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Type VI secretion TssK baseplate protein exhibits structural similarity with phage receptor-binding proteins and evolved to bind the membrane complex

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The type VI secretion system (T6SS) is a multiprotein machine widespread in Gram-negative bacteria that delivers toxins into both eukaryotic and prokaryotic cells. The mechanism of action of the T6SS is comparable to that of contractile myophages. The T6SS builds a tail-like structure made of an inner tube wrapped by a sheath, assembled under an extended conformation. Contraction of the sheath propels the inner tube towards the target cell. The T6SS tail is assembled on a platform—the baseplate—which is functionally similar to bacteriophage baseplates. In addition, the baseplate docks the tail to a trans-envelope membrane complex that orients the tail towards the target. Here, we report the crystal structure of TssK, a central component of the T6SS baseplate. We show that TssK is composed of three domains, and establish the contribution of each domain to the interaction with TssK partners. Importantly, this study reveals that the N-terminal domain of TssK is structurally homologous to the shoulder domain of phage receptor-binding proteins, and the C-terminal domain binds the membrane complex. We propose that TssK has conserved the domain of attachment to the virion particle but has evolved the reception domain to use the T6SS membrane complex as receptor.

Delivery of bacterial effector proteins and toxins into target cells relies on trans-envelope nanomachines called secretion systems. These machines select and transport effectors in the milieu or directly into the target cell[1]. Most of these secretion systems evolved from efflux pumps or from machineries involved in conjugation or flagellar, twitching or gliding motility[1]. The type VI secretion system (T6SS) is a fascinating machine that uses a contractile mechanism similar to that of the bacteriophage or R-pyocin contractile tail[2–7]. The T6SS delivers toxins and effectors in both eukaryotic and prokaryotic cells, and participates in bacterial pathogenesis and interbacterial competition[8,9]. By eliminating competing bacteria, the T6SS confers an increased ability to colonize a niche[10–16].

The T6SS, in essence, can be viewed as a contractile tail oriented towards the target cell, and anchored to the cell envelope by a membrane complex (MC)[3,17]. The MC is evolutionarily related to a subcomplex associated with the type IVb secretion system[18,19], and is a 1.7 MDa trans-envelope structure composed of three conserved subunits: the TssJ outer membrane lipoprotein and the TssL and TssM inner membrane proteins[20–26]. In several cases, the MC is properly inserted and anchored to the cell wall by additional proteins with peptidoglycan hydrolysis and peptidoglycan binding properties[21,27–29]. The contractile tail is composed of an inner tube made of hexamers of the Hcp protein, stacked on each other[30,31], tipped by VgrG, and surrounded by a contractile sheath made of the TssBC proteins[30–32]. Polymerization of the tail tube/sheath tubular structure is initiated on an assembly platform—the baseplate (BP), the less characterized T6SS subcomplex—and is coordinated by the TssA protein[33–35]. The T6SS contractile tail shares functional and structural homologies with the tails of several bacteriophages[5,6,30,36–38]. Once the T6SS tail is assembled, the sheath contracts and propels the inner tube/spike needle complex towards the target cell[39–42], and it has been proposed that this needle complex traverses the cell envelope through the MC (ref. 26). A recent *in vivo* study identified five components of the BP: TssE, TssF, TssG, TssK and VgrG (ref. 34). Although TssA was also identified in this screen, later observations demonstrated that TssA is not a structural component of the BP *per se*[35]. TssE is a homologue of gp25, a bacteriophage T4 BP wedge protein[18,19,43]. By contrast, no tridimensional structure is available for TssF, TssG or TssK. *In silico* analyses recently predicted that TssF and TssG share limited homologies with gp6 and gp7, respectively[34] and controversies exist regarding TssK (refs 44, 45). Interestingly, T6SS BP subcomplexes could be isolated in *Serratia marcescens* and uropathogenic *Escherichia coli* (UPEC), including the TssKFG or TssKFGE complexes[33,45]. These complexes probably represent the equivalent of wedge complexes of phage BPs, which assemble around the central gp27–gp5 hub/spike[46,47]. In addition to appearing central for the assembly of the T6SS wedges, TssK is a key BP subunit mediating contacts with the cytoplasmic domains of MC components[35,48,49]. Hence, TssK is an essential BP component connecting the MC, BP and tail components. However, we still lack structural information on TssK. Although we have shown that TssK assembles trimeric complexes in enteroaggregative *E. coli* (EAEC)[48], a study has reported that it assembles

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