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From wall to wall: how the type 6 secretion system knows to stop growing

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Abstract

There is an inseverable link between the cell size the size of its subcellular components. The type 6 secretion system (T6SS) is no exception. In this issue of *Journal of Bacteriology*, Stietz et al. probe the T6SS when cell size is distorted to an extreme degree. This study and others investigating the regulation of T6SS filament polymerization have provided insight into how the T6SS apparatus matches its size to fit the cell that contains it.

Main Text

The type 6 secretion system (T6SS) is a bacterial nanomachine used by a wide variety of Gram-negative bacteria to deliver toxic effectors into adjacent prokaryotic or eukaryotic cells (1). The T6SS apparatus consists of a membrane bound baseplate complex which serves as the nucleation site for the polymerization of a central tube structure comprised of stacked rings of Hcp hexamers (2). Surrounding the Hcp tube is a contractile sheath comprised of TssB/TssC subunits (3). Additionally, the Hcp tube is sharpened at the baseplate end by a complex consisting of a VgrG trimer (4) and a PAAR domain-containing protein (5). The toxic effector substrates of the T6SS associate with the Hcp tube, the VgrG trimer or the PAAR protein either directly, through a coupling protein intermediate, or as protein fusions with Hcp, VgrG or the PAAR protein (1). Secretion occurs during discrete events when a conformation change in the TssB/C subunits results in a rapid contraction of the sheath structure (6). This contraction drives the expulsion of the Hcp tube along with VgrG and PAAR, as well as all associated effectors, through the membrane complex, out of the cell, and potentially across the membranes of adjacent cells.

Since this model for the mechanism of the T6SS was established, one of the outstanding questions for the field has been how the system regulates extension and termination of tube and sheath polymerization. More precisely, how are the Hcp tube subunits and the surrounding TssB/TssC sheath subunits recruited to the structure and what prevents the sheath structures from growing indefinitely? At first glance, T6SS apparatus growth appears to be regulated by the width of the cell as the T6SS apparatus in a number of different organisms appears to extend from the point of its biogenesis on one cell membrane until it reaches the distal side of the cell, where its growth stops. This extended structure then stalls for a period of time before eventually contracting.

The first mechanistic insight into how this process is regulated came with the characterization of TssA and its related proteins (7, 8). Members of this family assemble into dodecameric structures with individual subunits consisting of a conserved N-terminal domain (pfam accession PF06812) and a C-terminal extension (9). T6SSs from different organisms have different variants of this protein with distinct C-terminal domain architectures (10), which play different roles in T6SS apparatus assembly (11).

In *Escherichia coli* and *Vibrio cholerae*, TssA remains associated with the end of the Hcp/TssB/TssC filament distal to the baseplate and likely plays a role in stabilizing sheath polymerization, while a second related protein call TagA associates with the fully grown T6SS sheath. Although the C-terminal domains of TagA in these two organisms do not share much sequence similarity, they both carry a region rich in hydrophobic residues implying strong association with the cell membrane. In *E. coli*, TagA has been shown to associate with the distal end of the polymerized sheath only when it had spanned the width of the cell (12). Deletion of TagA resulted in excessive sheath polymerization with T6SS structures appearing to bend or even break upon reaching the opposite side of the cell, suggesting that the membrane-associated TagA plays a role in regulating T6SS structure length. Furthermore, TagA-less T6SS structures remain in their extended conformation for

significantly shorter periods of time than wild type cells and exhibited reduced efficiency of killing adjacent bacteria (12). Ultimately, these observations have led to a model where Hcp and sheath components polymerize from the baseplate complex facilitated by TssA until they reach the opposite side of the cell where they encounter membrane bound TagA, which stops the filament polymerization and stabilizes the extended T6SS structure. Without TagA, the sheath continues to grow until it runs out of room at which point the structure either breaks or contracts.

In a separate study, TagA was observed to not only stabilize the extended sheath, but actually attaches the T6SS structure to the distal membrane (13). This attachment was strong enough such that T6SS contraction events could actually lead to breakage of the T6SS filament and subsequent bi-direction contraction toward the both the baseplate end and the TagA ends of the structure. These so-called non-canonical contraction events accounted for approximately one third of all contraction events. Deleting TagA or disrupting the interaction between TagA and the growing end of the T6SS sheath prevented attachment of the T6SS sheath to the distal side of the cell and virtually eliminated the bi-directional contraction events. It still remains unclear how if at all the absence of these non-canonical contraction events contribute to the observed reduced T6SS killing activity. One possible explanation is that as the T6SS sheath over-extends, collision with the distal cell wall forces the T6SS apparatus to bend altering the angle at which the Hcp tube is ejected from the cell. These angled T6SS structures would then presumably leave the cell at a non-perpendicular angle, which could have a reduced ability to penetrate target membranes.

What happens then when the distal cell wall is moved farther away from the cell wall with the T6SS baseplate – in other words, what happens when the cells are wider than normal? Given the model for TagA function described above, the expectation should be that the T6SS will continue to grow until reaching the opposite side of the cell is reached. And indeed this appears to be the case. By treating *V. cholerae* cells with ampicillin, it is possible to generate rounded spheroplast cells (14). In these cells, fully functional T6SS filaments form and span the entire expanded cell width. In fact, these extended T6SS structures were even stable enough to conduct photobleaching analysis of growing T6SS sheaths, confirming that TssB/TssC subunits are added to the end of the sheath distal to the membrane-bound baseplate (14).

In a study {published in this issue}, Stietz et al. ask what happens to *V. cholerae* T6SS sheaths when similar spheroplast cells are allowed to grow to extreme sizes (multiple microns wide). With cells so big, one might expect that if anything the T6SS would fail to extend all the way across the cell. However, somewhat counterintuitively, when cells become big enough, the exact opposite appears to occur. T6SS structures do not just extend to the opposite side of the cell like they do in normal sized cells (6) and smaller rounded cells (14), but rather they continue to grow, eventually bending along with the curvature of the membrane. This T6SS overextension and eventual curving was extremely similar to the extended T6SS structures observed in TagA-deficient cells (13). Stietz et al. suggest that in these extremely large cells, TagA is effectively diluted out or otherwise destabilized to the point that the growing T6SS sheath never receives the stop signal that growing T6SS sheaths normally receive from TagA. Consistent with such an interpretation, over-expression of TagA results in fewer curved sheaths forming. Given that TagA functionality can effectively be diluted out of the cell, it suggests that TagA is acting as more than just a cap for T6SS sheath. Its presence is serving as a biological marker for the opposite side of the cell.

Interestingly, unlike the *E. coli* T6SS, in *V. cholerae* there is a negligible defect in T6SS function associated with deleting TagA in normal cells (11, 15) and minimal if any T6SS defects in the extremely large cells. This may be related to how tightly associated the extended sheath associates with TagA and may relate to how T6SS contraction is regulated in the two organisms. For example, in *E. coli* once the growing T6SS sheath reaches the opposite cell wall and associates with TagA, the

sheath does not continue growing. On the other hand, Stietz et al. observed that in their large *V. cholerae* cells, T6SS sheath that had their growth stalled could actually continue growing after a brief pause. The transient stall could result from momentary shortage of sheath-tube subunits or a weaker association with membrane-bound TagA. The latter could explain why bi-direction firing has not been observed in *V. cholerae.* It also makes one wonder just why the *E. coli* T6SS attaches so tightly via TagA. Both organisms exhibit a similar stall period between the completion of sheath extension and the eventual sheath contraction events, so it is unlikely that the tight association is necessary for holding the T6SS in its extended state. More likely, there are additional factors contributing to the triggering of T6SS sheath contraction that differ between the two organisms. Moreover, it is worth noting that some T6SSs, such as the H1-T6SS of *Pseudomonas aeruginosa* completely lack a TagA homolog. These T6SS exhibit completely different polymerization and contraction dynamics (8, 11, 16).

There are still a number of questions left outstanding regarding the regulation of T6SS sheath contraction. Perhaps the biggest one is what triggers sheath contraction? Although the T6SS sheath contraction may just be stochastic random events, perhaps there is some sort of signal that can control them. That the T6SS apparatus makes contact with the cell membrane at two locations – one at the baseplate and the other at the distal membrane via TagA – means that the T6SS structure can in principle sense lateral mechanical stresses applied to the cell wall. It would be very interesting if cell-to-cell contact in the context of a mixed species biofilm (conditions where T6SS killing readily occurs) could create such a stress. Such a mechanism would allow bacterial cells to only shoot their T6SS payloads when a potential target is in range. Ultimately, further studies visualizing T6SS activity under different cellular conditions will be needed to better resolve additional mechanistic details.

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