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Report

Cell Width Dictates Type VI Secretion Tail Length

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SUMMARY

The type VI secretion system (T6SS) is a multiprotein apparatus that injects protein effectors into target cells, hence playing a critical role in pathogenesis and in microbial communities [1–4]. The T6SS belongs to the broad family of contractile injection systems (CISs), such as *Myoviridae* bacteriophages and R-pyocins, that use a spring-like tail to propel a needle loaded with effectors [5, 6]. The T6SS tail comprises an assembly baseplate on which polymerizes a needle, made of stacked Hcp hexamers, tipped by the VgrG-PAAR spike complex and wrapped by the contractile sheath made of TssB and TssC [7–13]. The T6SS tail is anchored to the cell envelope by a membrane complex that also serves as channel for the passage of the needle upon sheath contraction [14–16]. In most CISs, the length of the tail sheath is invariable and is usually ensured by a dedicated protein called tape measure protein (TMP) [17–22]. Here, we show that the length of the T6SS tail is constant in enteroaggregative *Escherichia coli* cells, suggesting that it is strictly controlled. By overproducing T6SS tail subunits, we demonstrate that component stoichiometry does not participate to the regulation of tail length. The observation of longer T6SS tails when the apparatus is relocalized at the cell pole further shows that tail length is not controlled by a TMP. Finally, we show that tail stops its elongation when in contact with the opposite membrane and thus that T6SS tail length is determined by the cell width.

RESULTS AND DISCUSSION

T6SS Tail Sheath Length in EAEC

The length of bacteriophage and other contractile injection system (CIS) tails such as that of anti-feeding prophages is strictly controlled [17–23]. To determine whether this is also the case for the T6SS, we measured the length of T6SS sheaths in EAEC wild-type cells producing a functional fusion between the TssB tail subunit and the superfolder-GFP (TssB-sfGFP). The sfGFP-coding sequence was inserted on the chromosome, upstream the *tssB* stop codon. In this construct, the sfGFP sequence is in frame with the *tssB* gene, and the *tssB-sfGFP* fusion is under the control of the native *tssB* expression signals. Cells were grown in *sci1*-inducing medium (SIM), a defined synthetic medium that avoids batch-to-batch composition variability and induces the expression of EAEC T6SS genes [24]. In agreement with the localization of T6SS MC along the cell body with an under-representation at the poles [15, 25], we observed that T6SS sheaths assemble from one position on the cell body to the opposite membrane. To avoid measurements of the length of contracted sheaths or of sheaths under extension, time-lapse recordings were performed, and only sheaths for which the elongation has been completed (i.e., when the sheath holds >1 min with the same length) were considered (Figure 1A). The distal extremity of these extended sheaths always co-localized with the TagA stopper protein (Figure 1B). Quantitative measurements of these T6SS tail length showed low disparities, with a length mean of 0.76 ± 0.11 μm (n = 150) and a normal distribution (Figure 1C). We thus concluded that the length of the T6SS sheath is not randomly distributed and hence that the arrest of T6SS sheath elongation is controlled. Based on this conclusion, we hypothesize that T6SS sheath length might be determined by (1) the number of available T6SS tail subunits, (2) a tape measure protein (TMP), or (3) the cell width.

T6SS Tail Sheath Length Is Not Regulated by Tube/Sheath Component Stoichiometry

Several reports have demonstrated that the length of some pilus-like structures is limited by the number of available pilin subunits. For example, the T2SS uses a periplasmic pseudopilus to expel the substrates in the external medium such as a piston or an Archimedes screw [26]. By artificially increasing the number of pseudo-pilins, micrometer-long pili can be observed at the cell surface, suggesting that pseudo-pilus length is determined, in part, by the number of available subunits [27, 28]. A strict control of the number of subunits in the cell by finely tuned gene expression and protein stability levels would prevent the costly synthesis of unnecessary subunits. To test whether T6SS sheath length might be controlled by the number of available tail subunits, we modified the stoichiometry balance by deregulating the levels of tube/sheath subunits, Hcp, TssB, and TssC. TssA, which locates at the distal end of the growing sheath to coordinate the assembly of the tail tube/sheath [29–31], and the TagA stopper [31, 32] were not included in the study as single TssA and TagA complexes are responsible for promoting T6SS tail elongation and arrest. *tssB-sfGFP* and epitope-tagged *tssC* and *hcp* were cloned into the pTrc99A vector, under the isopropyl-β-D-thio-galactopyranoside (IPTG)-inducible p*trc*