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Piecing together the Type III injectisome of bacterial pathogens

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The Type III secretion system is a bacterial 'injectisome' which allows Gram-negative bacteria to shuttle virulence proteins directly into the host cells they infect. This macromolecular assembly consists of more than 20 different proteins put together to collectively span three biological membranes. The recent T3SS crystal structures of the major oligomeric inner membrane ring, the helical needle filament, needle tip protein, the associated ATPase, and outer membrane pilotin together with electron microscopy reconstructions have dramatically furthered our understanding of how this protein translocator functions. The crucial details that describe how these proteins assemble into this oligomeric complex will need a hybrid of structural methodologies including EM, crystallography, and NMR to clarify the intra- and inter-molecular interactions between different structural components of the apparatus.

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Introduction

Many Gram-negative bacterial pathogens share the remarkable ability to inject bacterial virulence factors directly into their eukaryotic host cells. These effector proteins subsequently subvert eukaryotic cellular processes in a variety of ways and enable the pathogen to directly modulate their host environment, making it susceptible for invasion and infection. The key component responsible for mediating the secretion of the bacterial virulence factors into the host cell is the Type III secretion system (T3SS). It has been identified in many Gram-negative organisms including *Pseudomonas*, *Yersinia*, *Salmonella*, *EPEC* and is essential for their virulence [1].

The T3SS is a structurally and functionally conserved, complex macromolecular machine, consisting of more than 20 different proteins [2–5]. Most of these proteins are involved in constructing a macromolecular complex that spans the inner bacterial membrane, the periplasmic space, the peptidoglycan layer, the outer bacterial membrane, the extracellular space and the host cellular membrane (Figure 1). This macromolecular assembly constitutes a regulated, continuous path for effector proteins from the bacterial cytosol into the host cell.

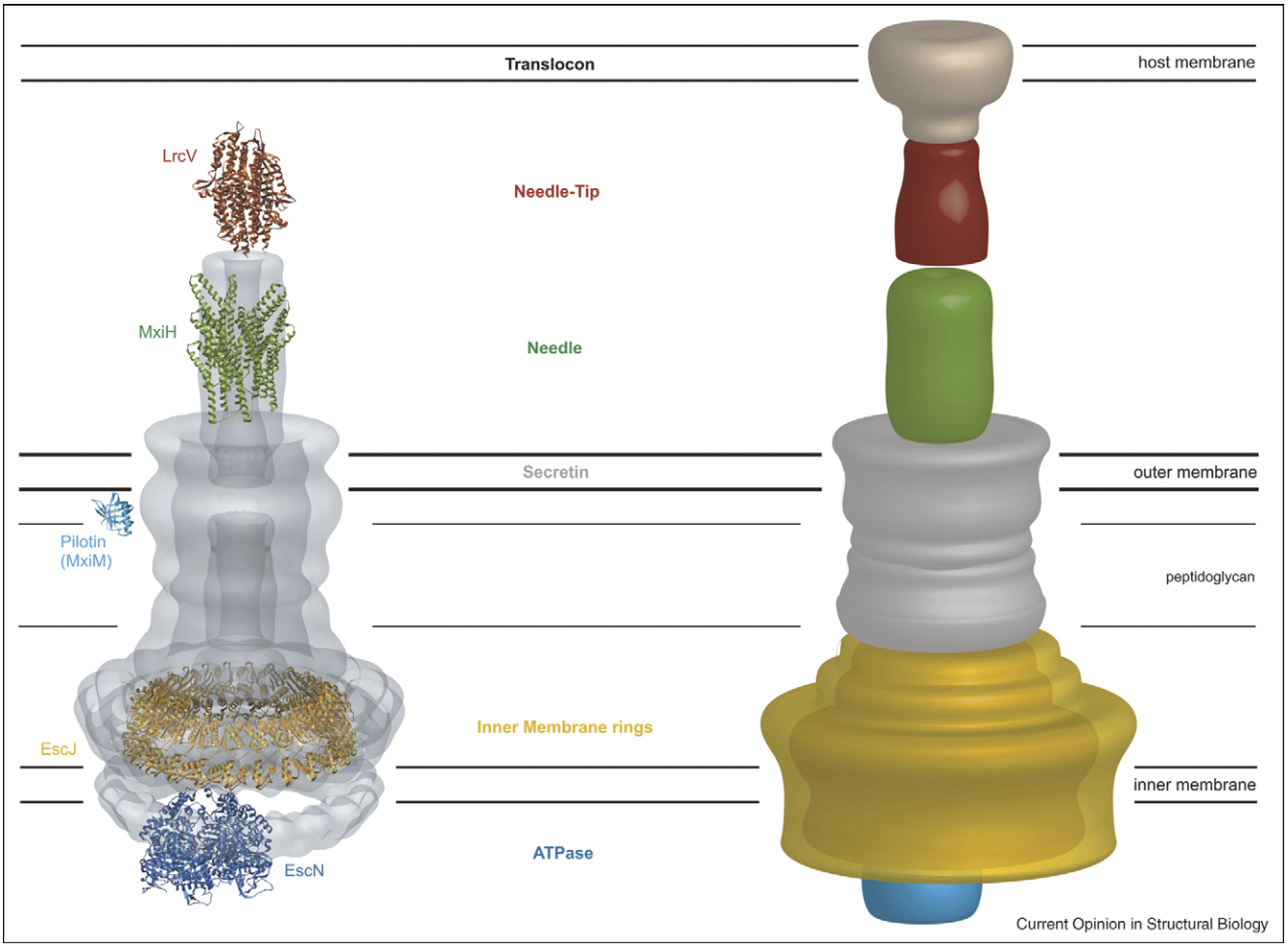
Since its first isolation in 1998, considerable progress in characterizing the T3SS apparatus has been made, but elucidation of the underlying principles of architecture and assembly of this macromolecular complex remains essential in understanding the processes of translocation and regulation. The complexity and membrane spanning nature of this sophisticated macromolecular assembly poses great challenges for structural biologists studying the molecular details of the individual components and the ultimate understanding of their individual functional roles within the context of the intact complex. In recent years, different approaches, including 3D EM reconstructions of the T3SS core at different stages of assembly and high-resolution crystal structures of some of the components, combined with molecular modeling techniques have advanced our understanding of the T3SS apparatus (Table 1).

In this review, we will highlight these recent structures and discuss their implications, in addition to a particular focus on the membrane spanning components of the needle complex.

Type III secretion system: the needle complex

The isolation of the core T3SS apparatus from native sources constituted a landmark achievement in the field and facilitated the characterization and visualization of the intact macromolecular complex [6]. 3D EM reconstructions of the purified complex revealed the so-called needle complex (NC) as the core T3SS assembly that spans both bacterial membranes and projects into the extracellular space (Figure 1) [7•,8,9]. This NC has been proposed to present a central channel, allowing passage of unfolded proteins in one step from the bacterial cytosol into the host. Utilizing cryo-EM and a combination of particle sorting and symmetry particle averaging, Marlovits *et al.* [10,11••] were able to further extend the resolution and show, that the NC consists of a cylindrical basal body and a hollow, extended needle like structure, which is incorporated into the base during assembly. Even though these EM reconstructions have provided excellent insight into the overall

Figure 1



Overall architecture of the Type III secretion apparatus. Crystal structures of known individual protein components [accession codes: LcrV(1r6f), MxiH (2v6l), MixM (1y9l), EscJ (1yj7) and EscN (2obl)] have been docked into a cryo EM Map of the *S. typhimurium* needle complex(3D EM Database accession code emd1100) alongside a model displaying the morphology of the T3SA. The predicted dimensions and locations of the three lipid bilayers and peptidoglycan layer are illustrated.

Table 1

Structurally characterized components of the T3SS

Functional component—location	Characterized homologs	Available structures	Refs.
Translocon	EspB, EspD (*EPEC*)	AFM	Ide *et al.* [40]
Needle—tip	LcrV (*Yersinia*)	X-ray	Derewenda *et al.* [33]
	IpaD (*Shigella*)	X-ray	Johnson *et al.* [32]
	BipD (*Burkholderia pseudomallei*)	X-ray	Johnson *et al.* [32]
Needle	PrgI (*S. typhimurium*)	NMR	Wang *et al.* [28]
	MxiH (*Shigella*)	X-ray	Deane *et al.* [27]
	BsaL (*Burkholderiapseudomallei*)	NMR	Zhang *et al.* [47]
Outer membrane secretin	InvG (*S. typhimurium*)	EM	Crago *et al.* [22]
	YscC (*Yersinia*)	EM	Burghout *et al.* [18]
Outer membrane pilotin	MxiM (*Shigella*)	X-ray	Lario *et al.* [24•]
Inner membrane rings	PrgK (*S. typhimurium*)	EM	Marlovits *et al.* [11••]
	EscJ (EPEC)	X-ray	Yip *et al.* [13••]
	PrgH (*S. typhimurium*)	EM	Marlovits *et al.* [11••]
ATPase	EscN (EPEC)	X-ray	Zarivach *et al.* [42•]

architecture of the T3SS, the intermediate resolution does not allow unambiguous localization of individual components and their interpretation is limited by the observed sample heterogeneity and the relevance of symmetry averaging to extend the resolution. Twenty-fold symmetry averaging of the whole NC might lead to an incomplete picture, since the NC is likely to be built up by oligomers with differing internal symmetries. Furthermore, the purified NCs lack a number of the inner membrane components that are embedded in the basal body and are essential for T3 secretion (Figure 2).

The basal body

Remarkably, the basal body of the T3SS, in which the needle like structure is anchored, consists of only three proteins. In *Salmonella typhimurium* this base is formed by the proteins PrgH and PrgK, that together form the inner membrane rings, and InvG, which is the only outer membrane component [6,7•,12].

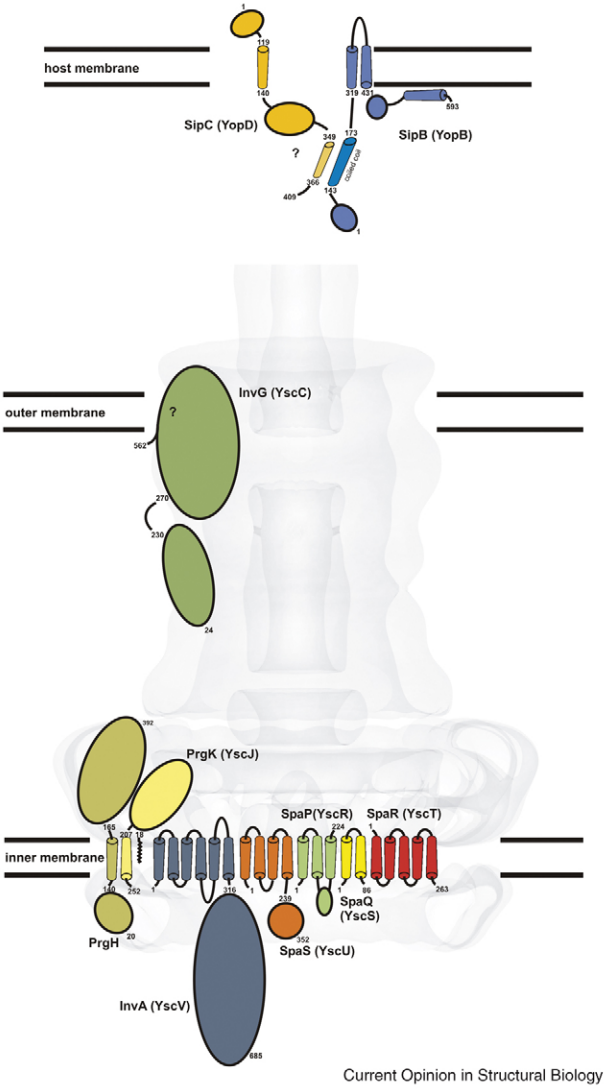
Inner membrane components

The inner membrane rings, constituted by the proteins PrgH and PrgK in *S. typhimurium*, exhibit a larger

Figure 2

Membrane Components of the T3SS:

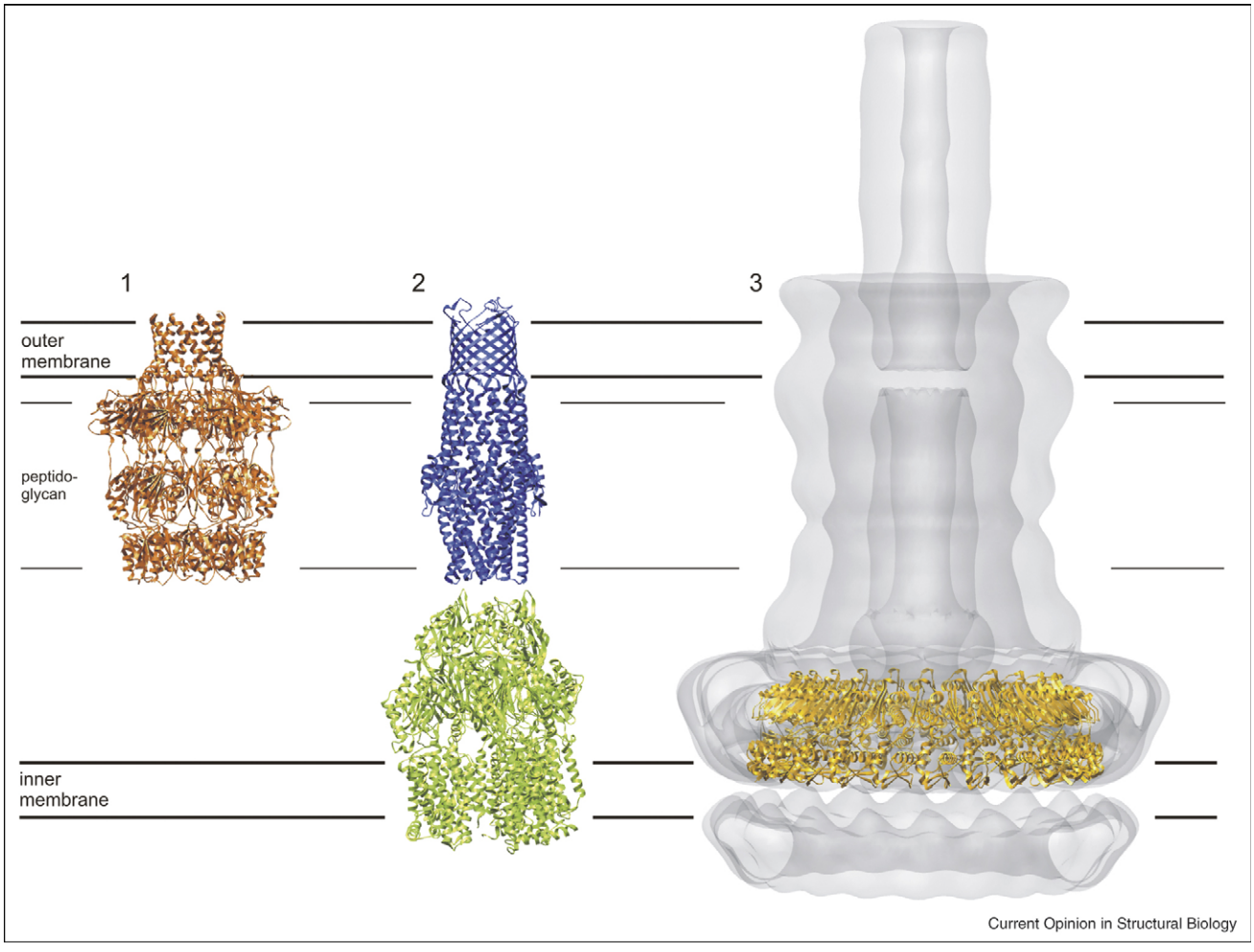
T3SS component Salmonella (Yersinia)	Flagella homolog	Localization/Function	Stoichiometry (approx.)	Assembly/ export system
Host Membrane Components:				
SipB (YopB)	-	Translocon	SipB:SipC - 6-8	T3SS
SipC (YopD)	-	Translocon	hetero-oligomers	T3SS
Outer Membrane Components:				
InvG (YscC)	-	Outer membrane ring / Secretin	12-14	Sec
Inner Membrane Components:				
PrgH	FliF	Inner membrane ring / basal body	18 - 20	Sec
PrgK (YscJ)	FliF	Inner membrane ring / basal body	24	Sec / N-terminal lipidation
SpaP (YscR)	FliP	Center of IM ring	?	?
SpaQ (YscS)	FliQ	Center of IM ring	?	?
SpaR (YscT)	FliR	Center of IM ring	?	?
SpaS (YscU)	FlhB	Center of IM ring / substrate specificity switch	?	?
InvA (YscV)	FlhA	Center of IM ring	?	?



Intergral membrane components of the T3SS. The table on the left side summarises the intergral membrane proteins of the T3SS, including the components in the inner and outer bacterial membrane and the translocon, which inserts into the host membrane. The illustration on the right shows a schematic diagram of the membrane proteins and their proposed localization, highlighting the transmembrane segments. The dimensions of the individual domains represent only a rough estimate to exemplify the overall architecture.

diameter than the outer membrane component. The protein PrgK belongs to the highly conserved YscJ family of periplasmic lipoproteins, which are thought to be anchored to the outer leaflet of the inner membrane by N-terminal lipidation and for many of the members in addition by a C-terminal transmembrane helix [7•,13••]. The atomic structure of EscJ, the YscC homolog from EPEC, has recently been solved [13••,14]. Intriguingly, in the crystal structure, individual molecules pack into a superhelical assembly with 24 subunits, indicating that EscJ might form corresponding symmetrical ring structures in the T3SS basal body. The proposed EscJ ring model has been supported by molecular modeling studies, confirming a symmetrical 24-subunit EscJ ring as a likely model [15]. This symmetrical 24 subunit EscJ model has similar overall dimensions to the IM rings, as visualized by EM and can be docked into the available EM map of the T3SS basal body from the homologous and highly conserved *S. typhimurium* (Figure 1). This appealing ring model is in agreement with biochemical data, since all of the typically C-terminal transmembrane anchors and N-terminal lipidation sites align in the same direction towards the membrane, allowing symmetrical anchoring of the complete ring to the inner membrane (Figure 3). The position of the model, lining the inside of the IM rings, is also supported by biotinylation data on the intact NC, indicating that the EscJ homolog PrgK is only partially accessible from the outside [13••]. In the currently proposed model, the periplasmic YscJ proteins form a 24 subunit symmetrical ring-structure, that is anchored to the outer leaflet of the IM, occupying the inner part of the upper IM ring as illustrated in Figures 2 and 3. In this localization within the T3SS, the PrgK ring might function as an initiator of NC assembly and present

Figure 3



Comparison of the T3SS NC to the T1SS (Type I Secretion System) and Wza, the translocon for capsular polysaccharides. **(1)** Crystal structure of the octameric 340 kDa outer membrane protein Wza, the *E. coli* translocon for capsular polysaccharide export (pdb accession code 2j58). **(2)** Crystal structures and proposed orientation of the T1SS components TolC and AcrB (pdb accession codes 1ek9 and 1iwg). **(3)** Proposed orientation of the T3SS NC. Shown are the 3D EM reconstruction of the NC (3D EM database code emd1100) and the docked EscJ ring model (pdb accession code 1yj7). Indicated are the positions of the outer and inner bacterial membrane and the approximate dimensions of the peptidoglycan layer.

a specialized membrane patch for recruiting other putative inner membrane embedded components of the T3SS (Figure 2).

In *S. typhimurium*, the second protein, which together with PrgK, constitutes the inner membrane rings is PrgH. The exact stoichiometry of PrgK:PrgH:InvG in the basal body is still a matter of debate, but it has been proposed to be close to 1.7:1.3:1.0 or 22 ± 1.7:17.4 ± 1.4:13 ± 1, assuming InvG with 12–14 subunits as reference [11••,13••]. Recent single particle cryo-EM studies using symmetry averaging have suggested a variable 19–21-fold symmetry at the inner membrane rings of the NC, with the majority of purified NCs having 20-fold symmetry [11••]. It has to be noted though, that 20-fold symmetry averaging over the whole NC will most likely not correctly reflect the components that do not follow 20-fold symmetry, like PrgK, InvG and needle subunits. Nevertheless, the 20-fold averaged EM map shows clear separation of individual subunits on the outside of the inner membrane rings, implicating an approximate 20-fold symmetry of this particular part of the inner membrane rings. Interestingly this correlates with the proposed stoichiometry and localization for PrgH and the hypothesis that the PrgK ring is encompassed by a PrgH oligomer [13••].

Taken together these studies present an initial hypothesis for the architecture of the IM rings of the T3SS apparatus. Continuative biochemical and structural analysis will be important to allow for more homogeneous and complete NC preparations, to confirm the stoichiometry of the subunits and to unambiguously determine the localization of the individual components and their symmetries.

Outer membrane component

The outer membrane component of the T3SS belongs to the secretin family of outer membrane proteins. The secretins are a group of unique integral membrane proteins that assemble into homomultimeric ring structures with large central pores and that function as the outer membrane component of a variety of multicomponent export systems, including Type III secretion, Type II secretion, Type IV pilus biogenesis and filamentous phage release. Secretins from different systems have been studied by low resolution EM, all showing a remarkably similar overall structure and organization, with 12–14 secretin subunits assembling into a homomultimeric pore structure, that crosses the outer membrane and protrudes into the periplasm layer [16–19,20••]. All secretins consist of two major domains: a C-terminal 'homology domain' that is embedded in the outer membrane and an N-terminal periplasmic domain [21]. Comparison of cryo-EM reconstructions of the full-length secretin PulD to its C-terminal domain only, revealed the approximate localization and dimensions of both domains within the overall low-resolution structure [20••]. The N-terminal periplasmic domain is only partially conserved among secretins of different export systems, but is highly conserved within each system and is thought to function in recognition and binding of other periplasmic or inner membrane components.

The outer membrane secretin requires, for efficient localization and assembly, the assistance of a specific lipoprotein called pilotin [18,22,23]. The recent crystal structure of MxiM, the pilotin from *Shigella*, has shed more light on how this protein might assist in proper insertion and assembly of the secretin [24•]. The pilotin binds the C-terminal helix of the secretin displacing a lipid-filled pocket allowing the pilotin to mediate the localization of the secretin to the outer membrane [24•]. In the absence of its pilotin (PulS), the Type 2 secretion system secretin PulD assembles in the inner bacterial membrane suggesting a targeting function for the pilotin. Furthermore in the presence of its pilotin, PulD does not require the essential outer membrane assembly factor YaeT for proper multimerization into the outer membrane [25•]. Taken together this suggests that the secretins are properly assembled and embedded independent of the classical, β-barrel OMP pathway and may be more similar in structure to the α-helical *Escherichia coli* Wza protein involved in capsular polysaccharide secretion [26•].

Extracellular components

The extracellular components of the T3SS consists of three distinct parts: the needle, a helical polymer made of a few hundred subunits of the YscF family; the needle extension, that forms a bell shaped cap or an extension depending on the bacterial species; and the translocation pore that consists of two membrane spanning proteins related to yopB and yopD family that hetero-oligomerize to create a channel in the host eukaryotic membrane [5].

The needle

Although its polymerized nature has precluded direct crystallographic analysis of the needle itself, the crystal structure of the monomeric MxiH [27•] and NMR models of monomeric BsaL (*Burkholderia pseudomallei*) and PrgI (*S. typhimurium*) [28] have provided recent insights into the atomic resolution structure of significant regions of the needle 'building block'. All the needle component structures share a two-helix bundle coiled–coiled motif that is linked by a conserved PxxP turn. This coiled–coil motif was subsequently docked into ~16 Å EM reconstructions of intact *Shigella* needles [29] providing the first atomic predictions of the intermolecular interactions that facilitate the helical needle architecture (Figure 1). Interestingly, the mutations in MxiH that lead to a constitutive secretion state or serve as defects in hemolysis or invasion [30] all map to a common region of the needle—within the contact surface between the head of one subunit in the polymer and the tail of the monomer packing above it. This corroborates the notion that the sensing feature of the needle is transmitted through the filament by mol-

ecular contacts between subunits thereby regulating secretion.

Tip/needle extension

Tip proteins are thought to orchestrate the correct insertion of the translocon, linking the needle to the eukaryotic membrane. The docking of MxiH into the EM reconstruction provided a platform for modeling the needle and tip proteins. By superimposing the C-terminal helices of MxiH with LcrV helices, five molecules of LcrV could be placed at the tip of MxiH polymer needle [27•] (Figure 1). This bell shaped complex at the tip of the *Yersinia pestis* needle has been shown to be LcrV [31] and recently NMR chemical shift mapping between MxiH and IpaD has further corroborated this interface [48]. Several structures of tip proteins (IpaD and BipD [32]) are now available to compare with the initial LcrV [33] and EspA [34•] structures. High-resolution structures of both IpaD and BipD from *Shigella flexneri* and *Burkholderia pseudomallei* have recently been elucidated [32,35], illustrating that they share a central coiled–coil domain with both LcrV and EspA. Both the N and C terminal domains vary to give specificity for the needle and translocon that they interact with. Of interest are the alpha helical coiled–coil structure and the N-terminal four helix domain of BipD and IpaD that mimics the T3SS chaperones with structural homology to the chaperone FliS that prevents premature association and oligomerization of secreted proteins.

The equivalent tip protein in EPEC, EspA, is bound to its chaperone CesA before being secreted through the needle complex. This feature was exploited to trap a monomeric and homogeneous form of the protein for crystallography analysis [34•]. Upon eruption from the needle, EspA forms a distinct and highly extended filament extension on the end of the EscF-needle, likely an adaptation to the required attachment of EPEC to host gut epithelial cells. Approximately 15 Å EM real space reconstruction of EspA filaments illustrates that the filament is polymorphic in its length, despite having a fixed twist of 5.6 subunits per turn, the axial rise varies between 3.6 and 5.8 Å [36•]. The mechanism to transmit a sensing signal from the tip of the extracellular domain though to the needle and then to the base may employ this method of polymorphic axial rise (although mutant needles that are constitutively active have the same average axial rise as the wildtype protein [37]). We await more atomic resolution details of EspA in an oligomeric form to further probe the details of this tip structure.

Translocon

The construction of the tip of the needle and its interaction with the membrane spanning translocon was recently addressed with STEM and quantitative immunobloting inferring that the tip of the needle structure is homo-pentameric [38•]. Because of species variation, it remains unclear weather the tip of the needle is a homopentamer [38•] or a heteropentameric where one of the tip protein IpaD molecules is replaced by a translocon protein such as IpaB. The heteropentameric complex of IpaD:IpaB(4:1) may serve to plug the end of the needle preventing the premature loss of translocon and effector proteins. Indeed there is evidence to demonstrate the regions of IpaD and IpaB are needed to form a functional needle complex [39].

What does this mean for the translocon's architecture and oligomerization? YopB (one of the *Yersinia* translocon proteins) interacts with LcrV (using its N-terminal domain) and this interaction is required for proper insertion of YopB into the host membrane, but the oligomerization state of the pore and the stiochiometry remains unclear. To date, there is only one structural report describing EM and AFM of the EPEC translocon embedded in a membrane (EspB and EspD within infected sheep red blood cells) suggesting a non-symetrical 6–8 subunit hetero-oligomeric stochiometry [40]. The predicted topology of the translocon proteins indicate key features including coiled–coiled domains, a single transmembrane domain in the SipC homologues and two transmembrane domains, coiled–coil and an amphipathic helix in the SipB homologues. The orientation of these two proteins with respect to the membrane is based on the interactions of the predicted coiled–coil domains with the needle and tip proteins (Figure 3). Obviously a huge void in determining how proteins are translocated across the eukaryotic host membrane is the lack of structural detail of any component of the translocation pore itself.

Regulation of assembly and translocation

The assembly of the T3SS is accomplished in a highly regulated stepwise manner, in which the formation of the basal body precedes the insertion of the needle [12,41]. The initial step is the separate assembly of the inner and outer membrane rings. How the outer and inner membrane components connect to form the basal body is still an unanswered question. After completion of the core basal body, the inner membrane rings are thought to provide a platform for localization of the essential inner membrane embedded proteins YscR, YscS, YscT, YscU, YscV (Figure 3) and associated ATPase.

ATPase

The conserved and essential ATPase is an inner-membrane component of the T3SS that serves to energize the export function and is involved in the initial stages of selective secretion. The recent structure of the EPEC ATPase, EscN [42•], and the flagellar ATPase, FliI [43], illustrates their structural homology to the F1 ATPase rather than the AAA ATPase disassembly apparatus that had once been inferred [44]. The monomeric structures are composed of three functional domains; an N-terminal six stranded β-barrel which may aid in the overall hex-

americ formation; an ATPase domain with the typical nucleotide binding α/β Rossman fold, and a walker A and B motif; and finally a C-terminal domain which has less structural homology to the F1-ATPase. The variant C-terminal domain is of interest as it may provide a docking interface for chaperone/effector complexes as first proposed in InvC through analysis of C-terminal mutants [44,45]. In comparison to the F1-ATPase, helices within the C-terminal domain of EscN are truncated or missing and could be complimented by helices present within the helical chaperones such as those present in CesT or SycN—suggested by Zarivach *et al.* [42•]. We believe that EscN sits as a hexamer in the C-ring aside from the main T3SS pore and that the ATPases' central cavity is filled by a stator that links it to the base of the needle complex. A modeled form of the hexameric ATPase (as verified by mutational and docking analysis) might be docked into the recently described ~16 Å resolution cyro-EM of the double hexameric ring formed by HrcN [46], once the EM maps are made available. Linking this structure to membrane is also a critical venture in order to clearly dictate how a stator like protein and/or an effector chaperone would associate. This would finalize debate over how EscN functions to disassemble and unfold effector–chaperone complexes and pipe them through the needle complex.

Conclusions

Over the past couple of years additional atomic details of the T3SS have been placed into the topology presented by the EM reconstructions. Still much more information needs to be assembled in order to understand how this macromolecular machine functions to direct virulence factors across three membranes. In addition to the high-resolution structures provided by NMR and crystallography and better isolation methods of needle complexes yielding more complete and hopefully even higher resolution EM maps, further biochemical methods need to be employed to identify protein-protein interactions and binding sites used to piece together this molecular apparatus. Together with molecular docking strategies an overall structural representation can then be assembled and the answers to how this injectisome functions as a macromolecular machine can be fully addressed.

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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. Coburn B, Sekirov I, Finlay BB: **Type III secretion systems and disease**. *Clin Microbiol Rev* 2007, **20**:535-549.

2. Cornelis GR: **The type III secretion injectisome**. *Nat Rev Microbiol* 2006, **4**:811-825.

3. Galán JE, Wolf-Watz H: **Protein delivery into eukaryotic cells by type III secretion machines**. *Nature* 2006, **444**:567-573.

4. Ghosh P: **Process of protein transport by the type III secretion system**. *Microbiol Mol Biol Rev* 2004, **68**:771-795.

5. Yip CK, Strynadka NC: **New structural insights into the bacterial type III secretion system**. *Trends Biochem Sci* 2006, **31**:223-230.

6. Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galan JE, Aizawa SI: **Supramolecular structure of the Salmonella typhimurium type III protein secretion system**. *Science* 1998, **280**:602-605.

7. • Blocker A, Jouihri N, Larquet E, Gounon P, Ebel F, Parsot C, Sansonetti P, Allaoui A: **Structure and composition of the Shigella flexneri 'needle complex', a part of its type III secreton**. *Mol Microbiol* 2001, **39**:652-663.
This paper describes 3D EM reconstructions of the *Shigella flexneri* Needle Complexes, visualized by low-dose EM of negatively stained samples. The resulting EM map shows a central channel of ~20–30 Å that extends from the inner membrane rings to the tip of the extracellular needle.

8. Sekiya K, Ohishi M, Ogino T, Tamano K, Sasakawa C, Abe A: **Supermolecular structure of the enteropathogenic Escherichia coli type III secretion system and its direct interaction with the EspA-sheath-like structure**. *Proc Natl Acad Sci U S A* 2001, **98**:11638-11643.

9. Tamano K, Aizawa S, Katayama E, Nonaka T, Imajoh-Ohmi S, Kuwae A, Nagai S, Sasakawa C: **Supramolecular structure of the Shigella type III secretion machinery: the needle part is changeable in length and essential for delivery of effectors**. *EMBO J* 2000, **19**:3876-3887.

10. Marlovits TC, Kubori T, Lara-Tejero M, Thomas D, Unger VM, Galan JE: **Assembly of the inner rod determines needle length in the type III secretion injectisome**. *Nature* 2006, **441**:637-640.

11. •• Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galan JE, Unger VM: **Structural insights into the assembly of the type III secretion needle complex**. *Science* 2004, **306**:1040-1042.
This article describes single particle cryo-EM studies of both the NC and the basal body from *S. typhimurium*. The EM reconstructions show differences between the assembled NC and the basal body in the interior of the complex, allowing further insight into the composition and assembly of the NC. Using a combination of particle sorting and 20-fold symmetry averaging the resolution of the EM maps could be extended to 17 Å. The approach of 20-fold averaging of the whole complex to extend the resolution is questionable though, since not all of the components of the NC have 20-fold symmetry and might therefore be underrepresented.

12. Kimbrough TG, Miller SI: **Contribution of Salmonella typhimurium type III secretion components to needle complex formation**. *Proc Natl Acad Sci U S A* 2000, **97**:11008-11013.

13. •• Yip CK, Kimbrough TG, Felise HB, Vuckovic M, Thomas NA, Pfuetzner RA, Frey EA, Finlay BB, Miller SI, Strynadka NC: **Structural characterization of the molecular platform for type III secretion system assembly**. *Nature* 2005, **435**:702-707.
This research paper describes the crystal structure of the protein EscJ, a member of the YscJ family. The crystal packing indicates that EscJ could form a 24-subunit ring structure, that is anchored to the outer leaflet of the inner membrane, lining the interior of the inner membrane rings of the NC. The hypothesis is supported by EM, labelling and mass spectrometry data analysing the stoichiometry, surface accessibiliy and membrane association of the homolog PrgK.

14. Crepin VF, Prasannan S, Shaw RK, Wilson RK, Creasey E, Abe CM, Knutton S, Frankel G, Matthews S: **Structural and functional studies of the enteropathogenic Escherichia coli type III needle complex protein EscJ**. *Mol Microbiol* 2005, **55**:1658-1670.

15. André I, Bradley P, Wang C, Baker D: **Prediction of the structure of symmetrical protein assemblies**. *Proc Natl Acad Sci U S A* 2007, **104**:17656-17661.

16. Collins RF, Frye SA, Kitmitto A, Ford RC, Tonjum T, Derrick JP: **Structure of the Neisseria meningitidis outer membrane PilQ secretin complex at 12 Å resolution**. *J Biol Chem* 2004, **279**:39750-39756.

17. Bitter W: **Secretins of Pseudomonas aeruginosa: large holes in the outer membrane**. *Arch Microbiol* 2003, **179**: 307-314.

18. Burghout P, van Boxtel R, Van Gelder P, Ringler P, Muller SA, Tommassen J, Koster M: **Structure and electrophysiological properties of the YscC secretin from the type III secretion system of Yersinia enterocolitica**. *J Bacteriol* 2004, **186**:4645-4654.

19. Opalka N, Beckmann R, Boisset N, Simon MN, Russel M, Darst SA: **Structure of the filamentous phage pIV multimer by cryo-electron microscopy**. *J Mol Biol* 2003, **325**:461-470.

20. •• Chami M, Guilvout I, Gregorini M, Remigy HW, Muller SA, Valerio M, Engel A, Pugsley AP, Bayan N: **Structural insights into the secretin PulD and its trypsin-resistant core**. *J Biol Chem* 2005, **280**:37732-37741.
This article illustrates the intermediate resolution 3D EM reconstructions of the T2SS secretin PulD in its full-length and proteolized form. Comparison of the EM maps reveals the overall dimensions and localization of both the C-terminal homology domain, sharded among all secretins and the N-terminal domain.

21. Genin S, Boucher CA: **A superfamily of proteins involved in different secretion pathways in gram-negative bacteria: modular structure and specificity of the N-terminal domain**. *Mol Gen Genet* 1994, **243**:112-118.

22. Crago AM, Koronakis V: **Salmonella InvG forms a ring-like multimer that requires the InvH lipoprotein for outer membrane localization**. *Mol Microbiol* 1998, **30**:47-56.

23. Daefler S, Russel M: **The Salmonella typhimurium InvH protein is an outer membrane lipoprotein required for the proper localization of InvG**. *Mol Microbiol* 1998, **28**: 1367-1380.

24. • Lario PI, Pfuetzner RA, Frey EA, Creagh L, Haynes C, Maurelli AT, Strynadka NC: **Structure and biochemical analysis of a secretin pilot protein**. *EMBO J* 2005, **24**:1111-1121.
This paper presents the first atomic structure of a secretin pilot protein. A deep hydrophobic cavity within the structure of MxiM provides a specific binding domain for the acyl chains of bacterial lipids. Isothermal titration analysis shows that the C-terminal domain of the secretin, MxiD525-570, hinders lipid binding to MxiM suggesting a mechanism for binding and localizing the secretin.

25. • Collin S, Guilvout I, Chami M, Pugsley AP: **YaeT-independent multimerization and outer membrane association of secretin PulD**. *Mol Microbiol* 2007, **64**:1350-1357.
See Ref. [26•].

26. • Guilvout I, Chami M, Engel A, Pugsley AP, Bayan N: **Bacterial outer membrane secretin PulD assembles and inserts into the inner membrane in the absence of its pilotin**. *EMBO J* 2006, **25**:5241-5249.
In this paper and the previous article, the localization and targeting for the type II secretin is addressed. The two papers describe the mislocalization and multimerization of the secretin into the inner membrane in the absence of its pilotin. The papers also describe that the functional secretin is assembled in the outer membrane independent of the YaeT OMP folding pathway.

27. • Deane JE, Roversi P, Cordes FS, Johnson S, Kenjale R, Daniell S, Booy 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.
This paper reports the crystal structure of the *Shigella flexneri* needle subunit MxiH and a model for the needle assembly built into a three-dimensional EM reconstruction of the needle. The model, combined with mutagenesis data, reveals that signaling of host-cell contact can be relayed through the needle via intersubunit contacts and suggests a mode of binding for the tip complex.

28. 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 PrgI, BsaL, and MxiH**. *J Mol Biol* 2007, **371**:1304-1314.

29. Cordes FS, Komoriya 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.

30. Kenjale R, Wilson J, Zenk SF, Saurya S, Picking WL, Picking WD, Blocker A: **The needle component of the type III secreton of Shigella regulates the activity of the secretion apparatus**. *J Biol Chem* 2005, **280**:42929-42937.

31. Mueller CA, Broz P, Müller SA, Ringler P, Erne-Brand F, Sorg I, Kuhn M, Engel A, Cornelis GR: **The V-antigen of Yersinia forms a distinct structure at the tip of injectisome needles**. *Science* 2005, **310**:674-676.

32. Johnson S, Roversi P, Espina M, Olive A, Deane JE, Birket S, Field T, Picking WD, Blocker AJ, Galyov EE *et al.*: **Self-chaperoning of the type III secretion system needle tip proteins IpaD and BipD**. *J Biol Chem* 2007, **282**:4035-4044.

33. Derewenda U, Mateja A, Devedjiev Y, Routzahn KM, Evdokimov AG, Derewenda ZS, Waugh DS: **The structure of Yersinia pestis V-antigen, an essential virulence factor and mediator of immunity against plague**. *Structure* 2004, **12**:301-306.

34. • Yip CK, Finlay BB, Strynadka NC: **Structural characterization of a type III secretion system filament protein in complex with its chaperone**. *Nat Struct Mol Biol* 2005, **12**:75-81.
This article demonstrates that EspA alone is sufficient to form filamentous structures and that CesA traps EspA in a monomeric state and inhibits its polymerization. Crystallographic analysis of the heterodimeric CesA-EspA complex reveals that EspA contains two long α-helices, which are involved in extensive coiled–coil interactions with CesA.

35. Erskine PT, Knight MJ, Ruaux A, Mikolajek H, Wong Fat Sang N, Withers J, Gill R, Wood SP, Wood M, Fox GC *et al.*: **High resolution structure of BipD: an invasion protein associated with the type III secretion system of Burkholderia pseudomallei**. *J Mol Biol* 2006, **363**:125-136.

36. • Wang YA, Yu X, Yip C, Strynadka NC, Egelman EH: **Structural polymorphism in bacterial EspA filaments revealed by cryo-EM and an improved approach to helical reconstruction**. *Structure* 2006, **14**:1189-1196.
In this paper cryo-EM of frozen-hydrated filaments of EspA demonstrate that these filaments have a fixed twist with 5.6 subunits per turn but an axial rise per subunit that varies from about 3.6–5.6 Å. The polymorphisms in the needle extension could be a mechanism for transmitting signals upon contacting host cell membrane through needle.

37. Cordes FS, Daniell S, Kenjale R, Saurya S, Picking WL, Picking WD, Booy F, Lea SM, Blocker A: **Helical packing of needles from functionally altered Shigella type III secretion systems**. *J Mol Biol* 2005, **354**:206-211.

38. • Broz P, Mueller CA, Müller SA, Philippsen A, Sorg I, Engel A, Cornelis GR: **Function and molecular architecture of the Yersinia injectisome tip complex**. *Mol Microbiol* 2007, **65**:1311-1320.
In this article, STEM and quantitative immunoblotting were used to analyze the molecular architecture of the tip protein LcrV showing that it caps the needle as a pentamer. Further tip proteins chimeras defined the base of the tip complex as critical for the functional insertion of translocation into the host cell membrane.

39. Veenendaal AK, Hodgkinson JL, Schwarzer L, Stabat D, Zenk SF, Blocker AJ: **The type III secretion system needle tip complex mediates host cell sensing and translocon insertion**. *Mol Microbiol* 2007, **63**:1719-1730.

40. Ide T, Laarmann S, Greune L, Schillers H, Oberleithner H, Schmidt MA: **Characterization of translocation pores inserted into plasma membranes by type III-secreted Esp proteins of enteropathogenic *Escherichia coli***. *Cell Microbiol* 2001, **3**:669-679.

41. Kubori T, Sukhan A, Aizawa SI, Galan JE: **Molecular characterization and assembly of the needle complex of the Salmonella typhimurium type III protein secretion system**. *Proc Natl Acad Sci U S A* 2000, **97**:10225-10230.

42. • 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.
This is the first high-resolution structural report of the type III secretion ATPase and demonstrates structural homology to the F1-ATPase family. Both *in vivo* and *in vitro* functional studies demonstrate the necessity of the

oligomeric form of the protein for function. The structure also suggests a binding site for chaperone/effector complexes that need to be dissociated by the ATPase before being translocated through the basal body.

43. Imada K, Minamino T, Tahara A, Namba K: **Structural similarity between the flagellar type III ATPase FliI and F1-ATPase subunits**. *Proc Natl Acad Sci U S A* 2007, **104**:485-490.

44. Akeda Y, Galán JE: **Chaperone release and unfolding of substrates in type III secretion**. *Nature* 2005, **437**:911-915.

45. Akeda Y, Galán JE: **Genetic analysis of the Salmonella enterica type III secretion-associated ATPase InvC defines discrete functional domains**. *J Bacteriol* 2004, **186**:2402-2412.

46. Müller SA, Pozidis C, Stone R, Meesters C, Chami M, Engel A, Economou A, Stahlberg H: **Double hexameric ring assembly of the type III protein translocase ATPase HrcN**. *Mol Microbiol* 2006, **61**:119-125.

47. 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.

48. Zhang L, Wang Y, Olive AJ, Smith ND, Picking WD, De Guzman RN, Picking WL: **Identification of the MxiH needle protein residues responsible for anchoring invasion plasmid antigen D to the type III secretion needle tip**. *J Biol Chem* 2007, **282**:32144-32151.