Gaming the competition in microbial cell–cell interactions

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It helps to pay attention to an opponent's prior actions when playing strategy games, be they chess, poker or rock-paper-scissors. In a thought provoking paper by Basler *et al* (2013), published in *Cell*, we have a chance to watch bacteria 'game' each other in a deadly match of life or death involving the deployment of a potent weapon called the Type VI Secretion System (T6SS; Basler *et al*, 2013). The results establish a paradigm for inter-bacterial

competition based on defensive counterattack with temporal and spatial precision.

T6SS organelles are encoded by about a quarter of all Gram-negative bacteria, including pathogens, commensals and environmental organisms. Contributions to virulence and host-cell injury have been established for a handful of these (Pukatzki *et al*, 2006; French *et al*, 2011), but it has now become apparent that this secretion system frequently targets

Figure 1 A model for T6SS aiming by *P. aeruginosa* in a duel with *V. cholerae*. T6SS attack from *V. cholerae* is sensed by the *P. aeruginosa* TagQRST signal-transduction system (yellow), which activates the PpkA kinase to phosphorylate Fha1 (red box). Phosphorylated Fha1 directs assembly of a T6SS apparatus near the point of attack. Contraction of the outer sheath (violet) fires the inner tube/spike complex (dark green), positioning the counterattack at the point of contact with the aggressor cell. Effector proteins delivered by the T6SS, such as the Tse1 peptidoglycan amidase, contribute to destruction of the target *V. cholerae*. Disassembly of the fired apparatus facilitated by the ClpV ATPase and dephosphorylation of Fha1 allows redeployment to new sites.

bacterial cells as well (Hood *et al*, 2010; MacIntyre *et al*, 2010). In this case, the system has been shown to kill ‘prey’ bacterial cells by forceful transfer of toxic effector proteins with muramidase or lipase activity (Russell *et al*, 2011; Dong *et al*, 2013). The T6SS organelle is a dynamic structure that is evolutionarily and functionally related to the contractile tails of bacteriophages (Figure 1), although it assembles in the cytoplasm and envelope of bacterial cells (Leiman *et al*, 2009; Basler *et al*, 2012). The firing of the apparatus correlates with contraction of a tail sheath-like structure and the sudden ejection of a phage tail tube-like structure tipped with a poison spike (Pukatzki *et al*, 2007; Basler *et al*, 2012). An ATPase called ClpV recognizes the contracted sheath and remodels it to re-cock the fired T6SS (Bonemann *et al*, 2009; Basler and Mekalanos, 2012). Biogenesis and cycling of the organelle is energetically expensive, so it is no surprise that expression and assembly are tightly regulated. Nonetheless, few of us would have guessed that the deployment of such a weapon might be regulated by the T6SS-mediated attack of a heterologous bacteria cell. In homage to Robert Axelrod’s treatise ‘The Evolution of Cooperation’ and the winning strategy of Anatol Rapoport for the ‘Prisoner’s Dilemma’ game (Axelrod and Hamilton, 1981), Mekalanos and colleagues entitled their paper ‘Tit-for-tat: Type VI secretion counterattack in bacterial cell-cell interactions’.

Previously, Basler and Mekalanos (2012) reported a phenomenon they termed ‘T6SS duelling’, in which sister cells of *Pseudomonas aeruginosa* could be observed in pitched battles when a fluorescent ClpV–GFP fusion protein was used to monitor the position of T6SS organelle firing. No apparent harm comes to sister cells in such duelling behaviour, as they encode immunity proteins to their own T6SS effectors (Hood *et al*, 2010). However, their stunning videos suggested that spatial and temporal T6SS activity in one cell could induce corresponding T6SS activity in other cells in direct contact. These observations prompted the present study, which addresses the regulatory mechanism and biological consequences of what appears to be a strategy for prey selection during interspecies interactions.

In their most recent report, Basler *et al* (2013) found that *P. aeruginosa* efficiently kills two other bacterial species, *Vibrio cholerae* and *Acinetobacter baylyi*, only if these organisms also deploy functional T6SS organelles. Imaging cellular mixtures confirmed that T6SS-competent *V. cholerae* cells were targeted for delivery of the *P. aeruginosa* toxic effector protein Tse1 30-fold more frequently than T6SS-defective *V. cholerae* mutants, even when all three strains were mixed together at the same time. This suggested that *P. aeruginosa* is capable of assembling, aiming and firing its T6SS apparatus at aggressive *V. cholerae*, while sparing peaceful bystanders. The lack of collateral damage in the midst of ongoing battle reveals an unanticipated precision of the *P. aeruginosa* T6SS counterattack, begging a molecular explanation. Basler *et al* (2013) went on to show that a signal-

transduction system, TagQRST–PpkA–PppA, is involved in sensing T6SS attack, presumably by detecting penetration of the *P. aeruginosa* cell surface. This membrane-associated complex was previously shown to mediate phosphorylation of a scaffold protein, Fha1, which controls T6SS organelle assembly (Mougous *et al*, 2007). It now appears that phosphorylated Fha1 also functions to position the T6SS apparatus at the precise point of engagement with an attacking bacterium. Furthermore, through cycles of phosphorylation and dephosphorylation, the signalling system facilitates spatial repositioning. Aiming T6SS counterattack in response to initial heterologous T6SS attack dramatically affects the accuracy of prey selection by *P. aeruginosa*. In contrast, other bacterial species such as *V. cholerae* and *A. baylyi* kill *Escherichia coli* K12 with their T6SS apparatus with apparently no requirement that this prey species is armed with a functional T6SS.

As with any paradigm-shifting study, the Basler *et al* (2013) report raises as many questions as it answers. How, exactly, does T6SS attack trigger the duelling counterattack response in *P. aeruginosa*? According to available data, the signal may be physical and positional (e.g., localized membrane perturbation) rather than enzymatic or chemical. What is the mechanism of T6SS organelle localization in *P. aeruginosa*, and how does spatially defined duelling differ, biologically and mechanistically, from the apparently random placement of sequential T6SS assembly sites by *V. cholerae* (Basler *et al*, 2012)? In a broader context, does the tit-for-tat response also characterize T6SS control in other bacterial species? As the ‘Prisoner’s Dilemma’ involves a defined reward system for which ‘tit-for-tat’ is the winning strategy, one wonders if evolutionary fitness is the reward *P. aeruginosa* receives by adopting this tactic in contrast to less discriminating (and more energetically costly) deployment of T6SS organelles?

Early results that contributed to this study were presented by John Mekalanos to a packed house at the President’s Forum of the 2012 General Meeting of the American Society for Microbiology, held in San Francisco last June. The tit-for-tat story was a highlight of the meeting and it clearly inspired many microbiologists to view bacterial cell–cell interactions in a new light. These discoveries point to a new mechanism that could determine, in part, the composition of the complex communities that make up the commensal microbiota, as well as microbial biofilms that characterize pathological environments such as the inflammatory milieu of the cystic fibrosis lung, acne pustules or the chronic inflammatory bowel. Pharmacological control of T6SS deployment may provide a new tool for controlling the composition of polymicrobial communities to promote health and mitigate disease.

Conflict of interest

The author is co-founder and chair of the scientific advisory board of AvidBiotics Inc., a biotherapeutics company in South San Francisco, CA, USA.

References

- Axelrod R, Hamilton WD (1981) The evolution of cooperation. *Science* **211**: 1390–1396
- 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
- Basler M, Mekalanos JJ (2012) Type 6 secretion dynamics within and between bacterial cells. *Science* **337**: 815
- Basler M, Ho BT, Mekalanos JJ (2013) Tit-for-tat: Type VI secretion system counterattack during bacterial cell-cell interactions. *Cell* **152**: 884–894

- Bonemann G, Pietrosiuk A, Diemand A, Zentgraf H, Mogk A (2009) Remodelling of VipA/VipB tubules by ClpV-mediated threading is crucial for type VI protein secretion. *EMBO J* **28**: 315–325
- Dong TG, Ho BT, Yoder-Himes DR, Mekalanos JJ (2013) Identification of T6SS-dependent effector and immunity proteins by Tn-seq in *Vibrio cholerae*. *Proc Natl Acad Sci USA* **110**: 2623–2628
- French CT, Toesca IJ, Wu TH, Teslaa T, Beaty SM, Wong W, Liu M, Schroder I, Chiou PY, Teitell MA, Miller JF (2011) Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade. *Proc Natl Acad Sci USA* **108**: 12095–12100
- Hood RD, Singh P, Hsu F, Guvener T, Carl MA, Trinidad RR, 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
- Leiman PG, Basler M, Ramagopal UA, Bonanno JB, Sauder JM, Pukatzki S, Burley SK, Almo SC, Mekalanos JJ (2009) Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proc Natl Acad Sci USA* **106**: 4154–4159
- MacIntyre DL, Miyata ST, Kitaoka M, Pukatzki S (2010) The *Vibrio cholerae* type VI secretion system displays antimicrobial properties. *Proc Natl Acad Sci USA* **107**: 19520–19524
- Mougous JD, Gifford CA, Ramsdell TL, Mekalanos JJ (2007) Threonine phosphorylation post-translationally regulates protein secretion in *Pseudomonas aeruginosa*. *Nat Cell Biol* **9**: 797–803
- Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ (2007) Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. *Proc Natl Acad Sci USA* **104**: 15508–15513
- 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 USA* **103**: 1528–1533
- Russell AB, Hood RD, Bui NK, LeRoux M, Vollmer W, Mougous JD (2011) Type VI secretion delivers bacteriolytic effectors to target cells. *Nature* **475**: 343–347