



# More Than Just a Spearhead: Diverse Functions of PAAR for Assembly and Delivery of Toxins of the Contractile Injection Systems

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**ABSTRACT** The type VI secretion system (T6SS) belongs to the evolutionarily related group of contractile injection systems that employ a contractile outer sheath to inject a rigid spear-like inner tube into target bacterial and eukaryotic cells. The tip of the rigid tube is often decorated by a PAAR-repeat protein as a key structural component. Many members of the PAAR protein family can also have additional and diverse functions by serving as toxins for those with extended domains or as carriers for interacting toxins. A plethora of toxin modules or modules of unknown functions have been bioinformatically predicted to be associated with PAAR either as a fused domain or as an interacting partner, and yet only a small number of PAAR proteins have been studied, highlighting the exciting and dire need for future research to better understand the diverse PAAR-mediated functions.

**KEYWORDS** protein secretion, interspecies interaction, effector, PAAR, T6SS, protein secretion

Microbes have to overcome not only the fierce competition from neighboring microbes in polymicrobial communities but also the formidable host defenses during infection. To meet the head-on challenges of their adversaries, microbes have evolved various forms of weapon-like mechanisms ranging from diffusible antimicrobial molecules capable of long-range effects to contact-dependent killing of their specific neighbors (1–5). Of these weapons, the type VI secretion system (T6SS) is one of the most effective that can deliver a plethora of toxic effectors into both prokaryotic and eukaryotic species (1, 6–8).

Based on structural similarity and protein conservation, the T6SS has been ascribed to a class of contractile injection systems (CIS) that also include the contractile phage tail, the R-type pyocin, the *Photorhabdus* virulence cassette, and the *Serratia* antifeeding prophage (Afp) (9, 10). They share a characteristic double-tubular structure with a rigid inner tube enclosed by an outer contractile sheath, and both tubes are made of stacking hexamers of their respective subunits (9). For the T6SS, the tube structure can span across the width, or less frequently the length, of a bacterial cell, and is anchored by a multicomponent base-plate and a transmembrane complex (Fig. 1) (11, 12). On the tip of the T6SS tube sits a spike complex composed of a VgrG-trimer and a cone-shaped PAAR which, together with the inner tube, are thrust out of the cell upon sheath contraction like a molecular spear. The released physical force is strong enough to enable the spear to puncture through the outer and inner membranes and the cell wall of Gram-negative cells (11, 13, 14). Excitingly, recent studies show that the T6SS spear can even penetrate and kill the relatively “tough” Gram-positive bacteria with much thicker cell envelopes (15, 16).

Because it is the effectors but not the physical puncture that kill bacteria (17, 18), the T6SS is not merely a spear but one smeared with deadly toxins. At the tip of the spear, PAAR-repeat proteins have been recognized as an important carrier for toxic effectors (19) or directly as

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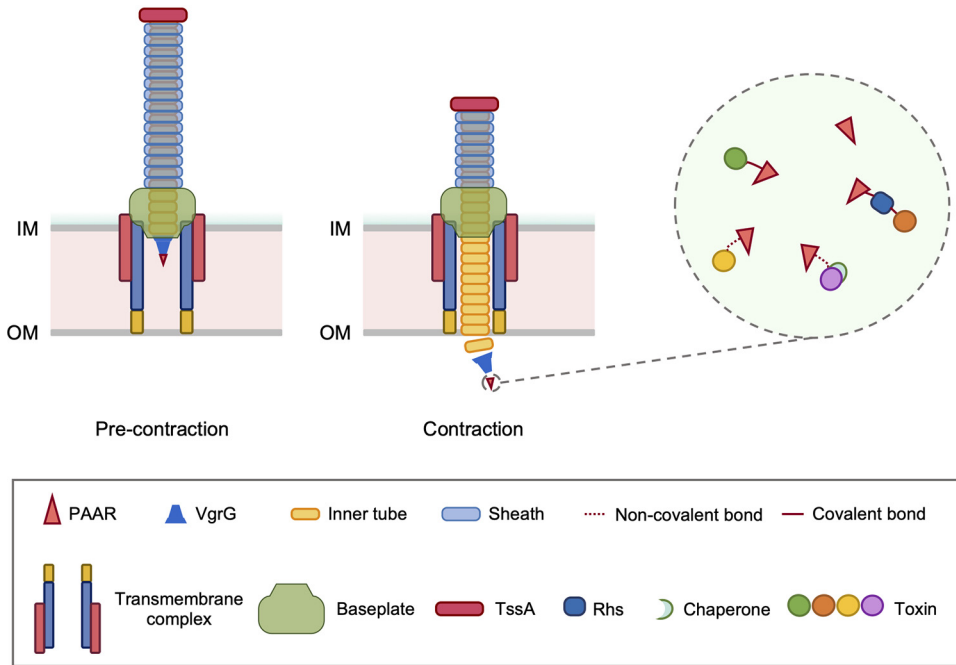
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**FIG 1** Schematic model depicting T6SS secretion. The rigid inner tube is wrapped around by the contractile outer sheath in a precontraction state. Upon sheath contraction, the inner tube and its tip spike complex consisting of VgrG-PAAR proteins are ejected out of the cell. The tip protein PAAR is highly diversified among T6SS species, whose homologs can be classified into several distinct classes, including PAAR-domain only, PAAR with a C-terminal tail that noncovalently binds to effectors and/or chaperones, extended PAAR with a C-terminal toxin domain, and extended PAAR-Rhs with or without a C-terminal toxin. IM, inner membrane; OM, outer membrane.

toxins for those PAARs with extended N- or C-terminal domains (20, 21). A recent study has done an extensive search for PAAR homologs in the NCBI RefSeq database (22), and the results are consistent with previous findings (20). A greater number of PAAR proteins (47,625 homologs) have been found, and it is estimated that 23% of bacterial genomes encode at least one PAAR gene, which is in general agreement with the distribution of T6SS in bacteria. Although it is known that genomes commonly contain multiple copies of PAAR genes, it is astounding that 35 copies are found in the strain *Chondromyces apiculatus* DSM 436 (22). There is also a strong positive correlation between PAAR and *vgrG* genes. Having learned the 35 copies of PAAR, one should be less surprised that *C. apiculatus* DSM 436 also carries 54 copies of *vgrG* (22). In addition, there are 1,300 PAAR-associated toxin-encoding genes, of which 40% are PAAR proteins with extended domains and the majority are predicted nucleases (22).

Among the identified PAAR-associated toxins, there is an important group possessing the Rhs domain (20–25). Compared with the PAAR proteins, the Rhs proteins are more broadly distributed with homologs including many toxins found in both Gram-positive and Gram-negative bacteria as well as teneurins of eukaryotic species (23, 25–27). The PAAR-Rhs toxins are featured with an N-terminal PAAR domain, a middle Rhs domain, and a variable C-terminal toxin domain (16, 24, 28–30). Therefore, it is a highly interesting question how PAAR and Rhs domains are combined through evolution, and yet multiple biochemical evidences suggest that some, if not all, PAAR-Rhs toxins are subject to autocleavage at both the N terminus and the C terminus in several species, resulting in separated PAAR, Rhs, and toxin domains (16, 28, 30). Both the mechanism of cleavage and its biological significance remain to be elucidated.

Lastly, why is a PAAR protein required for T6SS assembly in some but not all species? For example, deletion of all three PAAR genes abolishes T6SS secretion in *Acinetobacter baylyi* ADP1 but not in *Vibrio cholerae* V52 or *Aeromonas dhakensis* SSU (20, 31). Recent studies on the importance of effector proteins may provide some hints. T6SS secretion in

*V. cholerae*, *A. dhakensis*, *Agrobacterium tumefaciens*, and *Enterobacter cloacae* requires the presence of multiple VgrG-dependent effectors (17, 28, 31, 32). The previously known T6SS requirement for a heterotrimer VgrG spike in *V. cholerae* and in *A. dhakensis* is tightly linked to the specific structural effects of effectors because a pseudohotrimer VgrG spike made of VgrG chimeras, differing only by the C-terminal effector-loading tail, is functional for T6SS assembly (33). There is also direct interaction between effectors and the baseplate protein TssK and the assembly chaperone TssA (33). These findings not only suggest that the effector stuffing within the baseplate cavity is crucial for stabilizing T6SS assembly in these species but also lead to the hypothesis that the differential requirement for PAAR in different species may be dependent on PAAR-associated effectors. Supporting this hypothesis is a recent observation that, although the PAAR domain can stabilize the trimeric VgrG, only the full length of PAAR-containing effectors with the bulky Rhs domain can support efficient T6SS assembly in *E. cloacae* (28). Given the prevalence of PAAR proteins, it is foreseeable that more discoveries are to be made in understanding PAAR biochemical activities and ecological impacts in diverse T6SS species and complex interspecies interactions.

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### REFERENCES

- Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ. 2006. Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc Natl Acad Sci U S A* 103:1528–1533. <https://doi.org/10.1073/pnas.0510322103>.
- Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA. 2005. Contact-dependent inhibition of growth in *Escherichia coli*. *Science* 309:1245–1248. <https://doi.org/10.1126/science.1115109>.
- Brown ED, Wright GD. 2016. Antibacterial drug discovery in the resistance era. *Nature* 529:336–343. <https://doi.org/10.1038/nature17042>.
- Hibbing ME, Fuqua C, Parsek MR, Peterson SB. 2010. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 8:15–25. <https://doi.org/10.1038/nrmicro2259>.
- Riley MA. 1998. Molecular mechanisms of bacteriocin evolution. *Annu Rev Genet* 32:255–278. <https://doi.org/10.1146/annurev.genet.32.1.255>.
- Hood RD, Singh P, Hsu F, Güvener T, Carl MA, Trinidad RRS, Silverman JM, Ohlson BB, Hicks KG, Plemel RL, Li M, Schwarz S, Wang WY, Merz AJ, Goodlett DR, Mougous JD. 2010. A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* 7:25–37. <https://doi.org/10.1016/j.chom.2009.12.007>.
- Ho BT, Dong TG, Mekalanos JJ. 2014. A view to a kill: the bacterial type VI secretion system. *Cell Host Microbe* 15:9–21. <https://doi.org/10.1016/j.chom.2013.11.008>.
- MacIntyre DL, Miyata ST, Kitaoka M, Pukatzki S. 2010. The *Vibrio cholerae* type VI secretion system displays antimicrobial properties. *Proc Natl Acad Sci U S A* 107:19520–19524. <https://doi.org/10.1073/pnas.1012931107>.
- Taylor NMII, van Raaij MJ, Leiman PG. 2018. Contractile injection systems of bacteriophages and related systems. *Mol Microbiol* 108:6–15. <https://doi.org/10.1111/mmi.13921>.
- Basler M. 2015. Type VI secretion system: secretion by a contractile nanomachine. *Philos Trans R Soc Lond B Biol Sci* 370:20150021. <https://doi.org/10.1098/rstb.2015.0021>.
- Wang J, Brodmann M, Basler M. 2019. Assembly and subcellular localization of bacterial type VI secretion systems. *Annu Rev Microbiol* 73:621–638. <https://doi.org/10.1146/annurev-micro-020518-115420>.
- Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ. 2012. Type VI secretion requires a dynamic contractile phage tail-like structure. *Nature* 483:182–186. <https://doi.org/10.1038/nature10846>.
- Vettiger A, Basler M. 2016. Type VI secretion system substrates are transferred and reused among sister cells. *Cell* 167:99–110. <https://doi.org/10.1016/j.cell.2016.08.023>.
- Ho BT, Fu Y, Dong TG, Mekalanos JJ. 2017. *Vibrio cholerae* type 6 secretion system effector trafficking in target bacterial cells. *Proc Natl Acad Sci U S A* 114:9427–9432. <https://doi.org/10.1073/pnas.1711219114>.
- Le N-H, Pinedo V, Lopez J, Cava F, Feldman MF. 2021. Killing of Gram-negative and Gram-positive bacteria by a bifunctional cell wall-targeting T6SS effector. *Proc Natl Acad Sci U S A* 118:e2106555118. <https://doi.org/10.1073/pnas.2106555118>.
- Pei T-T, Kan Y, Wang Z-H, Tang M-X, Li H, Yan S, Cui Y, Zheng H-Y, Luo H, Dong T. 2021. Breaching the cell-envelope barriers of Gram-positive and fungal microbes by a type VI secretion system in *Acidovorax citrullii*. *bioRxiv* <https://www.biorxiv.org/content/10.1101/2021.05.31.446370v1>.
- Liang X, Kamal F, Pei T-T, Xu P, Mekalanos JJ, Dong TG. 2019. An onboard checking mechanism ensures effector delivery of the type VI secretion system in *Vibrio cholerae*. *Proc Natl Acad Sci U S A* 116:23292–23298. <https://doi.org/10.1073/pnas.1914202116>.
- Kamal F, Liang X, Manera K, Pei TT, Kim H, Lam LG, Pun A, Hersch SJ, Dong TG. 2020. Differential cellular response to translocated toxic effectors and physical penetration by the type VI secretion system. *Cell Rep* 31:107766. <https://doi.org/10.1016/j.celrep.2020.107766>.
- Burkinshaw BJ, Liang X, Wong M, Le ANH, Lam L, Dong TG. 2018. A type VI secretion system effector delivery mechanism dependent on PAAR and a chaperone/co-chaperone complex. *Nat Microbiol* 3:632–640. <https://doi.org/10.1038/s41564-018-0144-4>.
- Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ, Leiman PG. 2013. PAAR-repeat proteins sharpen and diversify the type VI secretion system spike. *Nature* 500:350–353. <https://doi.org/10.1038/nature12453>.
- Koskiniemi S, Lamoureux JG, Nikolakakis KC, t'Kint de Roodenbeke C, Kaplan MD, Low DA, Hayes CS. 2013. Rhs proteins from diverse bacteria mediate intercellular competition. *Proc Natl Acad Sci U S A* 110:7032–7037. <https://doi.org/10.1073/pnas.1300627110>.
- Zhang Z, Liu Y, Zhang P, Wang J, Li D, Li Y. 2021. PAAR proteins are versatile clips that enrich the antimicrobial weapon arsenals of prokaryotes. *mSystems* 6:e00953-21. <https://doi.org/10.1128/mSystems.00953-21>.
- Zhang D, de Souza RF, Anantharaman V, Iyer LM, Aravind L. 2012. Polymorphic toxin systems: comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. *Biol Direct* 7:18. <https://doi.org/10.1186/1745-6150-7-18>.
- Ma J, Sun M, Dong W, Pan Z, Lu C, Yao H. 2017. PAAR-Rhs proteins harbor various C-terminal toxins to diversify the antibacterial pathways of type VI secretion systems. *Environ Microbiol* 19:345–360. <https://doi.org/10.1111/1462-2920.13621>.

25. Jackson AP, Thomas GH, Parkhill J, Thomson NR. 2009. Evolutionary diversification of an ancient gene family (*rhs*) through C-terminal displacement. *BMC Genomics* 10:1–16.
26. Berns DS, DeNardo LA, Pederick DT, Luo L. 2018. Teneurin-3 controls topographic circuit assembly in the hippocampus. *Nature* 554:328–333. <https://doi.org/10.1038/nature25463>.
27. Busby JN, Panjikar S, Landsberg MJ, Hurst MRH, Lott JS. 2013. The BC component of ABC toxins is an RHS-repeat-containing protein encapsulation device. *Nature* 501:547–550. <https://doi.org/10.1038/nature12465>.
28. Donato SL, Beck CM, Garza-Sánchez F, Jensen SJ, Ruhe ZC, Cunningham DA, Singleton I, Low DA, Hayes CS. 2020. The  $\beta$ -encapsulation cage of rearrangement hot spot (Rhs) effectors is required for type VI secretion. *Proc Natl Acad Sci U S A* 117:33540–33548. <https://doi.org/10.1073/pnas.1919350117>.
29. Cianfanelli FR, Alcoforado Diniz J, Guo M, De Cesare V, Trost M, Coulthurst SJ. 2016. VgrG and PAAR proteins define distinct versions of a functional type VI secretion system. *PLoS Pathog* 12:e1005735. <https://doi.org/10.1371/journal.ppat.1005735>.
30. Pei T-T, Li H, Liang X, Wang Z-H, Liu G, Wu L-L, Kim H, Xie Z, Yu M, Lin S, Xu P, Dong TG. 2020. Intramolecular chaperone-mediated secretion of an Rhs effector toxin by a type VI secretion system. *Nat Commun* 11:1865. <https://doi.org/10.1038/s41467-020-15774-z>.
31. Liang X, Pei T-T, Wang Z-H, Xiong W, Wu L-L, Xu P, Lin S, Dong TG. 2021. Characterization of lysozyme-like effector TseP reveals the dependence of type VI secretion system (T6SS) secretion on effectors in *Aeromonas dhakensis* strain SSU. *Appl Environ Microbiol* 87:e0043521. <https://doi.org/10.1128/AEM.00435-21>.
32. Wu C, Lien Y, Bondage D, Lin J, Pilhofer M, Shih Y, Chang JH, Lai E. 2020. Effector loading onto the VgrG carrier activates type VI secretion system assembly. *EMBO Rep* 21:e47961. <https://doi.org/10.15252/embr.201947961>.
33. Liang X, Pei T-T, Li H, Zheng H-Y, Luo H, Cui Y, Tang M-X, Zhao Y-J, Xu P, Dong T. 2021. VgrG-dependent effectors and chaperones modulate the assembly of the type VI secretion system. *PLoS Pathog* 17:e1010116. <https://doi.org/10.1371/journal.ppat.1010116>.