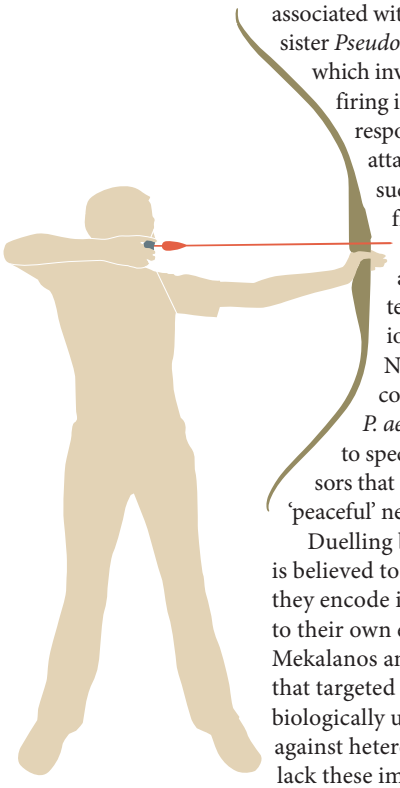
BACTERIAL PHYSIOLOGY

Ready, aim, fire!



The type VI secretion system (T6SS) of Gram-negative bacteria powers the secretion of effector proteins from one cell into another cell, resulting in antagonistic or even bacteriocidal activity between heterologous species. In addition, the system is associated with duelling between sister *Pseudomonas aeruginosa* cells, which involves directed T6SS firing into a target cell in response to T6SS-mediated attack. However, whether such targeted retaliatory firing occurs between heterologous species and the signalling system behind such behaviour were unknown. Now, Mekalanos and colleagues show that *P. aeruginosa* uses the T6SS to specifically target aggressors that attack first, but leaves 'peaceful' neighbouring cells intact.

Duelling between sister cells is believed to be harmless because they encode immunity proteins to their own effectors; however, Mekalanos and colleagues reasoned that targeted retaliation would be biologically useful if unleashed against heterologous species, which lack these immunity proteins. Using target cell rounding (caused by the T6SS effector Tse1) as a marker for T6SS activity, they found that T6SS$^+$ *P. aeruginosa* induced rounding of T6SS$^+$ *Vibrio cholerae* cells — mainly those in direct contact with *P. aeruginosa* — but not of T6SS$^-$ *V. cholerae* cells when these two *V. cholerae* strains were mixed individually with *P. aeruginosa* and also, remarkably, when all three strains were mixed. Similarly, in quantitative competition assays, the authors recovered more surviving T6SS$^-$ *V. cholerae* cells than T6SS$^+$ *V. cholerae* cells when these strains were competed individually with *P. aeruginosa*, indicating that *P. aeruginosa* T6SS activity targets cells that attack the bacterium first and does not affect 'innocent bystanders'.

The T6SS signalling cascade begins with the cell envelope-associated TagQRST proteins, which control phosphorylation of Fha1 (a T6SS scaffold protein) to trigger T6SS assembly. PppA-mediated dephosphorylation of Fha1 induces disassembly. In sister cell duelling assays, inactivation of *pppA* resulted in increased T6SS firing; however, this firing occurred at the same subcellular site and was not spatially or temporally aligned with an attack. The *pppA* mutant also showed no preferential targeting of T6SS$^+$ *V. cholerae* over T6SS$^-$ *V. cholerae*, suggesting that in the *pppA* mutant the T6SS machinery is recycled at the same location as it cannot be fully disassembled and reassembled at a different site. Random firing was also observed for a *tagT* mutant, indicating that the TagQRST cascade is involved in sensing T6SS attack by a neighbour (possibly through TagQRST sensing of membrane perturbations) to mark the site of T6SS reassembly and thus enable a targeted response.

> “*P. aeruginosa* T6SS activity targets cells that attack the bacterium first”

This contact-dependent retaliation also occurred in response to T6SS$^+$ *Acinetobacter baylyi* but not in response to the T6SS$^-$ species *Escherichia coli* and is therefore likely to be a general attack strategy used by *P. aeruginosa*. The authors propose that such precise deployment of T6SS effectors might have evolved to allow *P. aeruginosa* to kill harmful aggressors while sparing harmless, potentially helpful neighbours and to reduce unnecessary energy expenditure on T6SS firing.

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ORIGINAL RESEARCH PAPER Basler, M., Ho, B. T. & Mekalanos, J. J. Tit-for-Tat: type VI secretion system counterattack during bacterial cell-cell interactions. *Cell* **152**, 884–894 (2013)