Tagging the type VI secretion system

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Tagging the T6SS

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Standfirst

A powerful *in vivo* biotinylation approach identifies TagA as a binding partner of TssA, a central regulator of the assembly of a T6SS. TagA terminates assembly of the T6SS tail and tethers it to the membrane, acting as a crossbow latch that allows for efficient firing.

Main text

Bacteria use multiple mechanisms to secrete proteins across their envelope and gain access to resources in their niche. The type VI secretion systems (T6SS) specialize in injecting toxic protein effectors by direct contact, killing the target eukaryotic or bacterial cells[1]. The T6SSs share common origins with contractile tail bacteriophages, which attach to the cell surface and inject their DNA by puncturing the membrane with their tail spike. In T6SS, the tail-like structures are built inside the cytoplasm and inversely oriented, with their spike pointing outwards (Fig. 1). The T6SS tail is composed of an external layer – a contractile sheath – and a hollow inner tube that stores small protein effectors prior to secretion. Like an armed crossbow, the assembled T6SS remains inactive and ready to fire until it somehow senses a

prey cell within the firing range. This activates sheath contraction and exposes the spike, which penetrates the prey cell envelope and delivers its deadly cargo.

T6SS has emerged as one of the key weapons in the fierce warfare raging underneath the seemingly still surface or agar-grown bacteria. The fascinating revelation of T6SS dynamics and regulation has come from live microscopy of fluorescently tagged sheath structures[2]. On the macroscopic level, these wars result in contact-dependent inhibition, biofilm structuration and are key for inter-species competition in microbial communities. Despite tremendous recent progress in structural and functional dissection of T6SS nanomachines, the signals controlling their activation or dynamics are poorly understood.

In this issue of Nature Microbiology, Santin *et al.*[3] explore the late T6SS assembly steps. By applying a targeted proteomics analysis in enteroaggregative *Escherichia coli* (EAEC), they identified a novel T6SS component that could control the timing and efficiency of secretion. A component of the EAEC T6SS called TssA plays a central role during several stages of assembly[2]. Upon assembly of the membrane pore complex, binding of TssA facilitates assembly of the baseplate, and then orchestrates tail polymerization. TssA remains associated with the end of the growing tail, presumably acting as a chaperon to guide the addition of tube and sheath subunits at this site[4]. Live microscopy studies show that fluorescently labelled sheath polymerizes as a straight rod and extends across the bacterial cell until reaching the opposite membrane.

To characterize these late steps, the authors sought to identify TssA binding partners. For this, they employed a targeted proteomics approach recently developed and tested in eukaryotic systems. They fused TssA to a protein called APEX2, a variant of a small (28 kDa) heme-containing peroxidase developed as a tag for electron microscopy and spatially resolved proteomics in eukaryotic cells[5]. In live cells, in the presence of biotin-phenol, a short pulse of H_2O_2 generates short-lived biotinphenoxyl radicals that are transferred covalently onto proteins in close proximity, typically in a 20-nm radius. Purification of labelled proteins on streptavidin matrix and mass spectrometry provide an unbiased method of mapping protein interaction partners in a cellular process[5]. To test its possible use in bacteria, the authors demonstrated that TssA-APEX2 fusion was functional in secretion, even in presence of biotin phenol substrate.

Importantly, to avoid perturbing its expression level and cellular localization, the gene fusion was expressed from the native chromosomal locus. In these conditions, all the major TssA interacting partners were biotinylated, as well as a previously uncharacterized protein that the authors named TagA. TagA was not captured by biotinylation in EAEC mutants wherein T6SS assembly was arrested in earlier stages. Moreover, the authors went on to generate an APEX2 fusion to GspE, a cytoplasmic component of another EAEC complex, the type II secretion apparatus. This reaction captured known GspE interacting partners and none of the T6SS components, confirming the specificity of APEX2 labelling and showing its great potential to study bacterial protein complexes *ex vivo.*

Santin and coworkers studied TagA further and showed that it binds directly to TssA, but also to the cytoplasmic membrane. TagA sequence analysis revealed homology with TssA and the presence of an additional, hydrophobic domain that likely mediates its association to the membrane. Mutants lacking TagA showed some fascinating phenotypes. First, when sheath polymerization was visualized by time-lapse fluorescence microscopy, in many cases it did not stop at the opposite membrane, but rather continued, often bending and breaking or forming long sheaths. Furthermore, other tails that showed normal appearance would contract immediately upon assembly, suggesting that TagA promotes TssA binding to the membrane and stabilizing its extended high-energy conformation. Although these analyses show that tail assembly is only partially defective, *tagA* mutants were very significantly impaired in killing and retained only 1/10th of activity in a competition assay. Whether this defect is due to suboptimal angle of firing or to its reduced energy remains to be tested.

The T6SS device is often compared to a molecular crossbow, where tail assembly would be analogous to loading and pulling of the elastic string. Thanks to a latch on a crossbow, an archer can attach the string and keep it extended effortlessly, taking the time to aim and fire at the right moment. The TagA protein identified here could play a role of the latch, reinforcing this analogy. The fact that TagA is present only in some bacteria makes one wonder whether other proteins similarly control firing in other strains, and whether this correlates with their different behaviours in secretion. Can we measure forces that bacteria generate when firing their crossbow and how do they correlate with the observed biological activity? Is the

mechanical signal propagated across the membrane contact site to TagA? Addressing these questions may bring new functional insights into these fascinating nanomachines.

(920 words)

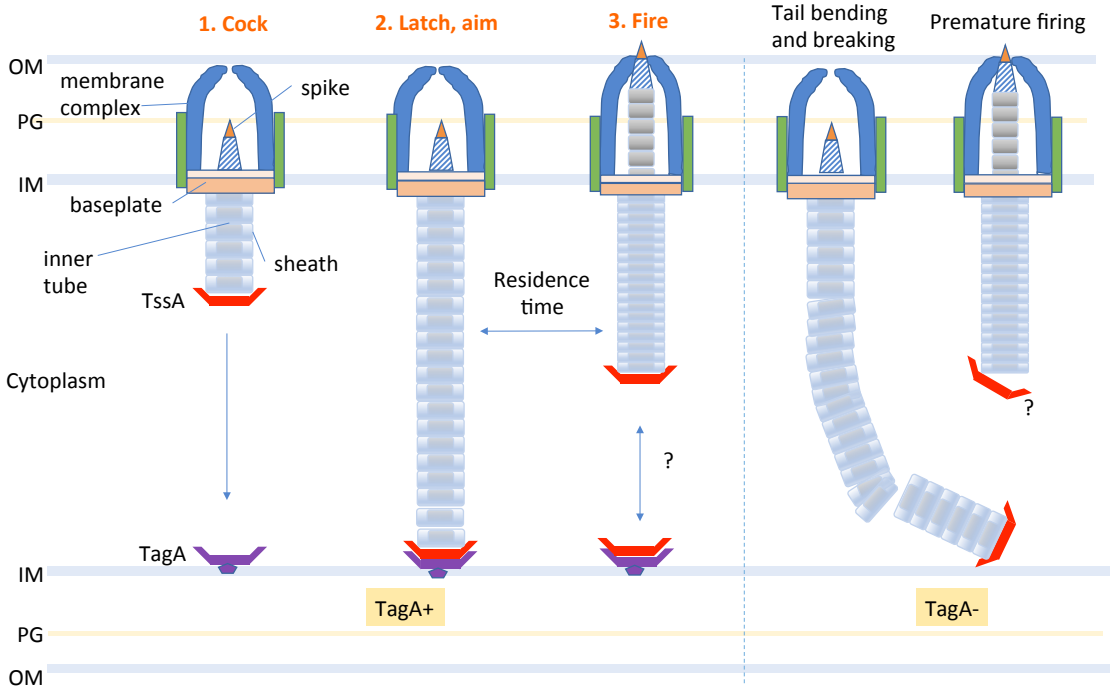

Figure 1. Role of TagA in terminating T6SS assembly, triggering and prey cell killing. Left: 1. In wild type cells, T6SS assembly begins with formation of the membrane complex, followed by the baseplate and tail polymerization. The TssA chaperon (shown in red) facilitates the cytoplasmic assembly steps by interacting with the baseplate and by capping the distal end of the tail. 2. A membrane-associated TssA homologue, TagA, captures the TssA-growing tail complex as it reaches the membrane, thereby terminating assembly. The TagA membrane clamp could stabilize the extended sheath/tube during a variable *residence time* period. 3. An unknown signal promotes sheath contraction, firing the spike and effectors into the target cell. An open question is whether TssA is released or remains associated with TagA upon contraction. Right: In *tagA* mutants, the sheath assembly does not terminate at the membrane, resulting in long, improperly oriented tails that often bend or break. Another observed defect is a rapid tail contraction upon assembly with reduced residence time. Killing efficiency is roughly reduced 10-fold in these strains.

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