supernatants [4; 6; 8]. As a result, DUF4123 proteins were proposed to load their cognate effector onto the secretion system without being secreted themselves (see Figure 1). With the adaptor gene deleted, the effector is no longer detected at the tip of the T6SS and vice versa, indicating that the two proteins interact prior to loading onto the tip [4; 7; 8]. Effectors loaded by the DUF4123 superfamily proteins interact with sites of VgrG proteins situated C-terminal to the

105 highly conserved core and distinct from the VgrG-site interacting with PAAR domain effectors
106 [4; 8]. Determining the structure and function of the various conserved DUF domains is needed
107 to better understand the differences between the superfamilies of adaptor proteins.

108 Strains of the same bacterial species can contain a repertoire of multiple effectors that differ in
109 their biochemical activity and the mechanism by which they are associated with the T6SS.
110 Bacteria such as *S. marcescens* produce various effectors that compete for a binding site at the
111 T6SS tip to be transported in the same translocation event [7]. Differences in the affinity of the
112 adaptor proteins to the T6SS tip might enable an adaptor protein with high affinity to load its
113 cognate effector onto the T6SS at higher frequency [7]. Another way by which the adaptor
114 protein might influence the effector set of a particular bacterium is if the adaptor protein-
115 encoding gene aids in the acquisition of a new effector protein-encoding gene. The effector
116 repertoire of a bacterial strain may change due to the acquisition of effector protein-encoding
117 genes in effector modules that are likely exchanged between strains via horizontal gene transfer
118 [12]. These effector modules contain all or part of an adaptor protein-encoding gene, an effector
119 protein-encoding gene, and an immunity protein-encoding gene [4]. The genetic linkage of genes
120 encoding DUF4123, DUF1795 and DUF2169 superfamily proteins with genes encoding their
121 cognate effectors located immediately downstream suggests that this genetic organization applies
122 to all three superfamilies [3; 4; 6; 8]. Homologous recombination at conserved sites in the center
123 of *tap-1* and an additional site flanking the 3'-end of the module would result in the formation of
124 chimeric adaptor proteins that are specific to their cognate effector and match the resident VgrG-
125 1 protein encoded upstream of the adaptor gene [4]. Adaptor proteins might thereby play a
126 crucial role for providing a system in which newly acquired effectors, which are encoded on

127 effector modules together with at least parts of the adaptor protein-encoding gene, are
128 successfully translocated by the resident T6SS.

129 The ability to secrete a variety of effectors makes the T6SS a versatile system, aiding bacteria in
130 competition with prokaryotic and eukaryotic competitors. Effector diversity is the basis for the
131 formation of compatibility groups: bacteria with the same set of effectors and immunity genes
132 are able to co-exist in direct contact; incompatible strains with differing effectors kill each other
133 due to the lack of cognate immunity proteins [12]. Multiple mechanisms to associate effectors
134 with the tip of the T6SS may increase the number of effectors and the variety of effector proteins
135 with differing toxic activities that can be translocated during the same translocation event.

136 DUF4123, DUF1795 and DUF2169 superfamily proteins are widespread among bacterial
137 species [3-6; 8] and are thus likely to make crucial contributions to the competitive fitness of
138 bacteria. Studying the basic mechanism of effector secretion has implications for our basic
139 understanding of microbial competition and could lead to the development of novel therapeutic
140 interventions that instruct pathogens to outcompete each other.

141

Figure 1. General model for the role of adaptor proteins in T6SS-mediated export.

The membrane-baseplate complex and the Hcp tube secreted upon contraction of the VipA and VipB sheath are depicted. Adaptor proteins with characteristic DUF domains stabilize cognate effector proteins and are required for effector proteins to locate to the tip of the T6SS. The current working model suggests that once located to the tip, the effector is getting exported via the T6SS whereas the adaptor protein stays behind. IM inner membrane, OM outer membrane.

References

- [1] Cianfanelli, F.R., Monlezun, L., and Coulthurst, S.J. (2016). Aim, Load, Fire: The Type VI Secretion System, a Bacterial Nanoweapon. *Trends in microbiology* *24*, 51-62.
- [2] Pukatzki, S., Ma, A.T., Revel, A.T., Sturtevant, D., and Mekalanos, J.J. (2007). Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proceedings of the National Academy of Sciences of the United States of America* *104*, 15508-15513.
- [3] Alcoforado Diniz, J., and Coulthurst, S.J. (2015). Intraspecies Competition in *Serratia marcescens* Is Mediated by Type VI-Secreted Rhs Effectors and a Conserved Effector-Associated Accessory Protein. *Journal of bacteriology* *197*, 2350-2360.
- [4] Unterweger, D., Kostiuk, B., Ojtjengerdes, R., Wilton, A., Diaz-Satizabal, L., and Pukatzki, S. (2015). Chimeric adaptor proteins translocate diverse type VI secretion system effectors in *Vibrio cholerae*. *The EMBO journal* *34*, 2198-2210.
- [5] Whitney, J.C., Quentin, D., Sawai, S., LeRoux, M., Harding, B.N., Ledvina, H.E., Tran, B.Q., Robinson, H., Goo, Y.A., Goodlett, D.R., *et al.* (2015). An interbacterial NAD(P)(+) glycohydrolase toxin requires elongation factor Tu for delivery to target cells. *Cell* *163*, 607-619.
- [6] Liang, X., Moore, R., Wilton, M., Wong, M.J., Lam, L., and Dong, T.G. (2015). Identification of divergent type VI secretion effectors using a conserved chaperone domain. *Proceedings of the National Academy of Sciences of the United States of America* *112*, 9106-9111.
- [7] Cianfanelli, F.R., Alcoforado Diniz, J., Guo, M., De Cesare, V., Trost, M., and Coulthurst, S.J. (2016). VgrG and PAAR Proteins Define Distinct Versions of a Functional Type VI Secretion System. *PLoS pathogens* *12*, e1005735.
- [8] Bondage, D.D., Lin, J.S., Ma, L.S., Kuo, C.H., and Lai, E.M. (2016). VgrG C terminus confers the type VI effector transport specificity and is required for binding with PAAR and adaptor-effector complex. *Proceedings of the National Academy of Sciences of the United States of America* *113*, E3931-3940.
- [9] Ma, L.S., Hachani, A., Lin, J.S., Filloux, A., and Lai, E.M. (2014). *Agrobacterium tumefaciens* deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition in planta. *Cell host & microbe* *16*, 94-104.
- [10] Miyata, S.T., Unterweger, D., Rudko, S.P., and Pukatzki, S. (2013). Dual expression profile of type VI secretion system immunity genes protects pandemic *Vibrio cholerae*. *PLoS pathogens* *9*, e1003752.
- [11] Silverman, J.M., Agnello, D.M., Zheng, H., Andrews, B.T., Li, M., Catalano, C.E., Gonen, T., and Mougous, J.D. (2013). Haemolysin coregulated protein is an exported receptor and chaperone of type VI secretion substrates. *Molecular cell* *51*, 584-593.
- [12] Unterweger, D., Miyata, S.T., Bachmann, V., Brooks, T.M., Mullins, T., Kostiuk, B., Provenzano, D., and Pukatzki, S. (2014). The *Vibrio cholerae* type VI secretion system employs diverse effector modules for intraspecific competition. *Nature communications* *5*, 3549.