Arming the Neighborhood

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Bacteria use type 6 secretion systems in antagonistic behavior to compete for resources with other bacteria. In a recent issue of *Cell*, Vettiger and Basler (2016) show that bacteria can also use these systems to arm neighboring cells and force them to pass on a signal in the bacterial population.

Bacteria live in harsh environments where competition for resources is a constant battle. In this competition with other cells, bacteria use multiple secretion systems to deliver toxic proteins to the extracellular milieu or directly to targeted cells. Type 6 secretion systems (T6SSs) are widespread and have been linked to antagonistic behaviors in many important pathogens, including *Pseudomonas* and *Vibrio* spp (Alcoforado Diniz et al., 2015). T6SSs are dynamic, multi-component machineries that deliver toxic effectors directly into the targeted prokaryotic or eukaryotic cells using a needle-like cell-puncturing device. Delivery can occur within as well as between species, and the delivered effectors result in cell lysis or arrest of cell growth (Russell et al., 2014). To protect themselves from auto-inhibition, bacteria express immunity proteins that bind and abolish the toxic effect of their cognate effectors. In a recent issue of *Cell*, Vettiger and Basler (2016) show for the first time that in addition to effectors, several components of the type 6 apparatus itself are delivered to targeted bacteria. Even more surprisingly, the targeted cell can use these acquired components to build its own machinery for effector delivery, propagating the attack potency.

The T6S apparatus is composed of 13 core proteins that span the cell envelope of Gram-negative bacteria. According to the current model, the components assemble into a structure similar to an inverted bacteriophage tail that is linked to the cell envelope (Figure 1). Contraction of an outer sheath composed of VipAB results in ejection of the cell-puncturing tube composed of Hcp with a cell-permease spike, VgrG (Figure 1). The effectors are delivered to the target cell either through the Hcp tube or through attachment to the VgrG tip (Basler, 2015). Now, Vettiger and Basler (2016) show that components other than effectors also reach the recipient cell and that these components can be used to build new secretion apparatuses. Using an imaging assay in which one of the sheath components (VipA) is tagged with fluorescent proteins of different color in the donor and recipient strains, the authors are able to visualize assembly of the apparatus in live cells using time-lapse microscopy. By excluding one component at a time in the recipient cells, the authors were able to determine which components of the type 6 machinery could be recycled by the targeted cell. Interestingly, the Hcp tube and the VgrG spike components of the apparatus, as well as three different effectors, were delivered into and reused by the target cell.

In wild-type *Vibrio* cells, the Hcp tube spans the entire cell before launch (Figure 1A). Vettiger and Basler (2016) show that the acquired Hcp can be used to assemble a new type 6 apparatus in cells unable to produce Hcp. However, only a part of the tube is delivered to the targeted cells, and as a result the acquired components are not sufficient to build a full-length tube. As a consequence, the tubes in these cells are short: 0.25 μm instead of 0.60 μm. To confirm that these shorter tubes are functional in delivering effectors, the authors used a setup in which cell lysis could be measured through release of β-galactosidase, an enzyme that converts a colorless substrate to a colored product. One of the effectors (TseL) is a peptidoglyconase, which can degrade the bacterial cell wall in cells lacking the cognate immunity (TsiVI), resulting in cell lysis. The authors set up an experiment in which the delivering cells lack TseL-TsiVI but produce β-galactosidase. Because β-galactosidase cannot escape intact cells, lysis of these cells can be measured as release of the enzyme. If lysis occurred when these reporter-donor cells were mixed with recipient cells lacking different components of the T6S apparatus, this suggested that the recipients could build a functional T6SS and use it to deliver effectors. Using this assay, the authors showed that the shorter tube is sufficient to deliver effectors to cells, demonstrating for the first time that a full-length tube is not required for effector delivery. However, the effectors delivered here have periplasmic/inner-membrane targets, and a remaining question is whether the shorter tube would also be long enough to deliver to the cytoplasm.

Whether effectors are delivered to the periplasm or cytoplasm of targeted cells has been an open question. Previous findings suggest that the Hcp tube capped with VgrG and effectors is assembled in the cell cytoplasm (Durand et al., 2015). Thus, when these components are delivered to the targeted cell and reused, they must end up in the cytoplasm prior to assembly. Vettiger and Basler (2016) show that a functional T6SS in the VgrG-lacking receiving cell can be assembled 2 min after the delivery of the VgrG component by the attacking cell. By the authors' logic, the rapidity of this response indicates direct delivery to the cytoplasm by the T6SS. On the other hand, toxins delivered by this system target processes in the periplasm and are not toxic when produced intracellularly (Brooks et al., 2013; Miyata et al., 2013), suggesting that these effectors must be delivered to the periplasm. Thus, the effectors are on one hand toxic, requiring delivery to the periplasm, and on
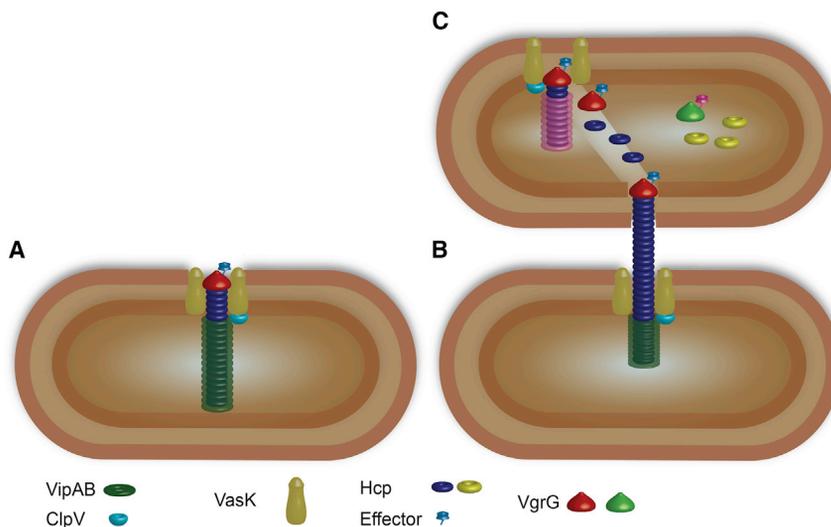
Figure 1. Composition of the Bacterial T6SS
Bacteria with a type 6 secretion system (T6SS) assemble a needle-like cell-puncturing device anchored in the cell envelope (A). Upon delivery (B), the outer sheath—composed of VipAB proteins—contracts, pushing the inner Hcp tube—spiked with the VgrG cell-permease—into the recipient cell. Hcp, VgrG, and effectors are translocated to the recipient cell and can be recycled and used to build up a new, shorter T6S apparatus (C).

the other hand being reused, which requires delivery to the cytoplasm. Perhaps the secretion system is able to deliver effectors to both periplasm and cytoplasm simultaneously, but how this could be achieved remains unclear.

A final question that arises from the new findings by Vettiger and Basler (2016) is why a system that delivers reusable components and effectors to target cells would evolve. Interestingly, the Hcp and VgrG components of the apparatus associate and bind specifically to different effectors (Silverman et al., 2013; Whitney et al., 2014). Thus, if the effectors were delivered to a cell lacking the correct

Hcp or VgrG, that particular effector could not be passed forward. However, simultaneous delivery of Hcp, VgrG, and effectors, as shown by the authors, ensures that the effectors can be propagated further (Vettiger and Basler, 2016). In addition, the authors show that cells targeted and affected by effectors that stop translation continue to deliver effectors to neighboring cells, suggesting that acquired components can be recycled and used for delivery even in dormant cells. If T6S is simply used for interbacterial battle, it seems disadvantageous to provide competitors with weapons that can be used in competition. Another system readily

used in antagonistic behavior among bacteria—contact-dependent growth inhibition—was recently shown to also mediate contact-dependent intraspecies signaling between immune cells (Garcia et al., 2016). If T6S effectors are used for intraspecies communication in addition to competition, recycling of components could allow dormant or unrelated cells to pass forward a signal in a bacterial population.

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