Towards a complete structural deciphering of Type VI secretion system

van Son Nguyen, Badreddine Douzi, Eric Durand, Alain Roussel, E. Cascales, Christian Cambillau

Towards a complete structural deciphering of type VI secretion system

Van Son Nguyen[1,2], Badreddine Douzi[1,2§], Eric Durand[1,2,3], Alain Roussel[1,2], Eric Cascales[3] and Christian Cambillau[1,2*]

[1] Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, Campus de Luminy, Case 932, 13288 Marseille Cedex 09, France.

[2] Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique (CNRS), Campus de Luminy, Case 932, 13288 Marseille Cedex 09, France.

[3] Laboratoire d'Ingénierie des Systèmes Macromoléculaires, Institut de Microbiologie de la Méditerranée, Aix-Marseille Univ. - Centre National de la Recherche Scientifique (UMR7255), 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France.

[*] corresponding author: Christian Cambillau (cambillau@afmb.univ-mrs.fr).

Running title: T6SS-

Keywords: Type VI secretion, baseplate, contractile tail, receptor binding protein, tail assembly, bacteriophage

[§] present address

The Type VI secretion system (T6SS) is a complex and dynamic nanomachine present in ~50% Gram-negative bacteria. Using a contraction mechanism similar to that of myophages, bacteriocins or anti-feeding prophages, it injects toxic effectors into both eukaryotic and prokaryotic cells. T6SS assembles three large ensembles: the trans-membrane complex (TMC), the baseplate and the tail. Within the two last years, the tail structure has been elucidated by cryo electron microscopy (cryoEM) both in extended and contracted forms. The structure of the trans-membrane complex has been deciphered using a combination of X-ray crystallography and EM. However, despite recent progress, the structural knowledge on the baseplate lags behind. Baseplate should therefore remain the target of future studies, leading then to a complete structural description of T6SS.

INTRODUCTION

The first report on components of the cluster taht would become T6SS appeared more that 15 years ago. It was only in 2006 that the identification of a secreted tube component, Hcp, established that the identified cluster was a secretion system [1]. It was coined Type VI Secretion System (T6SS) the following year [2]. The structure of Hcp together with the determination of the crystal structure of the tube tip, VgrG, established the common evolutionary origin of T6SS and myophages (e.g. T4 or Mu) contractile tail [1,3], leading to the vision that T6SS could be some kind of tamed phage. Later on, this hypothesis was completed when T6SS tail sheath proteins VipA and VipB (TssB and TssC) were found to share structural and functional homology with those of myophages, and were at the origin of tail's contraction [4]. Contrary to what occurs for all other contractile machineries, T6SS tail is recycled after contraction thaks to an ATPase, ClpV, that disassemble the tail sheat into monomeric components, TssB and TssC [5]. This tail sheath recycling constitutes a hall mark of T6S. A group of proteins were found to be attached or embedded in both IM or OM. One of them, TssM, shares sequence identity with a Tss4 component IcmF. This finding completed the view of T6SS as being a phage-like machinery, attached to a secretion membrane system. Between them, another ensemble constitutes the baseplate, which was found to share some similarity with that of myophages.

In 2012, a tomography electron microscopy (EM) examination of T6SS yielded the first complete topology and dynamics of the system. It made it possible to visualize the three domains of T6SS, TMC, baseplate and tail, and revealed that the tail crosses completely the cell's cytoplasm. Furthermore, analysis of tail's dynamics established that elongation takes tens of seconds , while contraction is very fast (<5 ms) [6].

T6SS is able to kill both prokaryotic or eukaryotic cells [7,8]. To this end, deadly effectors can be loaded at different positions: inside Hcp, docked to VgrG via PAAR module, or can be part of evolved Hcp or VgrGs. These effectors have been the subject of several reviews and are not part of this account [9,10].

The trans-membrane complex spans between cytoplasm and outer membrane

On the 13 proteins conserved in the T6SS cluster (Fig. 1a), four have been shown to be inserted in the cell membranes: TssL, TssL and TagL in the IM and TssJ, a lipoprotein, in the

OM [11]. TssL is a monotopic membrane protein with a large cytoplasmic domain. Its X-ray structure and further analysis allowed to determine that it forms an essential biological dimer [12]. The main component of the TMC, TssM, was early identied as a T4SS IcmF-like protein [1]. This ~1100 amino-acids large protein was shown to be formed of four structural segments: a large cytoplasmic domain (~250 aa) inserted in the IM through a three helix membrane domain, followed by a periplasmic segment that can be further divided in a large helical domain followed by a C-terminal beta domain [11,13]. This latter beta-domain was shown to interact with the TssJ OM lipoprotein that binds TssM and brings its C-terminus in the vicinity of the OM [13]. Together with TssL [12] and TagL [14,15], TssM and TssJ form the TMC [16]. Despite this knowledge, the functionning of T6SS TMC remained obscure, letting open questions about the presence of an eventual OM pore and its opening.

In 2015, the TCM biogenesis, structure and function were deciphered using an converging biophysical and biochemical techniques [17] (Fig. 1c-g). The negative stained EM (nsEM) structure of the ternary complex TssJ-TssL-TssM (TssJLM) was determined at ~12 Å resolution (Fig. 1f,g). It assembles 10 IM anchored TssJ-TssM pilars spanning the periplasm and contacting the OM. Five dimers of TssL are anchored via a trans-membrane helix (TMH) in the IM and point in the cytoplasm. Together with the cytoplasmic domain of TssM, they form a hydrophilic platform that serves to dock the baseplate and the rest of the system. Quite surprinsingly, the TCM complex obeys a C5 symmetry, forming 5 dimers that could be split between 5 internal and 5 external pilars [17] (Fig. 1f,g). Noteworthy, both the reduced size of the internal channel (15 Å) and the absence of pore did not allow the nsEM structure to elucidate the mysterious secretion mechanism. However, the X-ray structure of the C-terminal domain of TssM in complex with TssJ made it possible to identify loops at the extremity of TssM as candidates that could eventually cross the OM. Residues of TssM C-terminal domain were mutated in cysteines and assayed in a functional T6SS. Loops at the tip of TssM are labelled during T6SS secretion, establishing that they reach the cell exterior [17]. Furthermore, it was demonstrated that TssJ dissociates from TssM during the process. It was therefore proposed that the stroke of the Hcp/VgrG occuring during tail contraction pilum would lead the C-terminal TssM domain through the OM by brute force and drive it towards the cell exterior. The TCM would thus form a transient pore of sufficient size. T6SS TCM domain would thus oscillate between a closed rest state and a forced-open state during secretion.

The T6SS C5 symmetry was quite a surprise as the tail symmetry is C6 for the tail tube and the sheath (C3 for VgrG) (see below). Indeed, the immediate partner of TCM is the baseplate whose structure is unknown. However, due to faint similarity with myophages baseplates [18] it has been proposed that the overall baseplate structure may also obey a C6 symmetry. Therefore, a symmetry mismatch should certainly occur between the TssJLM 5-fold symmetry and its partner component.

Until a recent paper in 2016, TssA was the most intriguing and less documented component of T6SS. The nsEM map of TssA revealed that it is a dodecamer assembling two back-to-back C6 hexamers [19]. The crystal structure of the core C-terminal domain exhibits the shape of a donut, roughly the diameter of Hcp. Both nsEM and SAXS reveal the presence of 6 propeller-like N-terminal domains decorating each hexamer. Noteworthy, co-expressed TssA and TssJLM result in a complex that, examined by nsEM, exhibits a large volume at the base of TssJLM, most likely due to the attachment of TssA. Knowing the tridimensional structures of both partners spured us on to model their complex. This proved impossible to perform with the C5 TssJLM nsEM structure. A model was put up by assembling TssJLM membrane and cytoplasmic domains with C6 symmetry that exquisitely fits with TssA [20] (Fig. 1h,i). This suggest that TssA acts as a chaperone selecting C6 TssJLM, and deplacing C5 TssJLM and eventual other forms towards it. It was also found that TssA, after priming, located at the distal end of the tail where it acts as a stopper [19 ,20].

The tail, a highly dynamic and energetic system

T6SS tail is a part that attracted most interest in the last five years. Not a surprise, the first components for which the X-ray structure have been determined were Hcp and VgrG, suggesting the common origin of T6SS and myophage tails [1,3,21,22]. More recently another component of the tail, PAAR, was identified. Its position at the tip of VgrG allows to sharpen the puncturing device and to dock effectors [23].

A contractile tail sheath, composed of TssB and TssC (identified as VipA and VipB in *Vibrio cholerae*) enveloppes the Hcp/VgrG internal tube. The T6SS tail has been show to be micro-meters long in the metastable extended form, spanning the whole cytoplasm from cell-wall to cell-wall. The assembly of this tube involves TssA that acts both as a chaperone for the TCM as well as a “stopper” at the distal extremity of the tube [19]. Under an external and unknown trigger, TssBC changes conformation and contracts, thus propelling the Hcp/VgrG pilum

through TssJLM and to the inter-cellular space, and further into the nearby prey (REFs). Because the extended tail sheath is in a metastable state, the *in vitro* expression of TssBC leads exclusively to the contracted form. Three groups reported cryoEM structure of expressed (contracted) TssBC at medium or high resolution, resulting in atomic models of the tail sheath [24-26] (Fig. 2). VipAB/TssBC form a helix of 6 components per layer. The core domain of the sheath is maintained by four β-strands originating from three monomers, one VipA (TssB) and two VipB (TssC). The internal radius of the contracted sheath is larger that the Hcp diameter, allowing thus tube release during contraction (Fig. 2a,b). While the overall sheath structure resembles that of phage T4 [18], the external part is different. Not surprising, a VipB (TssC) exposed domain interacts with the ClpV ATPase, allowing VipAB recycling, a phenomenom not present in T4 [27]. This TssC domain is indeed hidden in the extended sheath [28].

Recently, electron cryotomography and sub-tomogram averaging of T6SS tail *in vivo*, yielded the low-resolution structure of the extended tail sheath loaded with Hcp, together with that of the contracted form [28]. A while after, the cryoEM structure of *Vibrio cholerae* extended tail was determined at ~3.5 Å resolution [29] (Fig. 2c,d). This extended form could be obtained thanks to a trick, the addition of three residues to the VipA N-terminal linker connecting sheath strands, blocked the tail in a metastable form. This remarkable work reveals the structure of the extended sheath with its internal Hcp tube, as well as the Hcp/Hcp contacts and the VipAB/Hcp interaction. Comparison of the extended and contracted structure reveals a shrink of the inter disk rise, from 38.0 Å to 21.8 Å (~42%) associated with an increase of the twist helical angle from 23.6 to 29.9 degree [29] (Fig. 2). The authors calculated that contraction of a 1 μm long sheath would release an energy of 10,000 kcal/mole, push the pilum by 420 nm towards the prey, while rotating at ~40,000 rpm: an incredible powerful machinery that indeed has no problem to open a passage in the TssJLM TCM complex.

The baseplate secrets

The baseplate is formed of TssE, F, G, H, and represents an adaptator between the TMC and the tail. Indeed, due to the drastic conformational changes between extended and contracted tail sheath, its role might be also to accommodate these changes in order to keep the system Strongly assembled.

160 The baseplate has been the least studied part of T6SS. Sequence analysis by bioinformatics
161 studies suggested that components of the baseplate – if not the complete baseplate – resemble
162 those of myophages, T4 or Mu [18,30,31]. TssE is clearly the counter part of gp25 that binds
163 strongly the tail sheath. TssF is similar with T4 gp6 or Mu ORF47 [31], and it has been
164 proposed that TssF and TssG assembly resemble that of gp6-gp53 in T4 [18,30].

165 The nsEM TssK structure reveals that it is a trimer [32]. It and intracts both with TMC and
166 tail [32]. Subsequently, TssK has been shown to be part of a definite TssKFG sub-complex
167 that may assemble into different multimers [33]. Later on, a larger complex was identified
168 that assemble TssE, F, G, K and VgrG. This complex is recruted at the IM by the TMC. K is
169 the first component to dock the TMC, and it further recruits FG [34]. Finally, TssBC and Hcp
170 elongate with the help of TssA, bolstered by the baseplate. In another report on phage T4
171 baseplate structure, purification of the TssKFG sub-complex was reported [18]. However, the
172 TssKFG soichiometry and the baseplate symmetry are still pending issues. SDS gel analysis
173 reported a clear 2:1 ration for TssF:TssG, comparable to that of myoviridae counterparts
174 [18,33]. However, contradictory results are reported concerning TssK, proposed to belong to a
175 4:2:1 complex [33] or a 3:2:1 complex [18].

176 We recently determined the X-ray structure of full-length TssK in complex with camelid
177 nanobodies [35] and analyzed its attachment to TssFG [36]. Each monomer of TssK trimer
178 has three domains: shoulders, neck and head (Fig. 3). The N-terminal shoulder domain of
179 TssK is structurally similar to the shoulder domain of siphophage receptor binding proteins,
180 such as phages p2 and 1358. Nanobodies competition assays showed that TssK binds to
181 TssFG using the bottom of the shoulders domain. The C-terminal head domain bind to the
182 TMC. The crude stoichiometry of the TssKFG complex returned from SDS gel analysis
183 reports K:F:G values of 4.04/1.86/1 and 4.6/1.97/1. The high ratio of K suggests that the
184 TssKFG complex stoichiometry might be either K_3:F_2:G (K_3)$_2$:F_2:G, i.e. each TssK binding a
185 TssK trimer.

186 From an evolutionary viewpoint, these results suggest that even if T6SS tail and baseplate
187 originates from Myophages, the TssK N-terminal domain may have derived from a
188 *Siphoviridae* component to provide the ability to bind the TMC, pointing thus to a complex
189 evolutionary process leading to T6SS in which components of different phage types were
190 combined.

191

The T6SS classes

T6SS is not a totally homogeneous system. Two classes have be distinguished on the base of functional co-evolution between TssB, TssC, TagJ/HsiE, and ClpV [37], while three classes have been identified within human gut bacteroidales [38]. However, in these classes, most of the 13 components of the T6SS cluster are conserved. Recently, the complete low-resolution structure of a contractile seccretion system from *Amoebophilus asiaticus*, has been determined by cryo-tomography [39].This system possesses a T6SS-like tail, a baseplate, an IM anchored module, but no *bona fide* TCM. The tail is shorter than in T6SS, as it is controlled by a tape measure protein (TMP), a feature found in all contractile systems but T6SS. This tail is not recycled by a ClpV ATPase. No effector has been isolated to date, and the action could be only due to mechanical effects. Contrary to T6SS, the system is not isolated and forms packed arrays at the IM surface. The *Amoebophilus asiaticus* secretion system cluster do not share any homology with those of T6SS and its components do not share any sequence identity with T6SS components, but share a strong identity with anti-feeding prophages. Although the authors proposed to class this secretion system as the 4th exemple of T6SS, this proposition seems fulsome and could be misleading for futures studies of T6SS.

Conclusion

The structural and functional knowledge of T6SS has made recently huge progresses, thanks to the achievments of cryoEM/tomography. The structural data available to date make it possible to tentatively propose a topological model of T6SS (Fig. 4). In the extended model presented here, 12 proteins out of the 13 conserved have been localized, either based on strong data, or tentatively (TssEFG). The cytoplasmic ClpV ATP ase, the 13th conserved protein, interacts with the contracted tail to disassemble it. Obviously, we are not far of having the structures of all the components determined, alone or in groups. No doubt that sub-tomogram averaging of a complete T6SS in *vivo* will allow in a near future to visualize a complete T6SS and its interactions, and shed further light on its secretion mechanism.

Acknowledgements

We thank the members of the Cambillau/Roussel and Cascales laboratories for insightful discussions. Prof. G. J. Jensen is gratefully acknowledged for providing us with a high resolution view of T6SS tomogram. This work was supported by the Centre National de la

Recherche Scientifique and the Aix-Marseille Université, and grants from the Agence Nationale de la Recherche (ANR-14-CE14-0006-02) and the French Infrastructure for Integrated Structural Biology (FRISBI). V.S.N was supported by a fellowship from the French Embassy in Vietnam.

References

Legend to Figures

Figure 1. Structure of the T6SS trans-membrane complex. (**a)** The cluster of conserved T6SS genes in the genome of *E. coli* EAEC. (**b)** Tomographic slice of an extended *V. clolerae* T6SS structure (adapted from ref. [6]). (**c)** Crystal structure of the TssM26Ct–TssJ complex represented as ribbons [17]. TssJ is coloured orange, while TssM26Ct is coloured cyan (α-domain) and blue (β-domain). The C-terminal α5-helix and the extended stretch are coloured magenta. (**d)** Orthogonal relative to (c). (**e)** View of the TssM26Ct–TssJ complex represented as surface representation (same orientation and colour as in (d)). (**f**) Segmentation of the TssJLM complex reconstruction [17]. Each pillar within the TMC is segmented in five different domains (shown in different colours). Domains 3,4 and TssJ correspond to the TssM26Ct–TssJ complex. (**g**) TssM26Ct–TssJ structure docked into the EM volume corresponding to TssJ and the TssM periplasmic domains. (**h**) View of the T6SS TMC (grey) modelled with 6/12 instead of 5/10 symmetry, forming a complex with TssA dodecamer (blue, 6 fold symmetry) (adapted from [20] and [19]). (**i**) Same model as in (h), but viewed from the cytoplasm, showing the fit of TssA arms in between the protruding TssL dimers.

Figure 2. **Structure of T6SS tail.** (**a)** Top ribbon view of the *V. clolerae* T6SS contracted tail sheath. Hcp hexamers have been modelled in the internal TssBC pipe in order to evidence that they do not interact with the sheath. (**b)** View rotated by 90° (lateral view). (**c)** Top ribbon view of the *V. clolerae* T6SS extended tail sheath, formed of TssBC (VipAB) hexamers, and containing stacked Hcp hexamers forming the tube [26]. (**d)** View rotated by 90° (lateral view). (a-d): One protofilament of the sheath is colored orange. The TssC recycling domain (that recognized by ClpV) is colored cyan. The Hcp inner tube is colored red.

Figure 3. Crystal structure of the TssK trimer. Ribbon view of TssK structure evidencing the topology of this trimer formed of shoulder, neck and head domains [36]. The binding of TssK to nbK27 (that occupies the binding site of TssFG) is reminiscent of the binding of the Dit arm and hand extension of the phage p2 Dit molecule [40].

Figure 4. Topology model of extended T6SS. (**a**) The cluster of conserved T6SS genes in the genome of *E. coli* EAEC. (**b**) Tomographic slice of an extended *Myxococcus xanthus*

T6SS structure (adapted from ref. [28]). (**c**) The position of the different modules with known structures have been tentatively assigned in the tomographic slice. Those with unknown structure (TssFGE) have been assigned according to ref. [28]. From the 13 conserved proteins forming T6SS (see (a), 12 have been tpologically assigned. The 13th, ClpV, is soluble cytoplasmic ATPase involved in contracted tail disassembling.

References

1. Mougous JD, Cuff ME, Raunser S, Shen A, Zhou M, Gifford CA, Goodman AL, Joachimiak G, Ordonez CL, Lory S, et al.: **A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus**. *Science* 2006, **312**:1526-1530.
2. Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ: **Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin**. *Proc Natl Acad Sci U S A* 2007, **104**:15508-15513.
3. Leiman PG, Basler M, Ramagopal UA, Bonanno JB, Sauder JM, Pukatzki S, Burley SK, Almo SC, Mekalanos JJ: **Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin**. *Proc Natl Acad Sci U S A* 2009, **106**:4154-4159.
4. Bonemann G, Pietrosiuk A, Diemand A, Zentgraf H, Mogk A: **Remodelling of VipA/VipB tubules by ClpV-mediated threading is crucial for type VI protein secretion**. *EMBO J* 2009, **28**:315-325.
5. Kapitein N, Bonemann G, Pietrosiuk A, Seyffer F, Hausser I, Locker JK, Mogk A: **ClpV recycles VipA/VipB tubules and prevents non-productive tubule formation to ensure efficient type VI protein secretion**. *Mol Microbiol* 2013, **87**:1013-1028.
6. Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ: **Type VI secretion requires a dynamic contractile phage tail-like structure**. *Nature* 2012, **483**:182-186.
7. Basler M, Mekalanos JJ: **Type 6 secretion dynamics within and between bacterial cells**. *Science* 2012, **337**:815.
8. Brunet YR, Espinosa L, Harchouni S, Mignot T, Cascales E: **Imaging type VI secretion-mediated bacterial killing**. *Cell Rep* 2013, **3**:36-41.
9. Durand E, Cambillau C, Cascales E, Journet L: **VgrG, Tae, Tle, and beyond: the versatile arsenal of Type VI secretion effectors**. *Trends Microbiol* 2014, **22**:498-507.
10. Russell AB, Peterson SB, Mougous JD: **Type VI secretion system effectors: poisons with a purpose**. *Nat Rev Microbiol* 2014, **12**:137-148.
11. Cascales E, Cambillau C: **Structural biology of type VI secretion systems**. *Philos Trans R Soc Lond B Biol Sci* 2012, **367**:1102-1111.
12. Durand E, Zoued A, Spinelli S, Watson PJ, Aschtgen MS, Journet L, Cambillau C, Cascales E: **Structural characterization and oligomerization of the TssL protein, a component shared by bacterial type VI and type IVb secretion systems**. *J Biol Chem* 2012, **287**:14157-14168.
13. Felisberto-Rodrigues C, Durand E, Aschtgen MS, Blangy S, Ortiz-Lombardia M, Douzi B, Cambillau C, Cascales E: **Towards a structural comprehension of bacterial type VI secretion systems: characterization of the TssJ-TssM complex of an Escherichia coli pathovar**. *PLoS Pathog* 2011, **7**:e1002386.
14. Aschtgen MS, Gavioli M, Dessen A, Lloubes R, Cascales E: **The SciZ protein anchors the enteroaggregative Escherichia coli Type VI secretion system to the cell wall**. *Mol Microbiol* 2010, 10.1111/j.1365-2958.2010.07028.x.
15. Aschtgen MS, Thomas MS, Cascales E: **Anchoring the type VI secretion system to the peptidoglycan: TssL, TagL, TagP... what else?** *Virulence* 2010, **1**:535-540.

16. Zoued A, Cassaro CJ, Durand E, Douzi B, Espana AP, Cambillau C, Journet L, Cascales E: **Structure-Function Analysis of the TssL Cytoplasmic Domain Reveals a New Interaction between the Type VI Secretion Baseplate and Membrane Complexes**. *J Mol Biol* 2016, **428**:4413-4423.
17. Durand E, Nguyen VS, Zoued A, Logger L, Pehau-Arnaudet G, Aschtgen MS, Spinelli S, Desmyter A, Bardiaux B, Dujeancourt A, et al.: **Biogenesis and structure of a type VI secretion membrane core complex**. *Nature* 2015, **523**:555-560.
18. Taylor NM, Prokhorov NS, Guerrero-Ferreira RC, Shneider MM, Browning C, Goldie KN, Stahlberg H, Leiman PG: **Structure of the T4 baseplate and its function in triggering sheath contraction**. *Nature* 2016, **533**:346-352.
19. *Zoued A, Durand E, Brunet YR, Spinelli S, Douzi B, Guzzo M, Flaugnatti N, Legrand P, Journet L, Fronzes R, et al.: **Priming and polymerization of a bacterial contractile tail structure**. *Nature* 2016, **531**:59-63.
20. *Zoued A, Durand E, Santin YG, Journet L, Roussel A, Cambillau C, Cascales E: **TssA: The cap protein of the Type VI secretion system tail**. *Bioessays* 2017, 10.1002/bies.201600262.
21. Osipiuk J, Xu X, Cui H, Savchenko A, Edwards A, Joachimiak A: **Crystal structure of secretory protein Hcp3 from Pseudomonas aeruginosa**. *J Struct Funct Genomics* 2011, **12**:21-26.
22. Douzi B, Spinelli S, Blangy S, Roussel A, Durand E, Brunet YR, Cascales E, Cambillau C: **Crystal structure and self-interaction of the type VI secretion tail-tube protein from enteroaggregative Escherichia coli**. *PLoS One* 2014, **9**:e86918.
23. Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ, Leiman PG: **PAAR-repeat proteins sharpen and diversify the type VI secretion system spike**. *Nature* 2013, **500**:350-353.
24. Kube S, Kapitein N, Zimniak T, Herzog F, Mogk A, Wendler P: **Structure of the VipA/B type VI secretion complex suggests a contraction-state-specific recycling mechanism**. *Cell Rep* 2014, **8**:20-30.
25. Clemens DL, Ge P, Lee BY, Horwitz MA, Zhou ZH: **Atomic structure of T6SS reveals interlaced array essential to function. *Cell* 2015, **160**:940-951.
26. Kudryashev M, Wang RY, Brackmann M, Scherer S, Maier T, Baker D, DiMaio F, Stahlberg H, Egelman EH, Basler M: **Structure of the type VI secretion system contractile sheath. *Cell* 2015, **160**:952-962.
27. Douzi B, Brunet YR, Spinelli S, Lensi V, Legrand P, Blangy S, Kumar A, Journet L, Cascales E, Cambillau C: **Structure and specificity of the Type VI secretion system ClpV-TssC interaction in enteroaggregative Escherichia coli**. *Sci Rep* 2016, **6**:34405.
28. Chang YW, Rettberg LA, Ortega DR, Jensen GJ: **In vivo structures of an intact type VI secretion system revealed by electron cryotomography. *EMBO Rep* 2017, **18**:1090-1099.
29. Wang J, Brackmann, M., Castaño-Díez, D., Kudryashev, M., Kenneth, Goldie, K., Maier, T., Stahlberg, H. and Basler, M.: **CryoEM structure of the extended Type VI secretion system tube-sheath complex. . *Nat Microbiol* 2017, **in press**.
30. Leiman PG, Shneider MM: **Contractile tail machines of bacteriophages**. *Adv Exp Med Biol* 2012, **726**:93-114.
31. Buttner CR, Wu Y, Maxwell KL, Davidson AR: **Baseplate assembly of phage Mu: Defining the conserved core components of contractile-tailed phages and related bacterial systems**. *Proc Natl Acad Sci U S A* 2016, **113**:10174-10179.
32. Zoued A, Durand E, Bebeacua C, Brunet YR, Douzi B, Cambillau C, Cascales E, Journet L: **TssK is a trimeric cytoplasmic protein interacting with components of both**

phage-like and membrane anchoring complexes of the Type VI secretion system. *J Biol Chem* 2013, **288** 27031–27041.

33. English G, Byron O, Cianfanelli FR, Prescott AR, Coulthurst SJ: **Biochemical analysis of TssK, a core component of the bacterial Type VI secretion system, reveals distinct oligomeric states of TssK and identifies a TssK-TssFG sub-complex**. *Biochem J* 2014, 10.1042/BJ20131426.

*34. Brunet YR, Zoued A, Boyer F, Douzi B, Cascales E: **The Type VI Secretion TssEFGK-VgrG Phage-Like Baseplate Is Recruited to the TssJLM Membrane Complex via Multiple Contacts and Serves As Assembly Platform for Tail Tube/Sheath Polymerization**. *PLoS Genet* 2015, **11**:e1005545.

35. Desmyter A, Spinelli,S., Roussel,A. and Cambillau,C.: **Camelid nanobodies: killing two birds with one stone**. *Curr. Opin. Struct. Biol.* 2015, **32** 1-8.

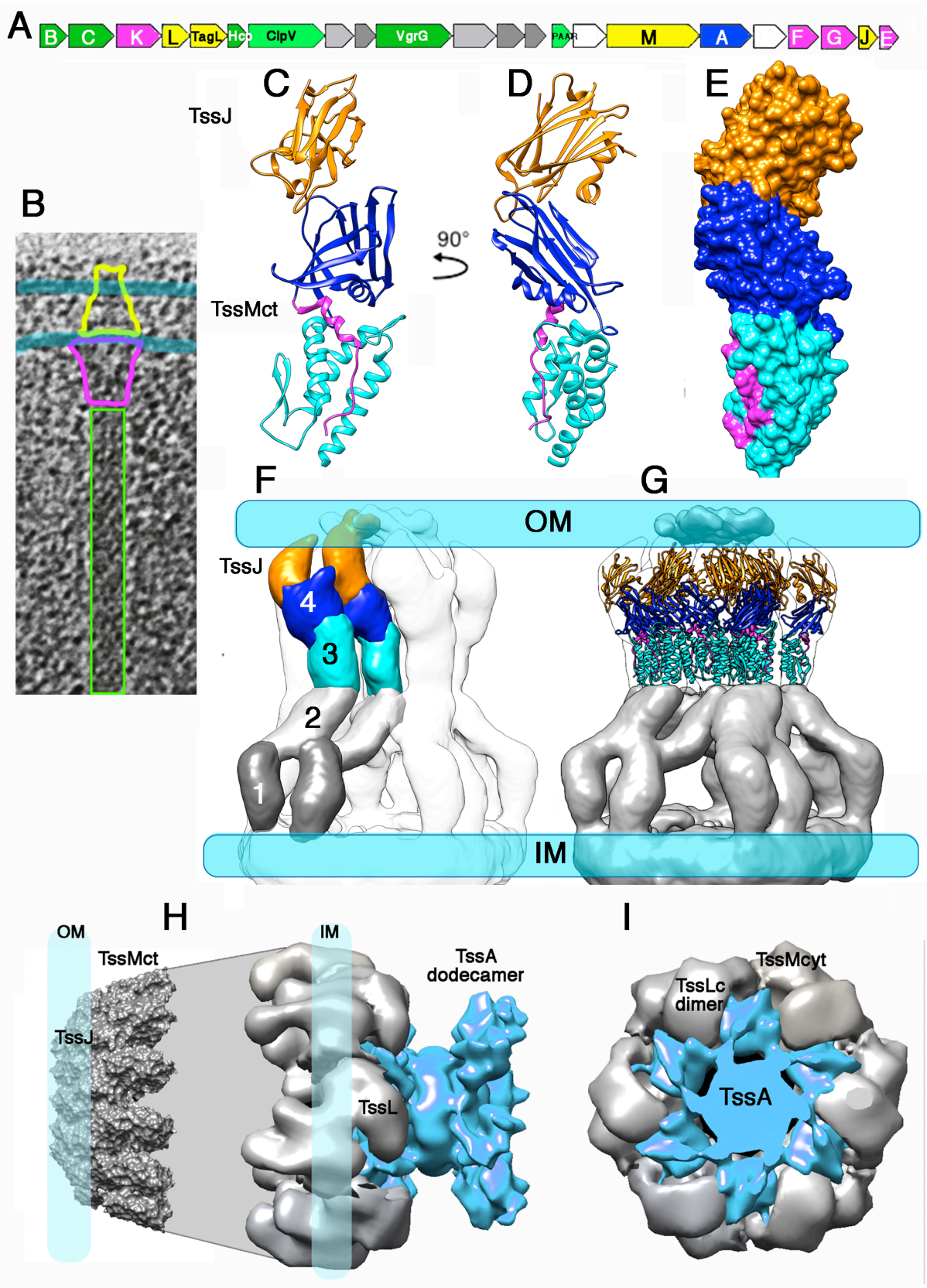
36. Nguyen VS, Logger L, Spinelli S, Legrand P, Huyen Pham TT, Nhung Trinh TT, Cherrak Y, Zoued A, Desmyter A, Durand E, et al.: **Type VI secretion TssK baseplate protein exhibits structural similarity with phage receptor-binding proteins and evolved to bind the membrane complex. *Nat Microbiol* 2017, **2**:17103.

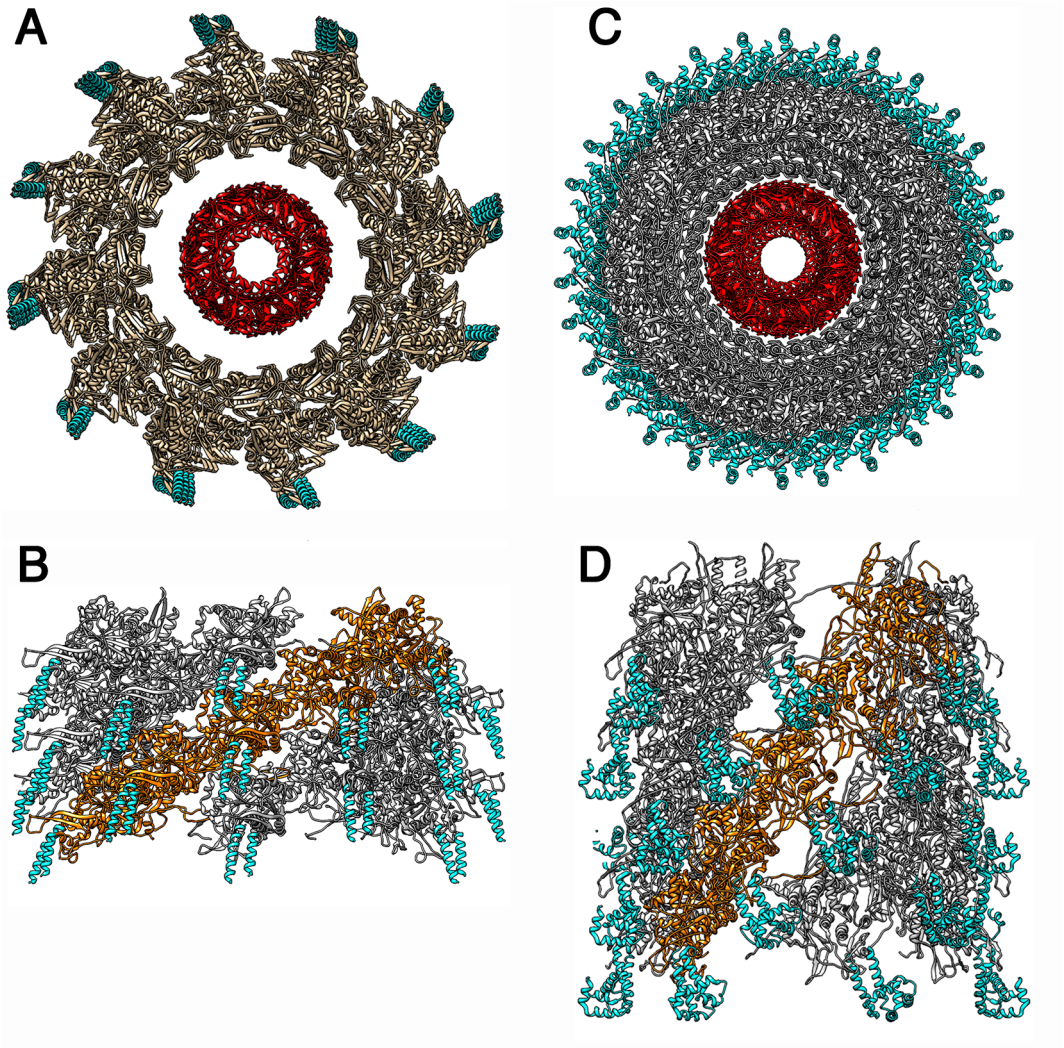
37. Forster A, Planamente S, Manoli E, Lossi NS, Freemont PS, Filloux A: **Coevolution of the ATPase ClpV, the sheath proteins TssB and TssC, and the accessory protein TagJ/HsiE1 distinguishes type VI secretion classes**. *J Biol Chem* 2014, **289**:33032-33043.

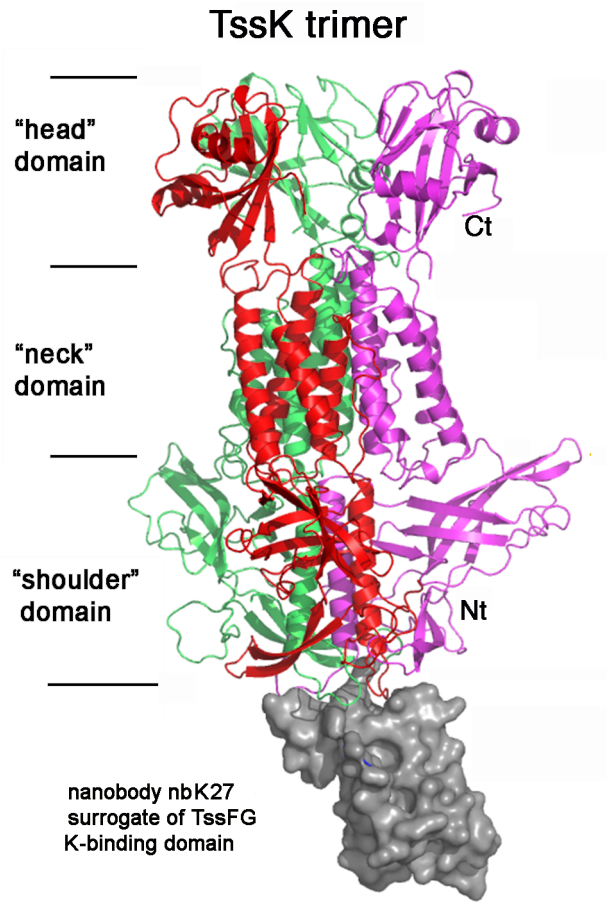
38. Coyne MJ, Roelofs KG, Comstock LE: **Type VI secretion systems of human gut Bacteroidales segregate into three genetic architectures, two of which are contained on mobile genetic elements**. *BMC Genomics* 2016, **17**:58.

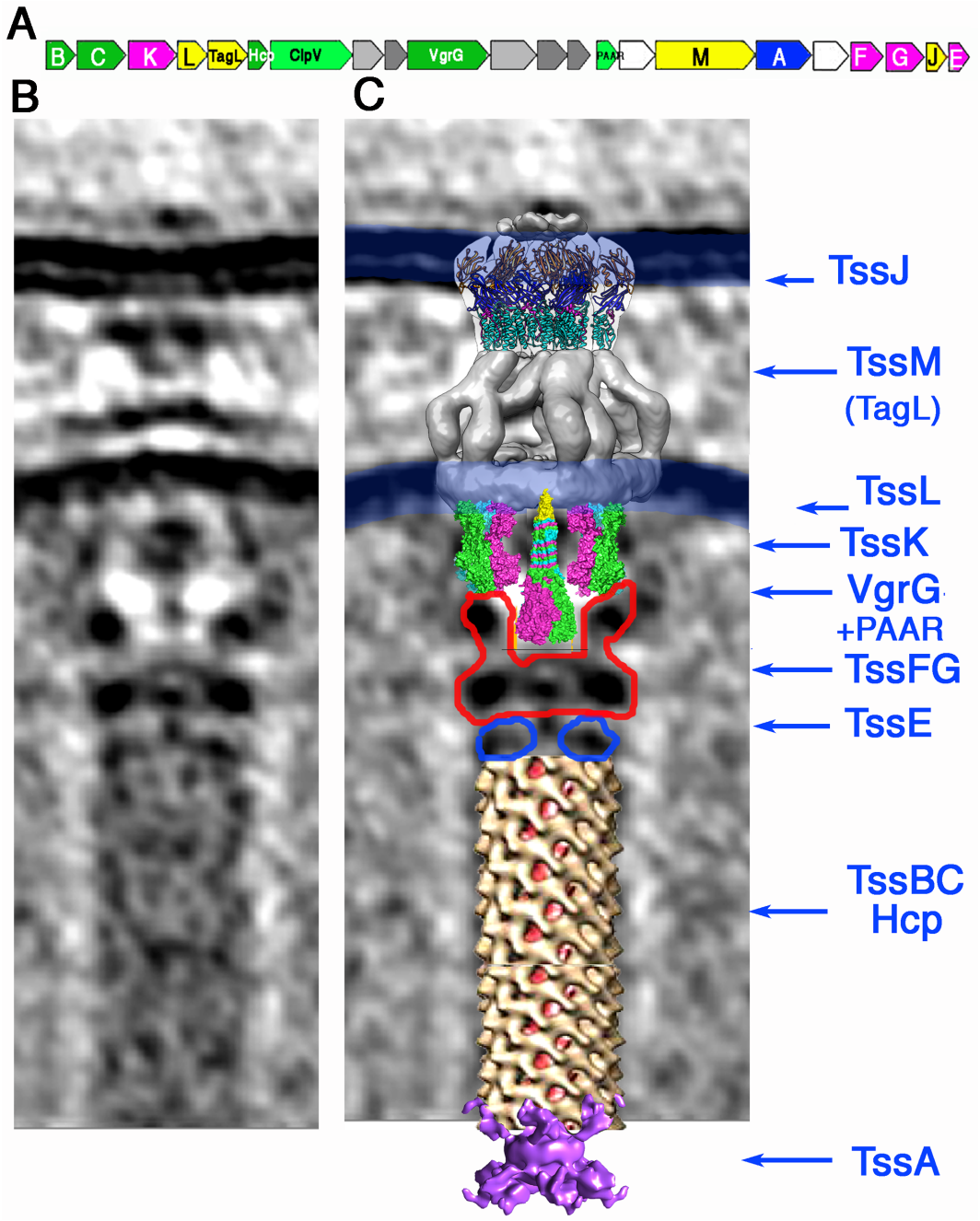
39. *Bock D, Medeiros JM, Tsao HF, Penz T, Weiss GL, Aistleitner K, Horn M, Pilhofer M: **In situ architecture, function, and evolution of a contractile injection system**. *Science* 2017, **357**:713-717.

40. Sciara G, Bebeacua C, Bron P, Tremblay D, Ortiz-Lombardia M, Lichiere J, van Heel M, Campanacci V, Moineau S, Cambillau C: **Structure of lactococcal phage p2 baseplate and its mechanism of activation**. *Proc Natl Acad Sci U S A* 2010, **107**:6852-6857.


A
B
C
TssJ
TssMct
90°
D
E
F
G
OM
TssJ
4
3
2
1
IM
H
OM
TssMct
TssJ
IM
TssA
dodecamer
TssL
I
TssMcyt
TssLc
dimer
TssA


A
C
B
D


TssK trimer
"head" domain
"neck" domain
"shoulder" domain
Ct
Nt
nanobody nbK27
surrogate of TssFG
K-binding domain


A
B
C
B
C
K
L
TagL
Hcp
ClpV
VgrG
PAAR
M
A
F
G
J
E
TssJ
TssM
(TagL)
TssL
TssK
VgrG
+PAAR
TssFG
TssE
TssBC
Hcp
TssA