Type 6 Secretion Dynamics Within and Between Bacterial Cells

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The bacterial type 6 secretion system (T6SS) is a dynamic apparatus that translocates proteins from predator to prey cells by a mechanism analogous to phage tail contraction (1–3). In Vibrio cholerae, two proteins (VipA and VipB) build a phage tail sheath-like tubular structure in the cytosol of predator cells that exists in two conformations, extended and contracted (3). Contraction of the extended VipA/VipB sheath is thought to drive the T6SS spike and inner tube complex out of the effector or ‘predator’ cell and into an adjacent target or ‘prey’ cell (3). Disassembly of the cytoplasmic contracted sheath requires ClpV in vitro (3), a AAA+ adenosine triphosphatase (ATPase) that binds VipA/VipB tubules in vitro and can remodel these structures in the presence of ATP (4, 5). In Pseudomonas aeruginosa, ClpV1-GFP localizes to discrete foci that depend on T6SS function (6). Although ClpV binds VipA/VipB tubules in vitro (4, 5), the ability of this protein to interact with other T6SS components has not been demonstrated in vivo. Accordingly, we imaged ClpV localization in intact cells to examine its possible association with dynamic T6SS structures in vivo such as extended and contracted T6SS sheaths and base plates.

We used time-lapse fluorescence microscopy to follow ClpV localization in live V. cholerae 2740-80 cells. Functional ClpV-superfolder GFP (sfGFP) and mCherry2 fusion proteins assembled at random times into short structures that disappeared in 10’s of seconds. In the ΔVipA background, ClpV was evenly distributed in cytosol suggesting that the short ClpV structures were dependent on T6SS sheaths (fig. S1, movie S1). We used functional VipA-sfGFP (3) and ClpV-mCherry2 fusions to image ClpV and T6SS sheaths simultaneously. Extended VipA-sfGFP containing sheaths were not co-localized with ClpV-mCherry2, while contraction of a sheath led to immediate co-localization of ClpV-mCherry2 with the whole contracted sheath (Fig. 1, movies S2 and S3). Half of the ClpV associated with the contracted sheath between 683 ms and 1273 ms (average 952 ms, standard deviation 164 ms, n = 10, fig. S2, movie S4). The disassembly of the contracted sheath required between 22 and 46 s (average 32.5 s, standard deviation 6.1 s, n = 40), measured from the moment of contraction to the moment when both ClpV and VipA signal were no longer co-localized to one spot (movies S2 and S3).

The Y664A mutation in the pore of ClpV blocks VipA/VipB disassembly but still allows binding of ClpV to VipB in vitro (4) while the F87R mutation of N-terminal domain of ClpV blocks VipB recognition in vitro (5). We found that in vivo ClpV-Y664A-mCherry2 were co-localized with VipA-sfGFP to short non-dynamic structures that were likely contracted T6SS sheaths (fig. S3, movie S5). In contrast, ClpV-F87R-mCherry2 was distributed uniformly in the cytosol with only VipA-sfGFP localized into contracted non-dynamic sheaths (fig. S3, movie S5). Localization of these ClpV mutants and the change in the dynamics of VipA-containing structures is consistent with published in vitro biochemical data (4, 5) and suggest that in vivo, the N terminus of VipB is exposed on the surface of the contracted sheath just prior to its disassembly.

In P. aeruginosa, mutation of the regulatory gene relS allows expression of one of its T6SS loci (6). To assess the dynamics of T6SS in P. aeruginosa we imaged a ClpV1-GFP fusion protein in a relS mutant (6) by time-lapse fluorescence microscopy. In contrast to V. cholerae, only a subset of P. aeruginosa cells actively formed and disassembled ClpV1-GFP containing complexes during the observation period; the formation and dynamics of these structures require the VipA homolog PA0083 (fig. S1, movies S6 and S7). ClpV1-GFP structures often assembled and disassembled repeatedly in apparently the same subcellular location (Fig. 1D, movie S7, segments 1–5) indicating that, in contrast to V. cholerae (3), multiple T6SS apparatuses assemble in close proximity, or more likely, T6SS ‘base plate components’ (3) are recycled by P. aeruginosa.

Interestingly, P. aeruginosa cells apparently responded to T6SS activity occurring in a neighboring sister cell with an increase in their own T6SS dynamics (Fig. 1D, movies S6 and S7). Over time the coincidence of T6SS activity between pairs of sister cells (termed “T6SS dueling”) became the dominant category of T6SS activity observable in the P. aeruginosa population (table S1, fig. S4). Spatially concurrent T6SS activity could not be documented between V. cholerae sister cells because this species exhibited much higher levels of T6SS activity in nearly all cells (fig. S4). The spatial and temporal coincidence of T6SS activity in adjacent P. aeruginosa cells strongly suggests that a signal was being transferred between cells precisely at the position of the initial T6SS activity. The P. aeruginosa T6SS is thought to transfer peptidoglycan-hydrolyzing T6SS substrates into sister cells that express immunity proteins to their action (7). We hypothesize that cellular attack by a T6SS apparatus mediated by translocation of T6SS components (e.g., the spike/inner tube complex, or effector proteins) into nearby adjacent sister cells induces local cell envelope alterations (e.g., membrane perturbation, mild peptidoglycan hydrolysis, or protein phosphorylation (7, 8)) that trigger the formation of a T6SS apparatus in the vicinity of such alterations (see fig. S5).

In conclusion, ClpV imaging provides evidence that P. aeruginosa likely recycles T6SS membrane base plate components and can sense T6SS activity in nearby cells. Because T6SS dueling events were spatially and temporally linked, we hypothesize that they likely mark the exact location of T6SS translocation of protein components (e.g., VgrG and/or effector proteins) between cells. Quorum sensing is a well-established means of cell-cell communication mediated by diffusible small molecules. Our results show that protein translocation between cells may also signal changes that alter the behavior of microbes and thus their social interactions with each other and their hosts.

References and Notes


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Supplementary Materials
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Materials and Methods
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Fig. 1. ClpV co-localizes with contracted sheath. 3x3 μm field of cells is shown. Bar in A is 1 μm and
applies to A-D. (A-C) *V. cholerae* ClpV-mCherry2 +
pBAD24-VipA-sfGFP. (A) Merge of ClpV-mCherry2
and VipA-sfGFP signals. (B) ClpV-mCherry2 signal.
(C) VipA-sfGFP signal. Additional frames and cells
are shown in Videos S2 and S3. (D) *P. aeruginosa*
ΔretS/ClpV1-GFP, additional frames and cells are
shown in movies S6 and S7.
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