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doi:10.1038/nature17182

Priming and polymerization of a bacterial contractile tail structure

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Contractile tails are composed of an inner tube wrapped by an outer sheath assembled in an extended, metastable conformation that stores mechanical energy necessary for its contraction. Contraction is used to propel the rigid inner tube towards target cells for DNA or toxin delivery. Although recent studies have revealed the structure of the contractile sheath of the type VI secretion system, the mechanisms by which its polymerization is controlled and coordinated with the assembly of the inner tube remain unknown. Here we show that the starfish-like TssA dodecameric complex interacts with tube and sheath components. Fluorescence microscopy experiments in enteroaggregative *Escherichia coli* reveal that TssA binds first to the type VI secretion system membrane core complex and then initiates tail polymerization. TssA remains at the tip of the growing structure and incorporates new tube and sheath blocks. On the basis of these results, we propose that TssA primes and coordinates tail tube and sheath biogenesis.

Contractile injection machines are nano-structures evolved to deliver macromolecules into target cells[1]. These machines have been elaborated for different purposes such as the injection of DNA into host cells in the case of bacteriophages, for the delivery of protein effectors into bacterial or eukaryotic cells in the case of R-pyocins, *Photorhabdus* virulence cassettes, anti-feeding prophages or type VI secretion systems (T6SS) or for inducing metamorphosis in invertebrates[1–6]. These machines include a tubular edifice called a tail[1,7,8]. The tail is essentially composed of a rigid inner tube wrapped by a contractile structure—the sheath—that is assembled in an extended conformation that stores mechanical energy necessary for its contraction and to propel the inner tube towards the target[8]. The tail is assembled on the baseplate that varies in terms of composition and number of subunits; however, a minimal baseplate consists of the hub protein surrounded by wedges[1,7,8]. The baseplate is not only the platform for the assembly of the tube/sheath, but also an important component of the signalling cascade that triggers sheath contraction[1,8]. Tails are usually completed by terminator proteins that stabilize the sheath and maintain tube and sheath together at the distal end to prevent energy dissipation during sheath contraction and to permit proper ejection of the inner tube[8–10].

The T6SS is composed of a contractile structure anchored to the cell envelope by the TssJLM membrane complex that serves as a docking station as well as a channel for the passage of the inner tube during sheath contraction[11–14] (Extended Data Fig. 1a). The contractile structure is composed of the tail tube made up of stacks of Hcp hexameric rings, wrapped by a sheath-like structure consisting of the TssB and TssC subunits (Extended Data Fig. 1a)[14]. During T6SS biogenesis, the assembly of the tube and sheath are coordinated: the insertion of a tube ring immediately preceding that of a sheath block[15]. This tail polymerizes on a baseplate-like complex composed of the VgrG hub and the TssE, TssF, TssG and TssK subunits[16–19] (Extended Data Fig. 1a). The TssBC sheath polymerizes in tens of seconds to build an ~600-nm long structure that contracts in a few milliseconds[17]. Contraction of the sheath propels the Hcp inner tube towards the target cell, like a 'nano-crossbow'[14], and is responsible for the delivery of toxin effectors, as it correlates with lysis of the competitor bacterium[20,21]. Recent cryo-electron microscopy studies have revealed the atomic structure of the T6SS sheath in its contracted conformation[22,23]. The sheath is a helical structure composed of 6-TssB/TssC heterodimer strands, each heterodimer being stabilized by an intra- and inter-strand handshake domain[23]. In addition, a cryo-electron microscopy study of the pyocin R2 has provided information regarding the atomic structure of this contractile nanotube in its extended conformation and on how it interacts with the inner tube[2]. Although the general mechanism of T6SS assembly and the structure of the T6SS sheath are now well documented, critical details are missing, such as how the polymerization of the sheath is controlled, how tube and sheath assembly is coordinated and how tail polymerization is stopped.

TssA initiates tail tube/sheath polymerization

During T6SS tail biogenesis, the recruitment and assembly of Hcp hexamers and TssBC sheath blocks should be coordinated and the tail tube and sheath should be firmly attached together at the distal end to allow proper tube throwing during contraction. We therefore hypothesized that at least one of the T6SS core proteins must be required to coordinate and/or terminate Hcp/TssBC tail assembly. Such candidate subunit(s) should interact with both the tube protein (Hcp) and with at least one component of the sheath (TssB and/or TssC). We therefore performed a systematic bacterial two-hybrid analysis in which Hcp, TssB and TssC were used as baits to identify prey partners within T6SS subunits. Extended Data Fig. 1b shows that a number of baseplate components (TssE, TssF, TssG and VgrG) interact with either Hcp or TssC. However, a unique protein, TssA (GenBank accession number: 284924261), interacts with both tube and sheath components. Recent

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