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Structure and Biophysics of Type III Secretion in Bacteria

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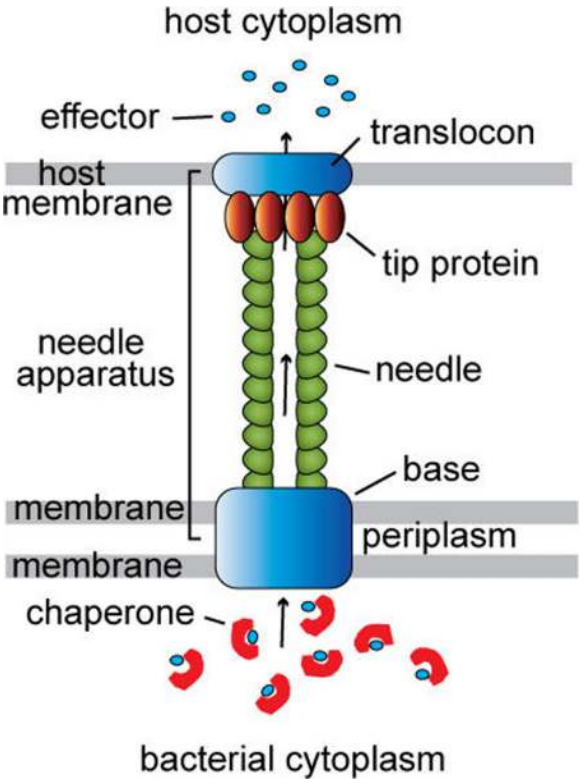
Abstract



Many plant and animal bacterial pathogens assemble a needle-like nanomachine, the type III secretion system (T3SS), to inject virulence proteins directly into eukaryotic cells to initiate infection. The ability of bacteria to inject effectors into host cells is essential for infection, survival, and pathogenesis for many Gram-negative bacteria, including Salmonella, Escherichia, Shigella, Yersinia, Pseudomonas, and *Chlamydia* spp. These pathogens are responsible for a wide variety of diseases, such as typhoid fever, large-scale food-borne illnesses, dysentery, bubonic plague, secondary hospital infections, and sexually transmitted diseases. The T3SS consists of structural and nonstructural proteins. The structural proteins assemble the needle apparatus, which consists of a membrane-embedded basal structure, an external needle that protrudes from the

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bacterial surface, and a tip complex that caps the needle. Upon host cell contact, a translocon is assembled between the needle tip complex and the host cell, serving as a gateway for translocation of effector proteins by creating a pore in the host cell membrane. Following delivery into the host cytoplasm, effectors initiate and maintain infection by manipulating host cell biology, such as cell signaling, secretory trafficking, cytoskeletal dynamics, and the inflammatory response. Finally, chaperones serve as regulators of secretion by sequestering effectors and some structural proteins within the bacterial cytoplasm. This review will focus on the latest developments and future challenges concerning the structure and biophysics of the needle apparatus.

OVERVIEW OF THE NEEDLE APPARATUS

More than a dozen different structural proteins assemble to form the type III secretion system (T3SS) needle apparatus. The needle complex, which consists of a basal structure and an external needle, was first visualized by electron microscopy of osmotically shocked *Salmonella typhimurium*.[1] Similar structures were also observed in other bacteria, such as *Shigella flexneri*,[2] *Yersinia pestis*,[3] *Escherichia coli*,[4] and *Pseudomonas aeruginosa*.[5] Following years of extensive genetic, biochemical, and biophysical research, the core components of the complex were identified. The ~3.5 MDa needle complex contains a basal structure spanning the periplasm that is embedded within the inner and outer bacterial membranes followed by a needle-like structure that protrudes into the extracellular space (Figure 1). In *Salmonella*, the basal structure is assembled from oligomeric rings of InvG, PrgK, and PrgH,[6] and an inner rod structure formed by PrgJ.[7] The basal structure supports the entire needle complex by anchoring the needle on the bacterial membranes. The needle itself is composed of protomers of PrgI arranged in a spiral symmetry around a central ~25 Å diameter channel to allow the passage of effector proteins.[8] At the needle tip, multiple copies of SipD form a platform for the translocon.[9] Upon contact with the host cell, the assembly of the entire needle apparatus is completed by the formation of the translocon, which is formed by the transmembrane proteins SipB and SipC.[9] Structural homologues of each protein are present in the T3SSs of other Gram-negative bacteria (Table 1). The assembly of the needle apparatus is a highly regulated process involving specific protein–protein, protein–membrane, and protein–small molecule interactions. Likewise, the order of protein secretion is also regulated;[10] for example, needle proteins pass through the nascent apparatus prior to the tip and the translocon proteins. Each protein plays a critical role in the proper assembly of the needle apparatus, as null mutations of any of the structural components render bacteria incapable of assembling a functional needle apparatus.[11] The following sections will focus on the structure, biophysics, and protein–protein interactions of the core components of the T3SS needle apparatus.

BASAL STRUCTURE

The basal structure anchors the needle to the bacterial membranes by traversing the inner membrane (IM), the periplasm, and the outer membrane (OM).[1] The base measures approximately 250 Å × 300 Å and is comparable in size to the flagellar basal body.[6] PrgH, PrgK, and InvG assemble into ringlike structures at the IM and OM in *Salmonella*.[6] The IM and OM rings enclose a tubelike inner rod structure formed by PrgJ[7] in *Salmonella*, MxiI[12] in *Shigella*, and YscI[13] in *Yersinia*. The atomic structure of the assembled inner rod is currently unknown; however, NMR and circular dichroism spectroscopy indicate that PrgJ in the monomeric form is a partially folded protein.[14] Further, yeast two-hybrid and GST pull-down experiments showed a direct binding interaction between the *Yersinia* inner rod protein YscI and the needle protomer YscF.[15]

In *Salmonella*, the IM ring structure is formed by PrgH and PrgK, which assemble into two concentric rings, with PrgK forming the smaller ring and PrgH forming the outer ring[6,12,16]

(Figure 2A,B). PrgK belongs to the highly conserved YscJ/EscJ family of periplasmic lipoproteins, and NMR results for the enteropathogenic *E. coli* (EPEC) EscJ revealed a two-domain structure connected by a flexible linker (Figure 2C).[17] These proteins are localized to the outer leaflet of the IM with their lipidated N-terminal domain and contain N-terminal signal sequences that are cleaved upon insertion in the membrane.[12,17,18] X-ray crystallography showed that EscJ packs into a 24-mer ring structure[18] with overall dimensions similar to those of the IM rings.[19] Homology modeling based on the EscJ template generated the atomic model of *Salmonella* PrgK, which was then fit into the EM density map of the *Salmonella* base.[20] The outer IM ring consists of 24 monomers of PrgH, a bitopic membrane protein with an N-terminal cytoplasmic domain and a large C-terminal periplasmic domain.[20,21] The crystal structure of PrgH (residues 170–362) showed a distinctive "boot-shaped" organization of its modular domains (Figure 2C).[21] The NMR structure of the N-terminal domain of *Shigella* MxiG (Figure 2C) (the homologue of *Salmonella* PrgH) supported the 24-subunit structure of the *Shigella* IM ring.[22]

The OM ring is formed by the secretin family of integral membrane proteins that includes *Salmonella* InvG,[20] *Yersinia* YscC,[23] *Shigella* MxiD,[24] and EPEC EscC.[21] A neck region spanning the periplasm connects the OM ring to the IM rings. The N-terminal domain of InvG forms the neck region of the base and is associated with the PrgH IM ring,[20] whereas the C-terminal domain forms the rest of the OM ring.[21] Electron microscopy of the *Shigella* base at 21–25 Å resolution showed an OM ring with a 12-fold symmetry.[24] Recently, the refined structure of the *Salmonella* base at 10 Å resolution showed a 15-fold symmetry for the OM ring (Figure 2B).[20] This higher-resolution structure of the base was achieved by selectively disassembling the basal components into stable IM and OM rings by pH treatment.[20] The structural model of the *Salmonella* InvG based on the crystal structure of EPEC EscC (Figure 2C) was fit into the EM density map of the *Salmonella* basal structure to generate an atomic model of the *Salmonella* OM ring.[21]

Secretins are transported to the OM with the help of pilotins, which are small lipoproteins that assist in the assembly of the OM ring.[25] The *Shigella* pilotin protein MxiM forms a pseudo-β-barrel with a hydrophobic pocket for binding lipids.[26] MxiM interacts with the secretin protein MxiD, and the binding interaction blocks the lipid-binding pocket in MxiM.[25] The pilotins also contain a characteristic signal peptide leader sequence with a conserved cysteine residue can be lipidated.[25] These features are thought to help in the membrane targeting of the secretin–pilotin complex.[25]

Secretion through the base is controlled by an export apparatus,[27] which serves as a platform for the assembly of the basal structure and controls substrate specificity.[27–29] The export apparatus is a complex of highly conserved integral membrane proteins: YscR, YscS, YscT, YscU, and YscV in *Yersinia*[28,29] and SpaP, SpaQ, SpaR, SpaS, and InvA in *Salmonella*.[27] In *Yersinia*, the assembly of the base and export apparatus is initiated independently at the outer and inner membranes.[28,29] In *Yersinia*, the OM ring of the base is formed by the secretin protein YscC (and assisted by the YscW pilotin), which then recruits the outer IM ring protein, YscD.[28] Simultaneously, the export apparatus proteins YscR, YscS, and YscT assemble at the inner membrane, which promote the polymerization of YscV.[29] These two pathways can independently recruit the inner IM ring protein, YscJ, which is thus thought to link these two substructures.[29] However, in *Salmonella*, the prior assembly of the export apparatus proteins (SpaP, SpaQ, and SpaR) at the IM is required for the assembly of the IM ring.[27]

The hierarchy of secretion of structural components and virulence effectors through the base is regulated in part by the export apparatus protein YscU in *Yersinia*,[30] SpaS in *Salmonella*,[31] Spa40 in *Shigella*,[32] and EscU in EPEC.[31] The overall structure of these

proteins consists of an N-terminal transmembrane domain that is connected to a C-terminal globular cytoplasmic domain by a flexible linker.[30,31] The cytoplasmic domain shows a conserved fold composed of a central five-stranded mixed β-sheet surrounded by four α-helices.[30–32] The cytoplasmic domain undergoes an autocatalytic cleavage at a highly conserved NPTH motif, resulting in an altered surface feature, which is important for substrate specificity switching.[32] The largest component of the export apparatus, the YscV[29] family of proteins (including *Salmonella* InvA[33] and *Shigella* MxiA[34]), forms a ring directly below the base. The atomic structures of the cytosolic domains of InvA,[33] MxiA,[34] and the flagellar homologue FlhA[35] show a conserved fold composed of four subdomains. The cytosolic domain of *Shigella* MxiA formed a nonameric ring, and residues lining the internal surface of this ring are important for secretion of T3SS substrates.[34]

An ATPase complex associated with the bacterial IM[36] is responsible for energizing the export of substrates through the needle apparatus. The ATPase complex couples ATP hydrolysis to the unfolding and release of effector proteins from their cognate chaperones prior to secretion through the needle apparatus.[34,37] The major component of the ATPase complex is a homohexamer formed by the EscN protein family (with homologues listed in Table 1), which is related to the α and β subunits of the F1 ATP synthase.[38]

STRUCTURE OF THE NEEDLE

The needle is assembled from multiple copies of a single protein, the needle protomer. Needle protomers are small polar proteins of <90 residues.[12] Early experiments with recombinant forms of these proteins impeded structure determination because of their tendency to aggregate in solution.[39] However, deletion of the last five residues at the C-terminus allowed for determination of the atomic structure of the *Shigella* needle protomer MxiH by crystallography,[40] and *Burkholderia* BsaL[41] and *Salmonella* PrgI[42] needle protomers by NMR spectroscopy (Figure 3A). Unfortunately, this deletion also inhibited polymerization of the needle and abolished host cell invasion.[43] Shortly after, the crystal and NMR structures of a soluble and functional V65A/V67A double mutant of full-length PrgI were determined (Figure 3A).[43] The monomeric forms of needle protomers adopt α-helical hairpin structures, essentially two α-helices joined by a four-residue PXXP motif (where X is any amino acid), containing a turn in the central region of the protein (Figure 3A). The *Pseudomonas* PscF, however, lacks a PXXP motif and instead contains an AXXP motif.[44] Complete structures of the *Yersinia* YscF and *Pseudomonas* PscF needle protomers are currently unknown; however, their partial structures in complex with their chaperones (YscE–YscG[45] and PscE–PscG,[44] respectively) are known. The structured regions of YscF (Figure 3A) and PscF in these complexes also form α-helices. Although YscF and PscF have chaperones, no chaperones have been reported for BsaL, MxiH, or PrgI.

Multiple copies of needle protomers assemble to form the T3SS needle. Electron microscopy and X-ray fiber diffraction of *Shigella* needles allowed the atomic scale modeling of a T3SS needle at 16 Å resolution.[46] This model revealed a structure measuring 500 Å in length and 70 Å in width enclosing an inner channel with a diameter of ~25 Å.[46] Subsequently, when the atomic structures of needle protomers became available, they were used to fit the electron density maps of the *Shigella*[40] and *Salmonella*[8] needles. The N-terminal region of MxiH lacked electron density in the crystal structure.[40] Therefore, it was modeled in the *Shigella* needle as an α-helix facing the lumen, based on the predicted α-helical propensities of residues within the region.[40] Similarly, in the *Salmonella* needle model, approximately half of the length of PrgI containing the flexible N- and C-termini was absent because of the conformational heterogeneity of the PrgI protomer in the NMR structure.[8] The *Shigella* needle was shown to contain ~5.6 subunits per turn with a helical

pitch of 24 Å,[40] while the *Salmonella* needle contained ~6.3 subunits per turn with a helical pitch of 26 Å.[8]

Recently, higher-resolution atomic models of the *Shigella*[47] and *Salmonella*[48] needles were reported. These models represent our current understanding of the atomic structures of the T3SS needles. Fujii et al. used cryo-electron microscopy (cryo-EM) to determine the structure of purified *Shigella* needles formed by the overexpression of recombinant full-length MxiH.[47] The atomic model of the *Shigella* needle was obtained by fitting the crystal structure of MxiH residues 26–51 (the "head" structure) into the 7.7 Å resolution electron density map.[47] In this model, the first 38 residues of MxiH form a straight α-helix facing the lumen and the exterior surface of the needle is formed by the C-terminus of MxiH, which contains a short α-helix (residues 44–50), a β-hairpin (residues 51–64), and a kinked helix (residues 65–83).[47] Intersubunit interactions are present between the β-hairpin and the PXXP motif, as well as the β-hairpin and the C-terminus of adjacent subunits. The needle is further stabilized by helix–helix interactions between adjacent subunits.

Loquet et al.,[48] on the other hand, used solid-state NMR spectroscopy (ssNMR) to determine the structure of *Salmonella* needles polymerized from full-length recombinant PrgI (Figure 3B). The use of [1-^{13}C]glucose or [2-^{13}C]glucose instead of the traditional uniform labeling with ^{13}C-labeled glucose[49] as the carbon source for bacteria expressing recombinant PrgI reduced the NMR signal complexity of the megadalton needle. Coupled with ^{15}N labeling, ssNMR allowed the identification of intra- and intersubunit ^{15}N–^{13}C distance restraints,[48] which were used in combination with Rosetta modeling[50] to generate the atomic structure of the *Salmonella* needle[48] (Figure 3B). According to the ssNMR model, the *Salmonella* needle is composed of PrgI protomers with an α-helical hairpin head structure similar to those that have been described previously.[42,43] However, unlike previously determined crystal and NMR structures, ssNMR secondary structure analysis excluded conformational heterogeneity at the N- and C-termini, except for Met1 and Ala2.[48] The *Salmonella* needle has an 80 Å outer diameter with a 25 Å lumen, similar to values previously described.[8,51] Unlike previous reconstructions,[8] however, the needle was shown to contain ~5.7 subunits per turn with a helical pitch of 24 Å.[48] The needle is held together by multiple intra- and intersubunit contacts. The intrasubunit contacts are primarily hydrophobic interactions between the two helices of the hairpin, and the intersubunit interactions are present on the lateral and axial surfaces of each subunit.[48]

There are two major differences between the ssNMR model[48] of the *Salmonella* needle and previous needle models.[8,40,47] The first difference is that in the ssNMR *Salmonella* needle model, the N-terminus of PrgI faces the exterior of the needle while the C-terminal tail faces the interior[48] (Figure 3B). Immunoelectron microscopy against the N-terminus of PrgI confirmed that nonconserved residues in the N-terminal region face the exterior of the needle, while highly conserved residues in the C-terminus are contained within the lumen.[48] In contrast, the EM models have reported that the N-terminus of the needle protomer faces the lumen while the C-terminus faces the exterior.[8,40,47] This was supported by secretion experiments in which the N-terminus of MxiH tolerated more substantial modifications than the C-terminus.[47] Another difference is that throughout the *Salmonella* needle protomer, no β-hairpin was detected, in contrast to the structure of the *Shigella* needle protomer.[47] Poyraz et al.,[43] however, reported that residues I71–I76 of PrgI V65A/V67A undergo a backbone α-helix–β-strand conversion upon polymerization. In the *Shigella* needle, the β-hairpin is hypothesized to regulate assembly and the positioning of the subunits within the needle.[47] ssNMR data of *Shigella* needles should clarify these differences.

NEEDLE TIP

On the distal end of the needle sits a tip protein complex that functions to sense the presence of eukaryotic cells, to serve as a platform for the assembly of the translocon, and to regulate the secretion of effector proteins into the host cell.[9,52] The T3SS tip proteins can be grouped into two classes based on their structures and properties. One class is formed by *Salmonella* SipD, *Shigella* IpaD, and *Burkholderia* BipD tip proteins, which have sequences that are 30–50% identical and are structural homologues with a Cα root-mean-square deviation of <1.5 Å.[53–56] Members of the SipD family of tip proteins share a more distant resemblance to another class of tip proteins, the *Yersinia* LcrV and the *Pseudomonas* PcrV tip proteins, with sequences that are ~37% identical.

The SipD, IpaD, and BipD[53–56] tip proteins contain eight α-helices and five β-strands, which are arranged into three distinct structural elements: an N-terminal α-helical hairpin, a long central coiled-coil, and a C-terminal region of mixed α-helices and β-strands (Figure 4A–D). The central coiled-coil is a distinct structural feature of all tip proteins and is responsible for their overall oblong shape. The coiled-coil motif, especially the C-terminal residues, is important for the interaction of the tip protein with the needle, and the needle protomer is expected to bind at multiple sites on the coiled-coil motif.[52,55–58] A three-stranded antiparallel β-sheet connects the central coiled-coil to the mixed α/β region.[53–55] The first 30–40 residues of the tip proteins are disordered in the crystal structures of SipD, IpaD, and BipD.[52] The N-terminal α-hairpin folds independently and functions as a self-chaperone by preventing the self-oligomerization of SipD and IpaD[53,56,59] and hinders the interaction between the needle protomer PrgI and the tip protein SipD.[56] Upon assembly of SipD at the needle tip, the α-hairpin is expected to be displaced by the needle protomer, which interacts with the coiled-coil domain.[53,56,58]

The 2.2 Å crystal structure of LcrV[60] (Figure 4) represents what is currently known about the atomic structure of the *Yersinia/Pseudomonas* LcrV/PcrV family of tip proteins. Similar to the SipD, the structure of LcrV also contains a long central coiled-coil, which is flanked by globular domains on the N- and C-termini, giving an overall "dumbbell shape" to LcrV.[60] LcrV, however, lacks the self-chaperoning α-helical hairpin found in SipD. Instead, a small cytoplasmic protein, LcrG, functions as the chaperone for LcrV.[11] Likewise, the LcrG homologue in *Pseudomonas*, PcrG, functions as a chaperone for the PcrV tip protein. In addition to binding their cognate tip proteins, LcrG and PcrG negatively regulate secretion of effectors[61,62] in *Yersinia* and *Pseudomonas*. Deletion of *lcrG* decreases the level of LcrV secretion,[63] and host cell contact with *Yersinia* upregulates the expression of LcrV, which then forms a tight binding complex with LcrG; this interaction relieves the negative block on effector secretion by titrating away LcrG.[61,62,64] The N-terminal regions of LcrG and PcrG are required for binding to their cognate tip proteins,[61,65,66] and the coiled-coil region of LcrV is involved in the interaction with LcrG.[65,67] The absence of an intramolecular chaperoning domain in LcrV and PcrV hints at divergent assembly processes at the needle tip between the *Salmonella*/*Shigella* and *Yersinia*/*Pseudomonas* species.

A major challenge in this field is the determination of atomic-resolution structures of the tip protein docked at the needle tip. Currently, low-resolution electron micrographs of the *Yersinia* LcrV tip[68] and the *Shigella* IpaD tip[69,70] as well as models made by docking the crystal structures of tip proteins on the needle[11,40] are available. Electron micrographs of the *Yersinia* needle tip show a well-defined tip complex that was estimated to be formed by an LcrV pentameric ring.[68,71] The *Yersinia* LcrV tip complex showed a distinct head, neck, and a base, and Broz et al.[71] concluded that the LcrV N-terminal globular domain forms the base, the C-terminal globular domain forms the head, and the coiled-coil region forms the

neck. The base was also shown to be responsible for the correct insertion of the *Yersinia* translocon protein YopB into the host cell membrane.[71]

Results from electron microscopy of the *Shigella* tip complex also estimated a stoichiometry of a pentameric IpaD at the needle tip.[70] However, the *Shigella* tip complex did not show the head, neck, and base morphology that was seen in the *Yersinia* needle tip. This difference was partly ascribed to the difference in the structure of IpaD and LcrV where the domains flanking the IpaD coiled-coil were more elongated in shape compared to the more globular domains flanking the LcrV coiled-coil. In the *Shigella* tip complex, the IpaD coiled-coil is aligned with the vertical axis of the tip complex with a 20° tilt and the tip complex forms an internal channel diameter from 40 Å at the middle to 22 Å at the top.[70] The most distinctive feature of this structure is that the IpaD mixed α/β domain undergoes a 165° rotation about the horizontal axis, forming a scepterlike structure at the top of the tip complex.[70] In support of this drastic rotation, tip complexes made of IpaD lacking the mixed α/β domain (residues 192–267) were shorter by ~37 Å and lacked electron density for the scepterlike structure.[70]

Prior to contact with the host cell membrane, the tip complex is present at the needle tip while the translocon is yet to be assembled.[9,69,72] The tip complex therefore controls the secretion of the translocon proteins, and this process is not well understood. It has been proposed that the pentameric tip complex forms a closed conformation that prevents protein secretion[11] and, upon receiving an extracellular signal in the presence of the host cell,[72,73] changes into an open conformation that allows protein secretion to complete the assembly of the needle apparatus. Bile salts, levels of which are elevated in the intestines, have been identified as extracellular signals that can affect type III secretion in the enteric pathogens *Shigella*[74–76] and *Salmonella*.[77,78] Bile salts bind to IpaD[72,73,75,76] and SipD;[56,76,79] however, despite the structural homology of IpaD and SipD, bile salts bind these proteins at different sites and affect their respective T3SSs in opposite ways. Bile salts increase the level of type III secretion and invasiveness of *Shigella*[74–76] but repress the transcription of T3SS proteins and decrease the invasiveness of *Salmonella*.[77,78] The mechanism of how extracellular signals affect the structure and conformation of the tip complex remains to be elucidated.

TRANSLOCON

The final stage in the assembly of the needle apparatus is the formation of the translocon, which forms a 20–30 Å pore on the host cell membrane to allow the passage of effectors into the host cell.[2,80–82] The translocon is assembled from two integral membrane proteins named SipB and SipC in *Salmonella*,[83] IpaB and IpaC in *Shigella*,[2,84] YopB and YopD in *Yersinia*,[82] and PopB and PopD in *Pseudomonas*.[85] The translocon proteins are important for the entry of bacteria into eukaryotic cells as demonstrated by the internalization of latex beads coated with IpaB and IpaC into HeLa cells.[86] While inside the bacterial cytosol, the translocon proteins are bound to their chaperones to maintain their intracellular stability. SipB and SipC are chaperoned by SicA in *Salmonella*.[87] The chaperones for the translocon proteins in other species are the *Shigella* IpgC,[88] *Yersinia* SycD (also named LcrH),[89] and *Pseudomonas* PcrH[90] proteins.

The major translocon proteins from *Shigella* and *Salmonella* (IpaB and SipB) are 62 kDa α-helical proteins that can bind to cell membranes.[91] The *Yersinia* YopB and *Pseudomonas* PopB share a higher degree of conservation and are ~40 kDa in size.[92] The N-terminal region of IpaB and SipB is responsible for their self-oligomerization.[91] Two transmembrane helices facilitate intimate attachment to lipid membranes.[91,93] The extreme C-terminus is predicted to form an amphipathic helix and is important for invasion of the host cell and regulation of effector secretion.[91,94,95] The crystal structures of the protease resistant

fragments in the N-terminal regions of IpaB (residues 74–224) and SipB (residues 82–226) revealed a trimeric coiled-coil domain formed by three antiparallel α-helices[96] (Figure 5A).

The *Shigella* IpaC,[52] *Salmonella* SipC,[9] *Yersinia* YopD,[97] and *Pseudomonas* PopD[98] are the minor translocon proteins and form complexes with the major translocon proteins, and this interaction is necessary for the attachment and entry into epithelial cells.[9] Incubation of *Shigella* with recombinant IpaC led to an increased level of invasion of epithelial cells,[99,100] and recombinant SipC can interact with actin.[101] Similar to other T3SS proteins, the N-terminus of these molecules is required for their secretion,[9,100,102,103] and a central hydrophobic region allows binding to lipid membranes.[104] The C-terminus, which is predicted to be helical, has been implicated in actin modulation[105–107] and hemolytic activity.[108] In addition, the conserved C-terminus is also involved in the homotypic oligomerization of IpaC.[100]

The chaperones of the translocon proteins belong to class II of T3SS chaperones and are responsible for partitioning the intracellular pools of the major and minor translocon proteins, thereby preventing their premature degradation.[87,109,110] Chaperones bind at the N-terminal regions of translocon proteins.[90,111–114] Cocrystal structures of chaperones bound to peptides derived from the chaperone-binding regions of the translocon proteins revealed how chaperones recognize their cognate translocon proteins (Figure 5B).[90,113,115] The chaperones form a homodimer of tetratricopeptide motifs composed of antiparallel α-helical repeats. In the cocrystal structure of the *Shigella* IpgC–IpaB peptide complex,[115] IpgC forms a dimer with each IpgC molecule forming a concave cleft ~10 Å in width to accommodate the IpaB peptide, and the IpgC–IpaB protein–protein contacts are mediated by conserved residues on both proteins. The 32 N-terminal residues of IpgC are responsible for its homodimerization, and this dimerization pattern is conserved in the *Salmonella* SicA[115] and *Yersinia* SycD[116] chaperones. Amino acid substitutions in the IpgC N-terminal region decreased the level of secretion of effectors and bacterial invasion.[115]

The major translocon proteins IpaB and SipB interact with lipids such as cholesterol and sphingomyelin,[117,118] and cholesterol is required for effector translocation in *Shigella*, *Salmonella*, and EPEC.[117,118] Further, the interaction of sphingomyelin and cholesterol with IpaB is proposed to induce the recruitment of IpaC to assemble the translocon.[118] In addition to their structural role in the assembly of the translocon, the translocon proteins are also delivered into the host cell where they interact with a number of cellular factors.[119] IpaB binds to caspase-1, and this leads to apoptosis of macrophages.[120,121] Furthermore, the C-terminus of IpaC induces actin polymerization and formation of filopodia and lamellipodia in fibroblast cells.[105,106] Cytoskeletal rearrangements induced by IpaC were dependent on the function of Cdc42 and Rac GTPases.[105,106] The C-terminus of SipC (within residues 200–409) could also induce actin polymerization in HeLa cells.[105,106]

CONCLUSION

Since the visualization of parts of the needle apparatus at low resolution 15 years ago, much progress has been achieved in determining atomic-resolution structures of components there-of from electron microscopy, crystallography, and NMR spectroscopy. However, much remains to be elucidated in understanding this macromolecular nanoinjector device. Among them are the atomic structures of the translocon and the tip complex. Because the needle apparatus plays a critical role in pathogenesis, understanding its assembly will contribute to the development of novel anti-infectives.

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ABBREVIATIONS

T3SS	type III secretion system
OM	outer membrane
IM	inner membrane
EPEC	enteropathogenic *E. coli*
NMR	nuclear magnetic resonance
ssNMR	solid-state NMR
EM	electron microscopy

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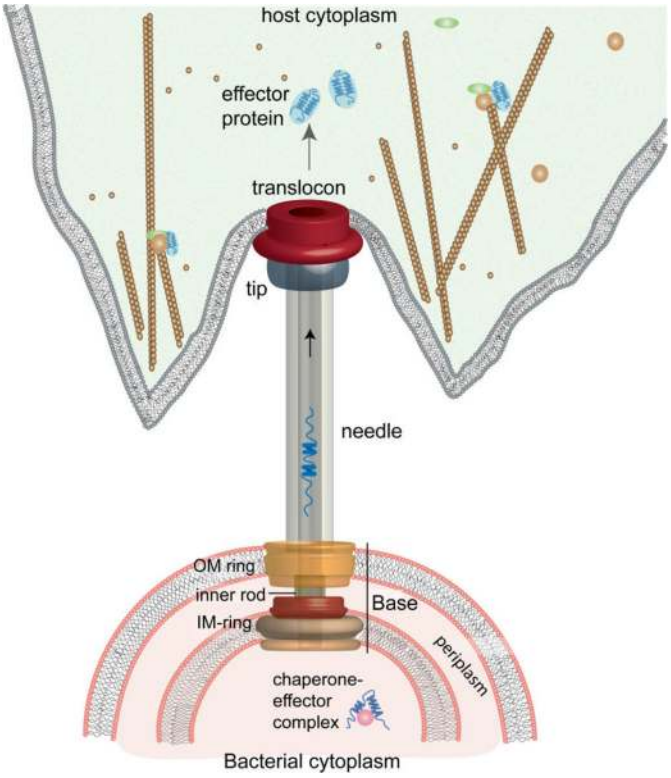

Figure 1.
Cartoon of the type III secretion system showing the needle apparatus in contact with the host cell.

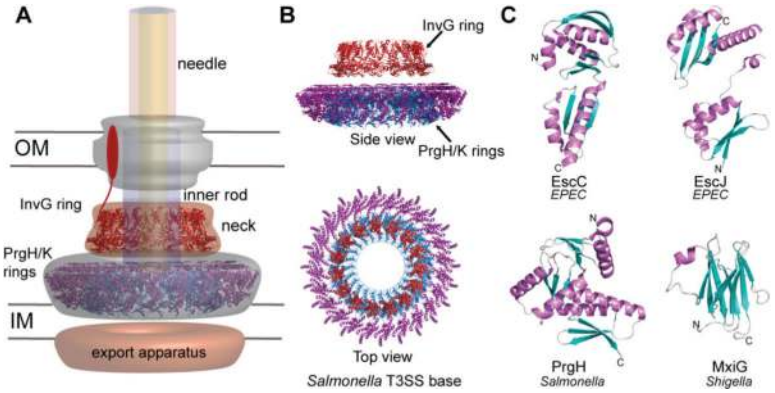

Figure 2.

(A) *Salmonella* T3SS basal structure that spans the outer membrane (OM), the periplasm, and the inner membrane (IM) and is formed by (B) rings of InvG (red), PrgH (purple), and PrgK (blue).[20] (C) Structures of proteins that form the T3SS basal body: EPEC EscC,[21] EPEC EscJ,[18] *Salmonella* PrgH,[21] and *Shigella* MxiG.[22]

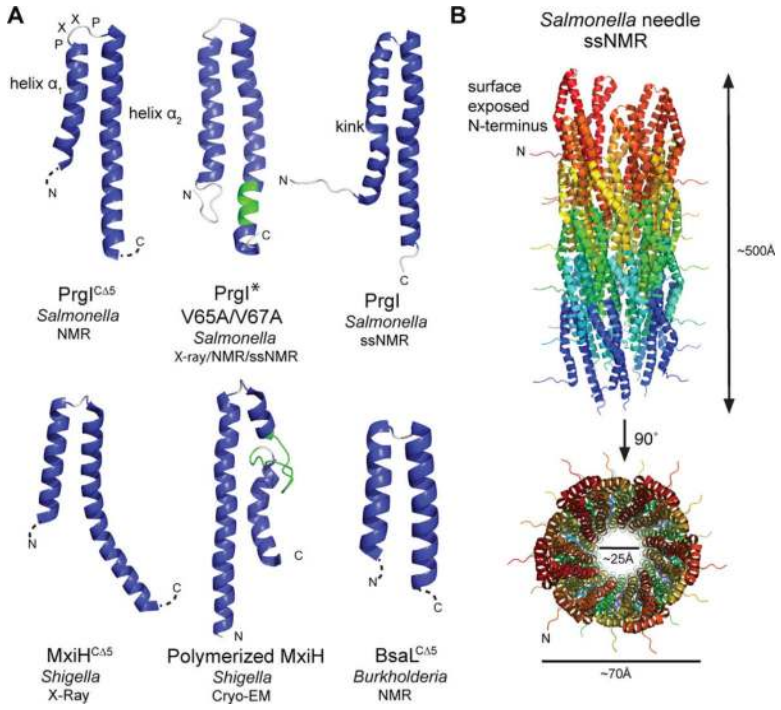

Figure 3.

(A) Structures of needle protomers *Salmonella* PrgI$^{C\Delta5}$ (CΔ5, deletion of five C-terminal residues),[42] PrgI* (a functional V65A/V67A double mutant),[43] PrgI polymerized into needles,[48] *Shigella* MxiH$^{C\Delta5}$,[40] MxiH polymerized into needles,[47] and *Burkholderia* BsaL$^{C\Delta5}$.[41] Regions that adopted β-strands upon needle assembly are colored green. (B) Atomic model of the *Salmonella* needle derived by solid-state NMR showing that the N-terminus of PrgI faces the exterior.[48]

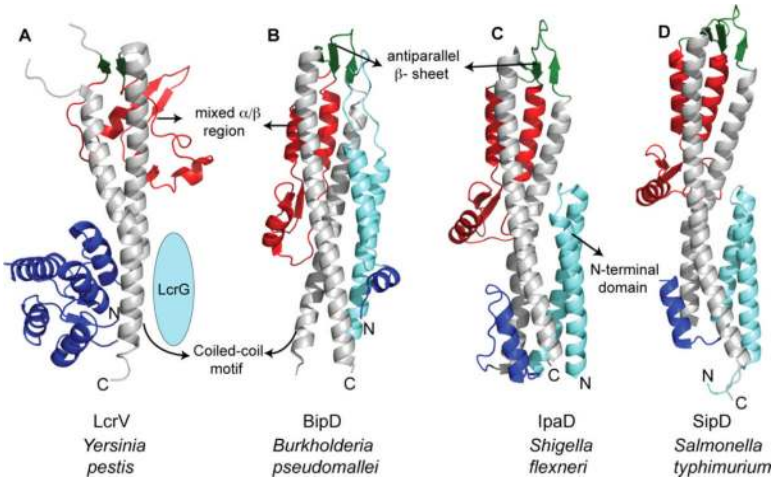

Figure 4.

Structure of tip proteins from (A) *Y. pestis*,[60] (B) *Burkholderia pseudomallei*,[54] (C) *S. flexneri*,[53] and (D) *S. typhimurium*.[55]

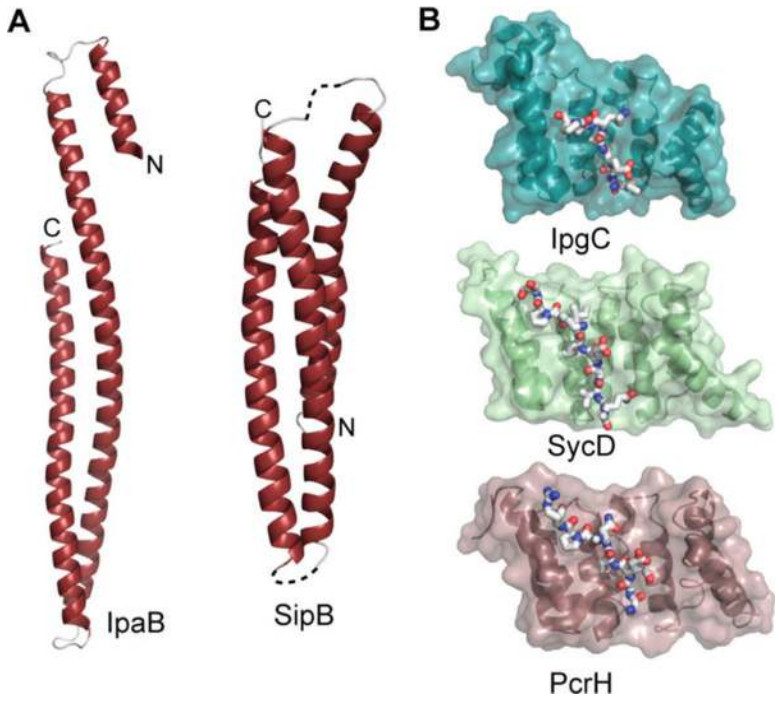

Figure 5.
(A) Structures of the N-terminal domains of the *Shigella* IpaB and *Salmonella* SipB translocon proteins.[96] (B) Cocrystal structures of IpgC,[115] SycD,[113] and PcrH[90] chaperones bound to peptides derived from their cognate translocon proteins.

Table 1

Structural Proteins of the T3SS Needle Apparatus

role	*Salmonella*	*Shigella*	*Yersinia*	EPEC	*Pseudomonas*
translocon	SipB	IpaB	YopB	EspB	PopB
translocon	SipC	IpaC	YopD	EspD	PopD
needle tip	SipD	IpaD	LcrV	EspA	PcrV
needle	PrgI	MxiH	YscF	EscF	PscF
OM ring	InvG	MxiD	YscC	EscC	PscC
inner rod	PrgJ	MxiI	YscI	EscI	PscI
IM ring	PrgK	MxiJ	YscJ	EscJ	PscJ
	PrgH	MxiG	YscD	EscD	PscD
export apparatus	SpaP	Spa24	YscR	EscR	PscR
	SpaQ	Spa9	YscS	EscS	PscS
	SpaR	Spa29	YscT	EscT	PscT
	SpaS	Spa40	YscU	EscU	PscU
	InvA	MxiA	YscV	EscV	PerD
ATPase	InvC	Spa47	YscN	EscN	PscN