Building a secreting nanomachine: a structural overview of the T3SS
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To fulfill complex biological tasks, such as locomotion and protein translocation, bacteria assemble macromolecular nanomachines. One such nanodevice, the type III secretion system (T3SS), has evolved to provide a means of transporting proteins from the bacterial cytoplasm across the periplasmic and extracellular spaces. T3SS can be broadly classified into two highly homologous families: the flagellar T3SS which drive cell motility, and the non-flagellar T3SS (NF-T3SS) that inject effector proteins into eukaryotic host cells, a trait frequently associated with virulence. Although the structures and symmetries of ancillary components of the T3SS have diversified to match requirements of different species adapted to different niches, recent genetic, molecular and structural studies demonstrate that these systems are built by arranging homologous modular protein assemblies.

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The core of both flagellar-and NF-T3SSs shows an evolutionary related architecture [1] consisting of a multi-ring basal structure embedded in both inner and outer bacterial membranes, with its proximal end connected to an export apparatus (EA) and to an ATPase complex in the cytosol. The T3SS is welded at its distal end to a needle to translocate virulence proteins directly into the host or a flagellar hook onto which polymerizes an extracellular filament dedicated to locomotion [2,3] (Figure 1a). In this review we focus on the recent breakthroughs about the supramolecular structure of NF-T3SS key subassemblies, highlighting analogies to the equivalent flagellar components, when appropriate. For clarity, we will refer to the proteins of the NF-T3SS according to their secretion and cellular translocation (Sct) abbreviation (Table 1), a unified naming system previously proposed [4].

One area in which major strides have been taken in the last two years is in the study of the needles of the NF-T3SS, the major extracellular component formed by the helical assembly of multiple copies of a single protein (SctF) in an analogous manner to the assembly of the flagellar hook and filament [5]. However, the small size of SctF (~9 kDa), combined with the natural propensity toward polymerization, has made high resolution structural studies challenging. Initial studies on the Shigella flexneri needle by negative stain EM demonstrated that it shared similar helical parameters (~5.5 subunits/turn; 4.6 Å axial rise/subunit) to the flagellar hook/filament [6]. Subsequently, X-ray crystallography and NMR [7–13] showed that SctF consisted of a conserved two helix coiled-coil fold, similar to the filament building D0 domains of the flagellar components. However, fitting of an X-ray crystallographic structure of the monomer into a 16 Å EM map produced a model for the needle in which the N-terminal helix lined the channel [7], at odds with the orientation of the D0 helices of the flagellar filament model built in a 4–5 Å cryo-EM map. Further confusion was introduced with the publication of two independent studies of the Salmonella typhimurium needle [14]. The first, using cryo-EM analyses, suggested very different helical parameters (~6.3 subunits/turn) to the S. flexneri needle and S. typhimurium flagellum. The second, using a combination of techniques, suggested that a double point mutant of S. typhimurium SctF underwent a structural rearrangement upon polymerization, whereby the C-terminal 25% of the protein went from α-helical to β-strand, again at odds with the polymerization of the flagellum [9].

Several elegant high resolution studies have now gone along way toward resolving these various inconsistencies. Early in 2012 a 7.7 Å cryo-EM map of the S. flexneri needle confirmed the flagellum-like parameters and demonstrated that 90% of the protein was α-helical [15]. Interestingly a novel, non-helical protrusion was observed and based on this the authors proposed an alternative model for the S. flexneri needle in which residues 51-65 of SctF were remodeled from the α-helix seen in the high
resolution structures to a β-hairpin structure (Figure 2a). Shortly after this, a new needle architecture was proposed for the *S. typhimurium* needle [16*]. Using a combination of solid state NMR and Rosetta modeling the authors produced a needle in which the C-terminal helix of SctF lines the needle channel, i.e., opposite to the previous models (Figure 2b). This orientation was validated by immuno-EM and was subsequently confirmed for the *S. flexneri* needle [17*]. Gratifyingly, the new model also fits well to the *S. flexneri* cryo-EM map, with the non-helical protrusion likely to be explained by the extended N-terminus (Figure 2c). These studies therefore return the NF-T3SS needle to being consistent with the flagellar filament, both in terms of helical parameters and orientation, with the highly conserved C-terminal helix lining the channel and forming the majority of the inter-subunit connections.

**Table 1**

Summary of names of homologous proteins in different type three systems including the unified, Sct naming system

| Functional name                  | Sct name | Yersinia | Shigella | *Salmonella* SPI-1 | Flagellar homologue |
|----------------------------------|----------|----------|----------|--------------------|---------------------|
| Needle filament protein          | SctF     | YscF     | MxiH     | PrgI               | –                   |
| Inner rod protein                | SctI     | YscI     | MxiI     | PrgJ               | –                   |
| OM secretin ring                 | SctC     | YscC     | MxiD     | InvG               | –                   |
| IM outer ring                    | SctD     | YscD     | MxiG     | PrgH               | –                   |
| IM inner ring                    | SctJ     | YscJ     | MxiJ     | PrgK               | FliF                |
| Minor export apparatus protein   | SctR     | YscR     | Spa24    | SpaP               | FliP                |
| Minor export apparatus protein   | SctS     | YscS     | Spa9     | SpaQ               | FliQ                |
| Minor export apparatus protein   | SctT     | YscT     | Spa29    | SpaR               | FliR                |
| Export apparatus switch protein  | SctU     | YscU     | Spa40    | SpaS               | FlihB               |
| Major export apparatus protein   | SctV     | YscV     | MxiA     | InvA               | FliA                |
| Accessory cytosolic protein      | SctK     | YscK     | MxiK     | OrgA               | FliG (?)            |
| C-ring protein                   | SctQ     | YscQ     | Spa33    | SpaO               | FliM + FliN         |
| Stator (ATPase regulator)        | SctL     | YscL     | MxiN     | OrgB               | FliH                |
| ATPase                           | SctN     | YscN     | Spa47    | InvC               | FliI                |
| Stalk                            | SctO     | YscO     | Spa13    | InvI               | FliJ                |
| Needle length regulator          | SctP     | YscP     | Spa32    | InvJ               | FliK                |

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As well as demonstrating the power of combining multiple high resolution structural techniques, this new needle model has implications for the structures of the periplasmic rod and distal tip assemblies. Structural information regarding the rod component SctJ is scant, with NMR studies showing that the monomeric protein is mostly unfolded in solution with a 15 residue stretch of α-helix in the C-terminal half [18*]. However, this combined with the homology to the needle monomer in the C-terminal helix suggests a model for the rod being assembled in much the same way, with the more variable N-terminal half decorating the outside. At the other end of the needle, despite a plethora of high resolution structures of tip protein monomers, confusion reigns over the structure of the tip assembly. A number of models have been proposed based on low resolution EM projections/maps and crystal structures [10,19,20], but they suffer from a lack of consistency even at the most basic level as to whether they display the helical symmetry of the needle or pure rotational symmetry. This is clearly another area where higher resolution information is required.

More recently, evidence from both high and low resolution structural techniques has demonstrated an overall threefold symmetry for the NF-T3SS, with an either 12-fold [21] or 15-fold [22**] outer membrane (OM) secretin ring through a 24-fold inner membrane (IM) ring [22**] to the 9-fold of the major component of the EA [23**] which is functionally coupled to the hexameric ATPase [24,25,26**]. Despite three-dimensional reconstructions from negative stain and cryo-EM, together with top views of selective disassembled basal body rings of isolated S. typhimurium and Yersinia enterocolitica T3SS, the symmetry of the OM ring remains ambiguous with genuine inter-species differences proposed as one resolution of the conflicts between different symmetric arrangements of SctG [21,22**,27]. However, the IM ring, which was previously debated as being 12-fold, 20-fold or 24-fold symmetric [28–30] has been revealed to be two concentric rings, each made of 24 subunits (the integral bitopic SctD outside and the lipided SctJ inside) (Figure 1b [22**]). Crosslinking and interface disruptive mutations also support some of the proposed symmetries and ring interactions [22**,31,32]. All three of the proteins that...
dominate the periplasmic regions are modular and contain one or more copies of two ‘ring forming motifs’ (α-β-β-α-β or β-α-β-β-α), through which the backbone of each ring is built [31,33]. More recently, the in situ tomographic reconstruction of the Y. enterocolitica T3SS, and the crystal structure of the equivalent SetD periplasmic domains [34*], seem to suggest that both the OM and IM components are capable of stretching vertically along the axis of the basal body. However the functional implications of such a mechanism are yet to be resolved. Understanding the molecular details of the symmetry mismatch in these assemblies and its effect on the formation and disruption of a web of non-covalent interactions among subunits is a crucial step to learn how the design of these nanomachines conjugates plasticity and integrity of the holostucture with its efficient assembly [35,36**].

Electron density maps also reveal that the SetD ring in the periplasmic region is less mobile than the ring formed by the SetD N-terminal cytosolic domain (SetD-N). The structure of this domain has been recently determined for several organisms [31,37,38*,39,40], revealing a forkhead-associated (FHA) fold with 4-stranded and 5-stranded β-sheets packing against each other in a globular structure that is conserved despite the low sequence identity amongst family members. However, all homologues lack the full-repertoire of highly conserved residues required for phospho-threonine binding, suggesting that any interactions with these FHA domains are occurring in a phosphorylation-independent manner. Although one study reported an interaction between S. flexneri SetD-N and phosphorylated peptides [39], other studies indeed indicate that these domains are unable to bind phosphorylated substrates and thus are likely to function as non-canonical FHA domains [31,37,38*,40].

Although a NF-T3SS C-ring has not yet been imaged, molecular evidence and sequence homology strongly suggest the existence of a structure analogous to the flagellar C-ring beneath the basal body [41]. This has been postulated to be involved in secretion substrate sorting through differential affinities for substrate–chaperone complexes [42]. As in the flagellar T3SS, the crucial role of the cytoplasmic IM ring could be to act as a scaffold in the formation of the putative NF-T3SS C-ring. Indeed, pull-down assays have indicated an interaction between S. flexneri SetD-N and the C-ring component SctQ [39,41], whilst deletion of either S. typhimurium SetD-N or SctQ results in the formation of basal bodies lacking the needle appendage [31,41], indicating that these proteins are acting at a similar point in T3SS assembly. The structural flexibility of the adjoining cytoplasmic SetD-N ring [22**] and observed dynamic nature of the flagellar C-ring [43] could explain why the NF-T3SS C-ring has thus far remained elusive in EM reconstructions.

SctQ, the essential component of this sorting platform, shows sequence homology to the two flagellar C-ring components: FliM, which is of an equivalent size, and FliN, which encompasses the C-terminal third of the protein sequence. Recently, it has been discovered that in the pathogenic NF-T3SS background an internal initiation site within the gene enables this class of proteins to be produced as two alternative translational forms [44*,45*], a full length and a shorter C-terminal variant. Although a chaperone role for the shorter variant has been proposed [45*], these proteins could also be directly equivalent to FliM and FliN respectively as integral structural components of the C-ring, suggesting that the molecular arrangement of the putative NF-C-ring could be more similar to the flagellar C-ring than previously anticipated. Indeed, structures of the C-terminal portion of NF-T3SS SctQ [44*,46] show an intertwined dimeric assembly highly similar to that already observed for FliN [47], where each protomer is made of 5 β-strands, with the first and the second strand undergoing domain swapping within the dimer. This homodimer binds to monomeric full-length SctQ to form a 1:2 complex that could be acting as the building block of the putative NF-C-ring, in contrast to the proposed 1:4 stoichiometry of the flagellar FliM-FliN building block [44*]. Furthermore, in the current model of the flagellar T3SS, the C-ring is connected to the basal body by FliG [3] and thus it is tempting to speculate that the proposed interaction between the SetD-N ring and C-ring is indirect. Given the requirement for the SctK family in C-ring localization [48], and its ability to interact with SctQ, assignment of a FliG-like role to these homologues could be hypothesized [49].

Finally, the combination of crystal structures with electron crytomography (ECT) has significantly advanced our understanding of the architecture of the T3SS EA and ATPase complex [23**]. The EA is made up of 5 integral IM proteins, with two of them (SctV and SctU) having globular C-terminal domains (SctV-C and SctU-C) protruding into the EA cytosol. It has been proposed to act as a secretion gate where substrates are gathered and uncoupled from their cognate chaperone to be routed out to the secretion path. Structurally the cytoplasmic domain of SctU is well characterized across species [50] and it is proposed to be mainly involved in export substrate selection in conjunction with SctP/FliK, a supposed molecular ruler for which structural information is only just becoming available [51]. Insights into the rest of the EA have advanced in the last few years. The structure of SctV-C is highly conserved in flagellar and non-flagellar homologues [52–55] and recently the crystal structure of the S. flexneri SctV-C demonstrated a homo-nonameric ring assembly [23**]. Crucially, not only did ring assembly mutations disrupt secretion, but variants containing mutations on the inner surface of the ring considerably affected protein export, suggesting that the route through
the SctV ring is the path toward secretion. Fitting this structure into a torus of density in ECT maps of flagellar motors [56**] has begun the process of defining the geometry of the export machinery, placing the SctV-C ring midway between the IM and the ATPase complex (Figure 3). Interestingly, the presence of the SctV-C ring is independent of the ATPase and vice-versa. The ATPase complex is made of three soluble components, SctN, SctO and SctL, and structures of SctN/FliI [24,57] and SctO/FliJ [26**,58] reveal homology with the \( \alpha/\beta \) and \( \gamma \) subunits of the \( F_0F_1 \)-ATP synthase, respectively. Although previously characterized as a general chaperone, it has now been shown that SctO/FliJ has a coiled-coil structure that inserts into the central cavity of the ATPase hexamer, in a manner directly analogous to the ATP synthase, and that it is capable of rotation within a chimeric \( V_1V_0A_1B_1J \) assembly [25]. In addition, the observed correlation between FliH length and ATPase/C-ring distance in flagellar motors [23**] combined with fluorescence data suggests that the ATPase complex is held underneath the SctV-C ring by SctL/FliH, which at its opposite end interacts with the hydrophobic patch at the dyad axis of the FliN dimer of the putative C-ring [59]. However, despite the recent progress in decoding the biophysical properties of its major components, the molecular architecture of the T3SS EA and C-ring clearly require further structural characterization in order to address mechanistic issues, such as the role of the protonotive force [60], and localization of the crucial, low copy number components.

In summary, cumulative work over the last few years has highlighted the architectural similarities between the flagellar-T3SS and NF-T3SS. Intriguingly, especially in light of the ATPase complex structures, a recent study has suggested a proton-motive force dependent rotation of the NF-T3SS needle filament, thereby drawing further parallels to the flagellum [61**] and the latest work proposing a general mechanism for flagellar export [62**] may therefore also provide insight into the NF-T3SS. Future research concerning the molecular mechanisms of assembly and secretion by these nanomachines will undoubtedly provide further insight into the extent of the diversification of T3SSs.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Abby SS, Rocha EP: The non-flagellar type III secretion system evolved from the bacterial flagellum and diversified into host-cell adapted systems. PLoS Genet 2012, 8:e1002983.
2. Buttner D: Protein export according to schedule: architecture, assembly, and regulation of type III secretion systems from plant- and animal-pathogenic bacteria. Microbiol Mol Biol Rev 2012, 76:262-310.
3. Erhardt M, Namba K, Hughes KT: Bacterial nanomachines: the flagellum and type III injectosome. Cold Spring Harb Perspect Biol 2010, 2:a000299.
4. Hueck CJ: Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev 1998, 62:379-433.
5. Blocker AJ, Deane JE, Veenendaal AK, Roversi P, Hodgkinson JL, Johnson S, Lea SM: What’s the point of the type III secretion system needle? Proc Natl Acad Sci USA 2008, 105:6507-6513.
6. Cordes FS, Korniyah K, Larquet E, Yang S, Egelman EH, Blocker A, Lea SM: Helical structure of the needle of the type III secretion system of Shigella flexneri. J Biol Chem 2003, 278:17103-17107.
7. Deane JE, Roversi P, Cordes FS, Johnson S, Kenjale R, Danieli S, Bovy F, Picking WD, Picking WL, Blocker AJ et al.: Molecular model of a type III secretion system needle: implications for host-cell sensing. Proc Natl Acad Sci U S A 2006, 103:12529-12533.

8. Zhang L, Wang Y, Picking WL, Picking WD, De Guzman RN: Solution structure of monomeric BsaL, the type III secretion needle protein of Burkholderia pseudomallei. J Mol Biol 2006, 359:322-330.

9. Poyraz O, Schmidt H, Seidel K, Delissen F, Ader C, Tenenboim H, Goosmann C, Laube B, Thunemann AF, Zychlinsky A et al.: Protein refolding is required for assembly of the type three secretion needle. Nat Struct Mol Biol 2010, 17:78-82.

10. Lunelli M, Hurwitz R, Lambers J, Kolbe M: Crystal structure of Prgl-SipD: insight into a secretion competent state of the type three secretion system needle tip and its interaction with host ligands. PLoS Pathog 2011, 7:e1002163.

11. Wang Y, Ouellette AN, Egan CW, Rathinavelan T, Im W, De Guzman RN: Differences in the electrostatic surfaces of the type III secretion needle proteins Prgl, BsaL, and MxiH. J Mol Biol 2007, 371:1304-1314.

12. Sun P, Tropea JE, Austin BP, Cherry S, Waugh DS: Structural characterization of the Yersinia pestis type III secretion needle protein YscF in complex with its heterodimeric chaperone YscE/YscG. J Mol Biol 2008, 377:819-830.

13. Guinaud M, Ple S, Job V, Contreras-Martel C, Simorre JP, Attree I, Dessain A: Structure of the heterotrimetric complex that regulates type III secretion needle formation. Proc Natl Acad Sci U S A 2007, 104:7803-7808.

14. Galkin VE, Schmied WH, Schradit O, Marlovtics TC, Egelman EH: The structure of the Salmonella typhimurium type III secretion system needle shows divergence from the flagellar system. J Mol Biol 2010, 396:1392-1397. Reports the highest resolution EM reconstruction of a NF-T3SS needle.

15. Loquet A, SGourakis NG, Gupta R, Gillet K, Riedel D, Goosmann C, Griesinger C, Kolbe M, Baker D, Becker AJ et al.: Atomic model of the type III secretion system needle. Nature 2012, 486:276-279. Uses in silico modelling to generate a model for the NF-T3SS needle topologically consistent with the flagellar models.

16. Demers JP, SGourakis NG, Gupta R, Loquet A, Gillet K, Riedel D, Laube B, Kolbe M, Baker D, Becker AJ et al.: The common structural architecture of Shigella flexneri and Salmonella typhimurium type three secretion needles. PLoS Pathog 2013, 9:e1003245. Extends the model from [16] to other species.

17. Zheng D, Lebebre M, Kaur K, McDowell MA, Gdowski C, Jo S, Wang Y, Benedict SH, Lea SM, Galen JE et al.: The Salmonella type III secretion system inner rod protein PrjG is partially folded. J Biol Chem 2012, 287:25303-25311. First structural studies of rod component and first evidence of inherent disorder in T3SS components.

18. Mueller CA, Broz P, Cornelis GR: The type III secretion system tip complex and translocon. Mol Microbiol 2008, 68:1085-1095.

19. Epler CR, Dickenson NE, Bullitt E, Picking WL: Ultrastructural analysis of IpA at the tip of the nascent MxiH type III secretion apparatus of Shigella flexneri. J Mol Biol 2012, 420:29-39.

20. Kowal J, Chami M, Ringler P, Muller SA, Kudryashov M, Castano-Diez D, Amstutz M, Cornelis GR, Stahlberg H, Engel A: Structure of the dodecameric Yersinia enterocolitica secretion YscC and its trypsin-resistant core. Structure 2013, 21:2152-2161.

21. Schradit O, Marlovtics TC: Three-dimensional model of Salmonella’s needle complex at subnanometer resolution. Science 2011, 331:1192-1195. Highest resolution imaging of the entire NF-T3SS assembly.

22. Abruini P, Vergara-Ingaray M, Johnson S, Beeby MD, Hendrixson DR, Roversi P, Friede ME, Deane JE, Jensen GJ, Tang CM et al.: Architecture of the major component of the type III secretion system export apparatus. Nat Struct Mol Biol 2013, 20:99-104. Evidence for the assembly and location of the ScvT homononameric ring.

23. Zarivach R, Vuckovic M, Deng W, Finlay BB, Strynadka NC: Structural analysis of a prototypical ATPase from the type III secretion system. Nat Struct Mol Biol 2007, 14:131-137.

24. Kishikawa J, Ibuki T, Nakamura S, Nakanishi A, Minamino T, Miyata T, Namba K, Konno H, Ueno H, Imada K et al.: Common evolutionary origin for the rotor domain of rotary ATPases and flagellar protein export apparatus. PLoS ONE 2013, 8:e64695.

25. Ibuki T, Imada K, Minamino T, Kato T, Miyata T, Namba K: Common architecture of the flagellar type III protein export apparatus and F- and V-type ATPases. Nat Struct Mol Biol 2011, 18:277-282. Identification of the gamma subunit of the T3SS ATPase.

26. Bayan N, Guilvout I, Pugliese AP: Secretins take shape. Mol Microbiol 2006, 60:1-4.

27. Hodgkinson JL, Horlsky A, Stabat D, Simon M, Johnson S, da Fonseca PC, Morris EP, Wall JS, Lea SM; Blocker AJ: Three-dimensional reconstruction of the Shigella T3SS transmembrane regions reveals 12-fold symmetry and novel features throughout. Nat Struct Mol Biol 2009, 16:477-485.

28. Marlovtics TC, Kubori T, Sukhan A, Thomas DR, Galen JE, Unger VM: Structural insights into the assembly of the type III secretion needle complex. Science 2004, 306:1040-1042.

29. Yip CK, Kimbrough TG, Felise HB, Vuckovic M, Thomas NA, Puetzten RA, Frey EA, Finlay BB, Miller SJ, Strynadka NC: Structural characterization of the molecular platform for type III secretion system assembly. Nature 2005, 435:702-707.

30. Bergeron JR, Worrall LJ, Sgourakis NG, DiMaio F, Puetzten RA, Felise HB, Vuckovic M, Yu AC, Miller SJ, Baker D et al.: A refined model of the prototypical Salmonella SPI-1 T3SS basal body reveals the molecular basis for its assembly. PLoS Pathog 2013, 9:e1003307.

31. Ross JA, Pianu GV: A C-terminal region of Yersinia pestis YscD binds the outer membrane secretin YscC. J Bacteriol 2011, 193:2276-2289.

32. Spreter T, Yip CK, Sanower S, Andre I, Kimbrough TG, Vuckovic M, Puetzten RA, Deng W, Yu AC, Finlay BB et al.: A conserved structural motif mediates formation of the periplasmic rings in the type III secretion system. Nat Struct Mol Biol 2009, 16:468-476.

33. Kudryashov M, Stenta S, Schmelz S, Amstutz M, Wiesand U, Castano-Diez D, Degiacomi MT, Munnoch S, Bleck CK, Kowal J et al.: In situ structural analysis of the Yersinia enterocolitica injectosome. eLife 2013, 2:e00792.

First tomograms of the NF-T3SS.

34. Loquet A, Habenstein B, Lange A: Structural investigations of molecular machines by solid-state NMR. Acc Chem Res 2013, 46:2070-2079.

35. Deeds EJ, Bachman JA, Fontana W: Optimizing ring assembly for the strength of weak interactions. Proc Natl Acad Sci U S A 2012, 109:2348-2353. Mathematical modelling demonstrates that weak interactions are key to correct assembly of highly symmetric homooligomers.

36. Loutos GT, Tropea JE, Waugh DS: Structure of the cytoplasmic domain of Yersinia pestis YscD, an essential component of the type III secretion system. Acta Crystallogr D Biol Crystallogr 2012, 68:201-209.

37. McDowell MA, Johnson S, Deane JE, Cheung M, Robach DD, Blocker AJ, McDonnell JM, Lea SM: Structural and functional studies on the N-terminal domain of the Shigella type III secretion protein MxiG. J Biol Chem 2011, 286:30606-30614. First structure of the cytoplasmic domain of the major component of the inner membrane ring.

38. Barnson J, Lambers J, Hurwitz R, Kolbe M: Interaction of MxiG with the cytosolic complex of the type III secretion system controls Shigella virulence. FASEB J 2012, 26:1717-1726.
A structural overview of the T3SS

40. Gamez A, Mukerjea R, Alayyoubi M, Ghassemian M, Ghosh P: Structure and interactions of the cytoplasmic domain of the Yersinia type III secretion protein YscD. J Bacteriol 2012, 194:5949-5958.

41. Morita-Ishihara T, Ogawa M, Sagara H, Yoshida M, Katayama E, Sasakawa C: Shigella Spa33 is an essential C-ring component of type III secretion machinery. J Biol Chem 2006, 281:599-607.

42. Lara-Tejero M, Kato J, Wagner S, Liu X, Galan JE: A sorting platform determines the order of protein secretion in bacterial type III systems. Science 2011, 331:1188-1191.

43. Brown MT, Delalez NJ, Armitage JP: Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor. Curr Opin Microbiol 2011, 14:734-740.

44. Bzymek KP, Hamaoka BY, Ghosh P: Two translation products of Yersinia YscQ assemble to form a complex essential to type III secretion. Biochemistry 2012, 51:1669-1677.

With [45], this study demonstrates that the NF-T3SS use alternate translation initiation to generate proteins homologous to FimF and FimN. This study also suggests a novel stoichiometry for the association of these homologues within the NF-T3SS.

45. Yu XJ, Liu M, Matthews S, Holden DW: Tandem translation generates a chaperone for the Salmonella type III secretion system protein SsaQ. J Biol Chem 2011, 286:36098-36107.

With [46], this study demonstrates that the NF-T3SS use alternate translation initiation to generate proteins homologous to FimF and FimN.

46. Fadouloglou VE, Tampakaki AP, Glykos NM, Hadden JM, Phillips SE, Panopoulos NJ, Kokkinidis M: Structure of HrcQB-C, a conserved component of the bacterial type III secretion systems. Proc Natl Acad Sci U S A 2004, 101:70-75.

47. Brown PN, Mathews MA, Joss LA, Hill CP, Blair DF: Crystal structure of the flagellar rotor protein FlmA from Thermotoga maritima. J Bacteriol 2005, 187:2890-2902.

48. Diepold A, Amstutz M, Abel S, Sorg I, Jenal U, Cornelis GR: Deciphering the assembly of the Yersinia type III secretion injectosome. EMBO J 2010, 29:1928-1940.

49. Johnson S, Blocker A: Characterization of soluble complexes of the Shigella flexneri type III secretion system ATPase. FEMS Microbiol Lett 2008, 286:274-278.

50. Deane JE, Abruscio P, Johnson S, Lea SM: Timing is everything: the regulation of type III secretion. Cell Mol Life Sci 2010, 67:1065-1075.

51. Mizuno S, Amida H, Kobayashi N, Aizawa S, Tate S: The NMR structure of FliK, the trigger for the switch of substrate specificity in the flagellar type III secretion apparatus. J Mol Biol 2011, 409:558-573.

52. Worrall LJ, Vuckovic M, Strynadka NC: Crystal structure of the C-terminal domain of the Salmonella type III secretion system export apparatus protein InvA. Protein Sci 2010, 19:1091-1096.

53. Saijo-Hamano Y, Imada K, Minamino T, Kihara M, Shimada M, Kitao A, Namba K: Structure of the cytoplasmic domain of FliA and implication for flagellar type III protein export. Mol Microbiol 2010, 76:260-268.

54. Moore SA, Jia Y: Structure of the cytoplasmic domain of the flagellar secretion apparatus protein FlhA from Helicobacter pylori. J Biol Chem 2010, 285:21060-21069.

55. Bange G, Kummerer N, Engel C, Bozkurt G, Wild K, Sinning I: FlhA provides the adaptor for coordinated delivery of late flagella building blocks to the type III secretion system. Proc Natl Acad Sci U S A 2010, 107:11295-11300.

56. Chen S, Biebuyck M, Murphy GE, Leadbetter JR, Hendrixson DR, Briegel A, Li Z, Shi J, Tocheva EI, Muller A et al.: Structural diversity of bacterial flagellar motors. EMBO J 2011, 30:2972-2981.

Survey using tomography to highlight conserved and varying structures making up the flagellar T3SS.

57. Imada K, Minamino T, Tahara A, Namba K: Structural similarity between the flagellar T3SS ATPase FliF and F1-ATPase subunits. Proc Natl Acad Sci U S A 2007, 104:485-490.

58. Lorenzini E, Singer A, Singh B, Lam R, Skarina T, Chirgadze NY, Savchenko A, Gupta RS: Structure and protein-protein interaction studies on Chlamydia trachomatis protein CT670 (YscO homolog). J Bacteriol 2010, 192:2746-2756.

59. Paul K, Harmon JG, Blair DF: Mutational analysis of the flagellar rotor protein FlmA: identification of surfaces important for flagellar assembly and switching. J Bacteriol 2006, 188:5240-5248.

60. Minamino T, Morimoto YY, Hara N, Namba K: An energy transduction mechanism used in bacterial flagellar type III protein export. Nat Commun 2011, 2:475.

61. Ohgita T, Hayashi N, Hama S, Tsuchiya H, Gotoh N, Kogure K: A novel effector secretion mechanism based on proton-motive force-dependent T3SS apparatus rotation. PASEB J 2013, 27:2862-2872.

This paper proposes that rotation of the NF-T3SS needle is required for secretion.

62. Evans LDB, Poulet S, Terentiev EM, Hughes C, Fraser GM: A chain mechanism for flagellum growth. Nature 2013, 504:287-290.

Proposes that a chaining mechanism underlies secretion through T3SS.