Published in final edited form as:

Mol Biosyst. 2008 December ; 4(12): 1176–1180. doi:10.1039/b808271p.

Structural Dissection of the Extracellular Moieties of the Type III Secretion Apparatus

Yu Wang^{1,#}, Lingling Zhang^{1,#}, Wendy L. Picking², William D. Picking^{1,2}, and Roberto N. De Guzman^{1,*}

¹Department of Molecular Biosciences, The University of Kansas, Lawrence, KS 66045

²The Higuchi Biosciences Center, The University of Kansas, Lawrence, KS 66045

Summary

Many Gram-negative bacterial pathogens use type III secretion systems (TTSSs) for subverting the normal cellular functions of their target eukaryotic cells. The type III secretion apparatus (TTSA) functions like a syringe to inject proteins through an external needle and into a target cell's membrane and cytosol. The TTSA basal body spans the bacterial inner and outer membranes, and the external needle is topped with a tip complex that controls the secretion and delivery of translocator and effector proteins. Recent structures of TTSA proteins have greatly advanced our understanding of shared themes in apparatus assembly and function. In this review, the structure-function of TTSA needle and tip complex proteins are described and common themes discussed.

Introduction

A common critical virulence factor for numerous Gram-negative bacterial pathogens of animals and plants is the type III secretion system (TTSS).^{1, 2} Among these are human pathogens such as enteropathogenic *Escherichia coli* (EPEC), *Salmonella* spp, *Yersinia* spp., *Shigella* spp., *Burkholderia* spp., and *Pseudomonas aeruginosa*. Illnesses caused by these pathogens range from opportunistic lung infections by *P. aeruginosa* to gastroenteritis caused by *Salmonella typhimurium*, *Shigella* and *Yersinia enterocolitica* to overwhelming systemic infections by *Yersinia pestis*, *Salmonella typhi*, and *Burkholderia pseudomallei*. In all cases, the TTSS contributes to infection by allowing prokaryote communication with eukaryotic cells. The outcome of this interspecies communication varies with pathogen and host cell type. The TTSA is a complex, protein-secretion nanomachine used to deliver bacterial effector proteins directly into a target eukaryotic hosts' membrane and cytosol. These effectors then display a variety of pathogen-specific roles that alter normal host cell regulatory events. Although effectors diverge from pathogen to pathogen, the TTSA, which is the multi-protein assembly for this translocating process, is structurally and functionally conserved.^{3, 4}

The external components of the TTSA

By 1992, it was recognized that Gram-negative bacteria use a unique secretion mechanism to deliver virulence factors directly into eukaryotic cells.^{1, 2, 5–8} Subsequently, the type III secretion apparatus (TTSA), which is a multi-protein assembly that resembles a syringe and needle (Figure 1), was visualized by transmission electron microscopy.⁹ The TTSA is

*Address correspondence to: rdguzman@ku.edu, Phone: (785) 864 4023.

#These authors contributed equally.

assembled from more than 20 different proteins, most of which are associated with the bacterial inner membrane which anchors the apparatus and provides the energy needed for the translocation of later structural units, translocator proteins and effectors.¹⁰ The entire basal body spans the bacterial inner and outer membranes with the three most abundant components forming the inner and outer membrane rings.¹¹ The extracellular portion of the TTSA consists of a needle and a tip complex that controls secretion. The needle is typically 50 to 60 nm in length, with a 7 nm outer diameter and 2 to 3 nm inner diameter.¹² Computer reconstruction of low-resolution electron microscopy images from the needle of *Shigella flexneri* shows that the needle monomers polymerize in a helical fashion, with 5.6 subunits per turn and 24-Å helical pitch.^{12, 13}

The complex at the tip of the TTSA needle was only recently described.¹⁴ LcrV, also called the V-antigen of *Yersinia* spp., was found located on top of *Y. enterocolitica* needle to form a tip complex.¹⁴ Shortly thereafter, IpaD of *S. flexneri* was also found to localize at the tip of the needle prior to induction of secretion.¹⁵ Although the precise sensing and triggering mechanisms for induction are unknown, it is clear that direct contact between the TTSA and some component in the host cell membrane leads to induction of TTS *in vivo*. In *Shigella*, host cell contact leads to the secretion of two translocator proteins, IpaB and IpaC, that are inserted into the host cell membrane where they form a pore-like structure called the translocon. Subsequently, effector proteins are then injected directly into the host cell cytosol through the needle and translocon pore. In *Shigella*, IpaD was shown to respond to small molecules which then trigger the secretion of IpaB through the needle and begin the process of assembling the translocon.¹⁶ This is the only stepwise demonstration of translocator recruitment to the bacterial surface observed thus far.¹⁶ Using atomic force microscopy, the pore size of the translocon from different species was shown to vary from 1.5 to 3.5 nm.¹⁷ The tip complex is believed to serve as a platform for the assembly of the translocon, which ultimately connects the bacterial pathogen to its eukaryotic host for the delivery of effector proteins.

Atomic structures of needle and tip proteins

The needle

The major extracellular portion of the TTSA is a needle assembled from the polymerization of approximately 120 copies of a single protein.¹¹ Analogous to bacterial flagellum, it is believed that needle monomers pass through the channel and polymerize at the growing distal end of the needle.³ Needle proteins are critical for virulence as null mutants are noninvasive and defective in secreting any virulence effectors.^{18–20} Needle proteins from a wide range of pathogens share sequence similarities. They are generally small (less than 90 residues), acidic (pI ~ 4.8), and contain a PxxP motif in the middle region.²¹ Two methods are currently used to prevent the self-polymerization and thus allow the structural determinations of needle monomers. First, truncation of the last five C-terminal residues yielded monomeric BsaL,²¹ MxiH,²² and PrgI,²³ the needle proteins of *B. pseudomallei*, *S. flexneri*, and *S. typhimurium*, respectively. Second, complex formation with chaperones prevented self-association of the needle proteins of *P. aeruginosa*, PscF,²⁴ and *Y. pestis*, YscF.²⁵

The NMR structure of BsaL²¹ revealed a two helix bundle in the middle region with the PxxP motif serving as a linker between the two helices. The N-terminal and C-terminal ends flanking the well-structured two helix bundle are not disordered but adopt partial helical features (Figure 2A). The two helix bundle is stabilized by hydrophobic interactions and the surface of this domain is lined with polar residues. The crystal structure of MxiH shows a similar two helix bundle with a longer C-terminal helix (Figure 2B).²² Similarly, the NMR structure of PrgI shows a two helix bundle with flexible ends (Figure 2C).²³ However, the

electrostatic surfaces of PrgI differ from those of BsaL or MxiH, suggesting that electrostatic packing interactions may be important in needle assembly and that packing interaction between needle monomers may be different among species. The importance of electrostatic interaction in needle assembly is corroborated by an earlier result showing that increasing pH, which is a factor in electrostatic contacts, from 8 to 10.5 disassembled the *Salmonella* needle without affecting the basal structure.¹¹

The crystal structures of PscF²⁴ and YscF²⁵ are different from the structures of BsaL, MxiH, and PrgI, which is probably due to the method used in preventing the self-polymerization of PscF and YscF. Deleting a small number of C-terminal residues does not lead to readily purified PscF and YscF monomers. The structure of PscF was only solved when it was bound to its chaperones PscE and PscG and when the first 54 residues of PscF were deleted.²⁴ The C-terminal 31 residues of PscF in the ternary complex form a short ordered region and a short helix similar to the C-terminal helix of MxiH (Figure 2D). Similarly, the crystal structure of YscF was determined in a ternary complex with its chaperones YscE and YscG.²⁶ In this ternary complex, the first 49 residues of YscF displayed no electron density and residue 50–87 formed an α -helical hairpin with a five-residue linker from Ile64 to Asn68 (Figure 2E). In both ternary complexes, more than half of PscF and YscF N-terminal residues, including the PxxP region, are missing or disordered in the crystals. The structures of PscF and YscF show how bacteria prevent the premature self-polymerization of needle monomers while inside the bacterial cytoplasm, however, no chaperones have been reported for BsaL, MxiH, or PrgI.

The tip

It was not until late 2005 that a tip complex was reported on top of the needle when the apparatus was in a resting state.¹⁴ This was quickly corroborated with the finding that IpaD formed a tip complex for newly made TTSA in *S. flexneri*.¹⁵ It is likely that these tip complexes are needed to sense environmental signals and control the secretion and insertion of translocators into host membranes to initiate translocon pore formation.^{16, 27, 28} Some known tip proteins have been distinguished into specific classes which share sequence similarity, especially within their C-terminal sequences.²⁹ For the tip proteins discussed here, there appear to be two main classes: a) those of the IpaD/SipD/BipD family, and b) those of the LcrV/PcrV family.

The X-ray structure of LcrV (Figure 2F) was the first reported in the LcrV/PcrV family.³⁰ LcrV has a dumbbell shape with a long central coiled-coil flanked by two globular domains.²⁹ The long central coiled-coil was found to be a signature of other tip proteins when the X-ray structures of BipD^{27, 31} and IpaD²⁷ became available. These proteins, however, possessed larger N-terminal domains (Figure 2G and 2H).^{27, 31} It is perhaps not surprising that the N-terminal domains of BipD and IpaD resemble the structure of the LcrV chaperone LcrG since homologs of this chaperone in *Shigella* and *Burkholderia* are absent and it has been suggested that IpaD and BipD may possess a self-chaperoning ability.²⁷

Assembly of the TTSA needle and tip

In general, TTSA from different species appear to have evolved into five or more different families. Here we have focused on the needle and tip assembly of the *Yersinia* Ysc/Psc family and *Shigella* Inv/Mxi/Spa family.³² Nevertheless, important themes have arisen in needle monomer and tip protein structure with likely implications in the assembly of needles and the retention of tip proteins at the distal end of these needle structures.

Needle assembly

The atomic structures of needle proteins has provided a basis for explaining biological data related to the phenotypes of needle proteins mutants,^{19, 25} however, empirical data on how needle monomers interact with each other for needle assembly and how relatively large proteins are able to traverse these needles are still lacking. Furthermore, how or even whether this portion of the TTSA is structurally altered during the process of protein secretion is still unclear. Nonuniformity in needle length, both for isolated needles and needles reconstituted *in vitro*, make crystallization of assembled needles unlikely.

A model of the assembled needle was generated by docking the crystal structure of MxiH into a 16 Å EM-derived electron density of the *Shigella* needle (Figure 3).²² In this model, the major axis of MxiH monomers was in line with the major axis of the needle. The orientation of this model is in agreement with the functionally- and structurally-related bacterial flagellar filament assembly.^{22, 33, 34} The PxxP motif, which forms part of the head domain of MxiH is pointed outwards the needle. The disordered N-terminal region of MxiH is modeled to form a regular alpha helix that lines the inner surface of the channel. The C-terminus of MxiH is buried within the needle wall and is in direct contact with three other MxiH monomers, thus showing how deletion of five C-terminal residues prevented the self-polymerization of MxiH. Published mutagenesis results agree with this model.¹⁹

Docking of the tip complex on the needle

Although the tip complex has been visualized on top of the needle,^{14, 15} how the tip protein assembles is not yet known. Based on immunoblotting and scanning-transmission electron microscopy (STEM), it was suggested that three to five LcrV molecules were needed to form the tip complex of the *Yersinia* needle.³⁵ Molecular modeling based on the crystal structures of MxiH and LcrV suggested a pentameric LcrV tip complex.²² Likewise, molecular modeling based on the crystal structures of MxiH and IpaD suggested a pentameric IpaD tip complex in *Shigella*.²⁷ The tip proteins have not, however, been found to self-assemble into pentamers *in vitro* during structure analyses which makes solving the true nature of the tip complex more challenging. Thus, how the tip monomers form the tip complex needs further investigation.

The positioning of the tip complex suggests that it directly interacts with host cells to trigger the delivery of translocon and effector proteins, respectively. Precisely how tip proteins interact with host cells and with needle monomers are currently unknown. The N-terminal domain of the IpaD tip protein has a two helix bundle that looks remarkably similar to the two helix bundle of the MxiH needle protein, suggesting a possible model for MxiH-IpaD interaction.³⁶ A model suggests that when IpaD is secreted and localized to the tip of the needle, its N-terminal domain swings away, leaving the binding interface of the long central coiled-coil accessible to MxiH.³⁶ MxiH thus mimics the IpaD N-terminus and binds to the lower part of the IpaD long central coiled-coil (Figure 4). Results of NMR mapping, fluorescence spectroscopy, mutagenesis, and microscopy support the suggested MxiH-IpaD binding interaction.³⁶ That the N-terminal domain of IpaD is not buried inside the needle but is exposed outwards the needle is consistent with the observation that anti-IpaD antibodies can neutralize some IpaD functions.²⁸ Furthermore, deletion of a small region at the C-terminus of IpaD led to a dramatic decrease in the ability of *S. flexneri* to infect cultured cells or to assemble IpaD at the needle tip.²⁸ In the model of MxiH-IpaD interaction (Figure 4A), the C-terminus of IpaD contacts MxiH. It is likely that other tip proteins will bind their respective needle partners in a similar manner based on the following observations: (i) the two helix bundle of BsaL can be fitted with the N-terminal two helix bundle of BipD (Figure 4B). (ii) BipD is also capable of binding the MxiH needle of *S. flexneri*,²⁷ and the C-terminal sequences of IpaD and BipD are similar. Likewise, the divergence of C-terminal

sequences for the LcrV/PcrV family and their inability to be retained at the *Shigella* MxiH needle tip (unpublished data) are also consistent with this model of MxiH-IpaD interaction (Figure 4A).

The interaction between the tip and translocon

When the bacterial TTSA comes into direct contact with the cognate host cell, the translocators are immediately secreted and inserted into the host cell membrane. The two translocators (IpaB and IpaC in *Shigella*) then form the translocon pore, thus opening a direct channel through which bacterial effectors can be injected into the host cell cytosol in a one-step process. In this scenario, the tip complex serves as a platform that connects the bacterial and host cell cytoplasm. The position of the tip complex suggests an interaction between tip proteins and translocators. Despite the high resolution structures of tip proteins, detailed structural information on the translocators is absent due to their overall hydrophobic nature. How the tip and translocon proteins interact with each other also remains to be determined.

Conclusion

The increasing number of structures of TTSA components is providing new insight into the assembly and function of this complex nanomachine. This knowledge could be exploited in developing novel drugs targeted against bacterial virulence.^{37–45} However, questions on how the needle and tip complex are assembled, how the tip complex recognizes extracellular signals, and how the tip complex interacts with the nascent translocon remain unknown. Many molecular, structural, and biophysical techniques have been applied to study this macromolecular system, but the protein dynamics and protein-protein interactions involved in TTSA assembly and function require further study and an additional blending of old and new approaches.

Acknowledgments

Funding was provided by the NIH (R01 AI067858 to WLP and AI074856 to RND and R03 AI072510 to WLP).

References

1. Salmond GP, Reeves PJ. Trends Biochem Sci. 1993; 18:7–12. [PubMed: 8438237]
2. Van Gijsegem F, Genin S, Boucher C. Trends Microbiol. 1993; 1:175–180. [PubMed: 8143135]
3. Blocker A, Komoriya K, Aizawa S. Proc Natl Acad Sci USA. 2003; 100:3027–3030. [PubMed: 12631703]
4. Blocker AJ, Deane JE, Veenendaal AK, Roversi P, Hodgkinson JL, Johnson S, Lea SM. Proc Natl Acad Sci USA. 2008; 105:6507–6513. [PubMed: 18458349]
5. Gough CL, Genin S, Zischek C, Boucher CA. Mol Plant Microbe Interact. 1992; 5:384–389. [PubMed: 1472716]
6. Rosqvist R, Magnusson KE, Wolf-Watz H. Embo J. 1994; 13:964–972. [PubMed: 8112310]
7. Woestyn S, Allaoui A, Wattiau P, Cornelis GR. J Bacteriol. 1994; 176:1561–1569. [PubMed: 8132449]
8. Allaoui A, Woestyn S, Sluiter C, Cornelis GR. J Bacteriol. 1994; 176:4534–4542. [PubMed: 8045883]
9. Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galan JE, Aizawa SI. Science. 1998; 280:602–605. [PubMed: 9554854]
10. Schroeder GN, Hilbi H. Clin Microbiol Rev. 2008; 21:134–156. [PubMed: 18202440]
11. Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galan JE, Unger VM. Science. 2004; 306:1040–1042. [PubMed: 15528446]

12. Cordes FS, Komoriya K, Larquet E, Yang S, Egelman EH, Blocker A, Lea SM. *J Biol Chem.* 2003; 278:17103–17107. [PubMed: 12571230]
13. Cordes FS, Daniell S, Kenjale R, Saurya S, Picking WL, Picking WD, Booy F, Lea SM, Blocker A. *J Mol Biol.* 2005; 354:206–211. [PubMed: 16243352]
14. Mueller CA, Broz P, Muller SA, Ringler P, Erne-Brand F, Sorg I, Kuhn M, Engel A, Cornelis GR. *Science.* 2005; 310:674–676. [PubMed: 16254184]
15. Espina M, Olive AJ, Kenjale R, Moore DS, Ausar SF, Kaminski RW, Oaks EV, Middaugh CR, Picking WD, Picking WL. *Infect Immun.* 2006; 74:4391–4400. [PubMed: 16861624]
16. Olive AJ, Kenjale R, Espina M, Moore DS, Picking WL, Picking WD. *Infect Immun.* 2007; 75:2626–2629. [PubMed: 17296762]
17. Ide T, Laarmann S, Greune L, Schillers H, Oberleithner H, Schmidt MA. *Cell Microbiol.* 2001; 3:669–679. [PubMed: 11580752]
18. Kubori T, Sukhan A, Aizawa SI, Galan JE. *Proc Natl Acad Sci USA.* 2000; 97:10225–10230. [PubMed: 10944190]
19. Kenjale R, Wilson J, Zenk SF, Saurya S, Picking WL, Picking WD, Blocker A. *J Biol Chem.* 2005; 280:42929–42937. [PubMed: 16227202]
20. Blocker A, Jouihri N, Larquet E, Gounon P, Ebel F, Parsot C, Sansonetti P, Allaoui A. *Mol Microbiol.* 2001; 39:652–663. [PubMed: 11169106]
21. Zhang L, Wang Y, Picking WL, Picking WD, De Guzman RN. *J Mol Biol.* 2006; 359:322–330. [PubMed: 16631790]
22. Deane JE, Roversi P, Cordes FS, Johnson S, Kenjale R, Daniell S, Booy F, Picking WD, Picking WL, Blocker AJ, Lea SM. *Proc Natl Acad Sci USA.* 2006; 103:12529–12533. [PubMed: 16888041]
23. Wang Y, Ouellette AN, Egan CE, Rathinavelan T, Im W, De Guzman RN. *J Mol Biol.* 2007; 371:1304–1314. [PubMed: 17617421]
24. Quinaud M, Ple S, Job V, Contreras-Martel C, Simorre JP, Attree I, Dessen A. *Proc Natl Acad Sci USA.* 2007; 104:7803–7808. [PubMed: 17470796]
25. Sun, P.; Austin, BP.; Tropea, JE.; Waugh, DS. *RCSB 2P58.* 2007.
26. Sun P, Tropea JE, Austin BP, Cherry S, Waugh DS. *J Mol Biol.* 2008; 377:819–830. [PubMed: 18281060]
27. Johnson S, Roversi P, Espina M, Olive A, Deane JE, Birket S, Field T, Picking WD, Blocker AJ, Galyov EE, Picking WL, Lea SM. *J Biol Chem.* 2007; 282:4035–4044. [PubMed: 17077085]
28. Picking WL, Nishioka H, Hearn PD, Baxter MA, Harrington AT, Blocker A, Picking WD. *Infect Immun.* 2005; 73:1432–1440. [PubMed: 15731041]
29. Espina M, Ausar SF, Middaugh CR, Baxter MA, Picking WD, Picking WL. *Protein Sci.* 2007; 16:704–714. [PubMed: 17327391]
30. Derewenda U, Mateja A, Devedjiev Y, Routzahn KM, Evdokimov AG, Derewenda ZS, Waugh DS. *Structure.* 2004; 12:301–306. [PubMed: 14962390]
31. Erskine PT, Knight MJ, Ruaux A, Mikolajek H, Sang NW, Withers J, Gill R, Wood SP, Wood M, Fox GC, Cooper JB. *J Mol Biol.* 2006; 363:125–136. [PubMed: 16950399]
32. Cornelis GR. *Nat Rev Microbiol.* 2006; 4:811–825. [PubMed: 17041629]
33. Samatey FA, Matsunami H, Imada K, Nagashima S, Shaikh TR, Thomas DR, Chen JZ, Derosier DJ, Kitao A, Namba K. *Nature.* 2004; 431:1062–1068. [PubMed: 15510139]
34. Yonekura K, Maki-Yonekura S, Namba K. *Nature.* 2003; 424:643–650. [PubMed: 12904785]
35. Broz P, Mueller CA, Muller SA, Philippsen A, Sorg I, Engel A, Cornelis GR. *Mol Microbiol.* 2007; 65:1311–1320. [PubMed: 17697254]
36. Zhang L, Wang Y, Olive AJ, Smith ND, Picking WD, De Guzman RN, Picking WL. *J Biol Chem.* 2007; 282:32144–32151. [PubMed: 17827155]
37. Muller S, Feldman MF, Cornelis GR. *Expert Opin Ther Targets.* 2001; 5:327–339. [PubMed: 12540268]
38. Kauppi AM, Nordfelth R, Uvell H, Wolf-Watz H, Elofsson M. *Chem Biol.* 2003; 10:241–249. [PubMed: 12670538]

39. Nordfelth R, Kauppi AM, Norberg HA, Wolf-Watz H, Elofsson M. Infect Immun. 2005; 73:3104–3114. [PubMed: 15845518]
40. Patel JC, Rossanese OW, Galan JE. Trends Pharmacol Sci. 2005; 26:564–570. [PubMed: 16182381]
41. Gauthier A, Robertson ML, Lowden M, Ibarra JA, Puente JL, Finlay BB. Antimicrob Agents Chemother. 2005; 49:4101–4109. [PubMed: 16189086]
42. Pan N, Lee C, Goguen J. Adv Exp Med Biol. 2007; 603:367–375. [PubMed: 17966433]
43. Aberg V, Almqvist F. Org Biomol Chem. 2007; 5:1827–1834. [PubMed: 17551629]
44. Clatworthy AE, Pierson E, Hung DT. Nat Chem Biol. 2007; 3:541–548. [PubMed: 17710100]
45. Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. Nat Rev Microbiol. 2008; 6:17–27. [PubMed: 18079741]

Figure 1.
The TTSA provides a conduit for the passage of effector proteins into the cytosol of targeted cells.

Figure 2. Ribbon structures of needle proteins (A) BsaL, (B) MxiH, (C) PrgI, (D) PscF and (E) YscF. Also shown are the structures of the tip proteins (F) LcrV (F), (G) BipD, and (H) IpaD. Figures were generated using the following PDB IDs: BsaL, 2G0U; MxiH, 2CA5; PrgI, 2JOW; PscF, 2UWJ; YscF, 2P58; LcrV, 1R6F; BipD, 2IZP; and IpaD, 2J0O.

Figure 3.

The crystal structure of MxiH monomer and electron microscopy of *Shigella* needles led to the first atomic model of a TTSA needle (PDB ID 2v6l).²² Shown are the (A) side view and (B) end-on view of the needle model.

Figure 4.

A model of MxiH-IpaD interaction is based on the structural similarity of these proteins. **(A)** The MxiH two helix bundle (green) can be superimposed with the N-terminal domain of IpaD (gray) with a C α root-mean-square deviation of 3.2 Å. Mutagenesis and results of *in vivo* invasion assays are consistent with this model.²¹ **(B)** Likewise, the BsaL two helix bundle (green) can be superimposed with the N-terminal domain of BipD.