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Structural Features Reminiscent of ATP-Driven Protein Translocases Are Essential for the Function of a Type III Secretion-Associated ATPase

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ABSTRACT

Many bacterial pathogens and symbionts utilize type III secretion systems to interact with their hosts. These machines have evolved to deliver bacterial effector proteins into eukaryotic target cells to modulate a variety of cellular functions. One of the most conserved components of these systems is an ATPase, which plays an essential role in the recognition and unfolding of proteins destined for secretion by the type III pathway. Here we show that structural features reminiscent of other ATP-driven protein translocases are essential for the function of InvC, the ATPase associated with a *Salmonella enterica* serovar Typhimurium type III secretion system. Mutational and functional analyses showed that a two-helix-finger motif and a conserved loop located at the entrance of and within the predicted pore formed by the hexameric ATPase are essential for InvC function. These findings provide mechanistic insight into the function of this highly conserved component of type III secretion machines.

IMPORTANCE

Type III secretion machines are essential for the virulence or symbiotic relationships of many bacteria. These machines have evolved to deliver bacterial effector proteins into host cells to modulate cellular functions, thus facilitating bacterial colonization and replication. An essential component of these machines is a highly conserved ATPase, which is necessary for the recognition and secretion of proteins destined to be delivered by the type III secretion pathway. Using modeling and structure and function analyses, we have identified structural features of one of these ATPases from *Salmonella enterica* serovar Typhimurium that help to explain important aspects of its function.

Type III secretion machines are essential for the interaction of many bacterial pathogens and symbionts with their respective hosts (1–3). They exert their functions by delivering into eukaryotic cells bacterial effector proteins with the capacity to modulate a variety of eukaryotic host cell functions (4). Although the effector proteins delivered by these machines are highly variable and customized for the biology of the bacteria that encode them, the constituents of the secretion machine are highly conserved (5). The core component of this machine is the needle complex, a supramolecular structure that mediates the passage of the type III secreted proteins through the bacterial envelope (6, 7). The needle complex is composed of a multiring base that is anchored to the bacterial envelope and a filament-like structure, the needle, which protrudes from the bacterial surface and is linked to the base by another substructure, the inner rod.

Assembly of the needle complex proceeds in a stepwise fashion such that upon completion of the base substructure, the secretion machinery subsequently engages the inner rod and needle filament protein subunits to build these structures. Once assembled, the type III secretion machine switches specificity and no longer engages the inner rod and needle filament subunits and begins the process of secreting effector proteins. These proteins must travel through a secretion channel with a diameter of ~20 Å, a size too narrow to fit a fully folded protein, which dictates that proteins moving through the channel must be completely or partially unfolded (8).

Type III secreted proteins are targeted to the secretion machine by at least two secretion signals (9). One of the signals resides at the extreme amino terminus of the secreted proteins, while the other is provided by customized chaperones that bind at a specific domain of their cognate effector protein, keeping it in an extended configuration (10). How the secretion machine recognizes substrates is incompletely understood, although it is clear that there is an order in which secreted proteins are engaged, a mechanism that involves a cytoplasmic sorting platform (11). Proteins that form a translocation pore within the eukaryotic cell membrane are secreted first, followed by the secretion of the effector proteins that are to be delivered into target cells.

A highly conserved cytoplasmic hexameric ATPase is essential for the recognition and targeting of type III secreted proteins to the secretion machine (12–14). Although structurally more closely related to the F_oF_1 family of ATPases (15–17), studies have identified functional similarities between this ATPase and other ATP-driven unfoldases. For example, type III secretion-associated ATPases have been shown to interact with chaperone-effector complexes (18, 19), a process thought to be essential in substrate

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recognition. In addition, *in vitro*, these ATPases have been shown to be able to disassociate the chaperone from its cognate effector and unfold the effector protein (18, 20, 21). Here through structure modeling and biochemical analyses of InvC, the ATPase associated with one of the type III secretion systems (T3SS) of *Salmonella enterica* serovar Typhimurium, we have identified structural features reminiscent of similar motifs identified in ATP-driven protein translocases. These features are a two-helix-finger motif located at the entrance of the predicted pore formed by the hexameric ATPases and a conserved loop located within the predicted lumen of the pore. We showed here by mutagenesis and functional analyses that these motifs are essential for InvC function. Our findings provide mechanistic insight into the function of this highly conserved component of type III secretion machines.

MATERIALS AND METHODS

Strains and plasmids. All bacterial strains used in this study are derived from *Salmonella enterica* serovar Typhimurium SL1344 and are listed in Table S1 in the supplemental material. The plasmids used in this study are listed in Table S2 in the supplemental material. Genetic modifications were introduced into *S.* Typhimurium by allelic exchange using R6K suicide vectors as previously described (22).

Structure modeling of InvC. The nucleotide-free and ATP-bound forms of InvC were modeled based on the crystal structures of EscN, a T3SS ATPase from enteropathogenic *Escherichia coli* (EPEC) (PDB no. 2OBM and 2OBL) with SWISS-MODEL (http://swissmodel.expasy.org). Hexameric forms were obtained by fitting the monomeric structures of InvC to a crystal structure of the F_1 ATPase hexamer (PDB no. 1BMF) with DeepView (Swiss-PdbViewer), and graphic representations were generated with PyMOL.

Type III protein secretion assay. Strains were grown to the late logarithmic phase in Luria broth supplemented with 0.3 M NaCl. Secreted proteins were obtained from supernatants of 5-ml cultures by trichloroacetic acid (TCA) precipitation as previously described (23). Bacterial cell lysates were prepared from 1 ml of culture by boiling the cell pellets in SDS-PAGE sample buffer. The presence of proteins in the different samples was probed by Western immunoblotting with antibodies specific to the relevant proteins or the epitope tags that had been attached to them.

Preparation and EM analyses of the needle complex. Needle complexes were purified from the relevant strains as described before (6, 24). Samples were applied to glow-discharged carbon-coated Cu grids followed by staining with 2% phosphotungstic acid (pH 7.0) and imaging by electron microscopy (EM). Images were acquired with a Tecnai Biotwin transmission electron microscope (FEI Company, Hillsboro) at 80 kV using the Morada Soft Imaging system and 6M pixel charge-coupled device (CCD) camera (Olympus, Munster, Germany).

Protein purification. His_{10}-InvC-3×Flag/OrgB-3×Flag complexes were expressed in *E. coli* BL21(DE3) harboring pET26-based plasmids. Bacterial cultures were grown at 30°C, and cells were harvested by centrifugation, resuspended in lysis buffer (20 mM HEPES-KOH [pH 7.4], 200 mM KCl, 10% glycerol, 10 mM imidazole, 1 mg/ml lysozyme, 10 μg/ml DNase I, 0.5 mM $MgSO_4$, cOmplete EDTA-free [Roche]), and lysed in a French press. The lysates were spun down, and the supernatants were mixed with Ni-nitrilotriacetic acid (NTA)–agarose and incubated for 2 h at 4°C with gentle shaking. The beads were washed with lysis buffer containing 40 mM imidazole, and bound proteins were eluted with the same buffer containing 500 mM imidazole and further purified on a Superose 6 column (GE Healthcare Life Sciences).

ATPase activity assay. The ATPase activity assay was carried out as described previously (14). Briefly, reactions were carried out at 37°C for 30 min in mixtures containing 20 mM HEPES (pH 7.4), 200 mM KCl, 10% glycerol, 0.5 mg/ml bovine serum albumin (BSA), 4 mM ATP, 4 mM $MgCl_2$, 2.5 mM dithiothreitol (DTT), and 0.01 mg/ml InvC. Reactions were stopped by adding malachite green-ammonium molybdate reagent and citric acid. The optical density at 660 nm was measured after 60 min of incubation at room temperature, and a standard curve was generated using various amount of KH_2PO_4.

RESULTS

Modeling of the InvC homohexamer reveals structural features reminiscent of ATP-driven protein translocases. Type III secretion-associated ATPases form an hexameric ring (25). Although the crystal structures of the monomeric forms of several type III secretion ATPases have been solved (15–17), the atomic structure of their hexameric form is not available. We therefore modeled the atomic structure of the InvC monomer using the crystal structure of its close homolog EscN (both its nucleotide-free and nucleotide analog-bound forms) (16) and subsequently modeled its hexameric form by fitting the monomeric structure into the hexamer of the F_1 ATPase (Fig. 1). We examined the modeled structure for features that have been shown to be important for the function of other ATP-driven protein translocases and unfoldases. Structure and function studies have shown that a helix-loop-helix motif, sometimes referred as a "two-helix finger," is critical for the function of SecA and other ATP-driven protein translocases (26, 27). This motif is composed of a tyrosine or other bulky hydrophobic residues positioned within a loop that is bounded by two helixes (26–28). Residues within the loop are thought to make contact with the protein substrates and direct them to a protein translocation pore (in the case of SecA) or to the center of the hexameric ring for translocation to the protease compartment (in the case of AAA+ protein translocating/unfolding ATPases). We located a putative "two-helix-finger" motif at the entrance of the modeled central pore of the InvC hexamer (Fig. 1). The motif is highly conserved among type III secretion-associated ATPases, including those associated with the flagellar assembly apparatus. In addition to the tyrosine residue (Tyr385 in InvC) located at the center of the loop, other residues within the loop are highly conserved, such as Gly383, Glu384, and Gly388.

In addition to the two-helix-finger motif, we found a conserved loop emerging from each InvC monomer and protruding into the central pore of the hexameric InvC ring (Fig. 1). This loop is reminiscent of equivalently positioned loops in AAA+ ATPases, which are thought to drive translocation and unfolding of their substrates (29). The loop consists of several acidic residues, some of which are conserved across family members (e.g., E308 and D312), while others exhibit similar charges.

In other protein translocases/unfoldases, it is thought that the movement of the loop driven by conformational changes triggered by nucleotide hydrolysis is ultimately responsible for substrate translocation or unfolding (28, 30–34). To ascertain whether the identified domains in InvC could potentially undergo equivalent ATP hydrolysis-driven conformational changes, we compared the modeled hexameric ring of InvC in its nucleotide analog-bound and native configurations. We found that the modeled structures predict that the side chain of the tyrosine within the loop of the "two-helix-finger" domain would undergo a significant conformational change by moving around the axis of the loop (Fig. 1E; see Video S1 in the supplemental material). Such movement would be consistent with equivalent conformational changes observed in other ATPases that use this domain to carry out protein translocation or unfolding functions. Similarly, modeling predicts that the loop located within the lumen of InvC would also undergo conformational changes as a consequence of

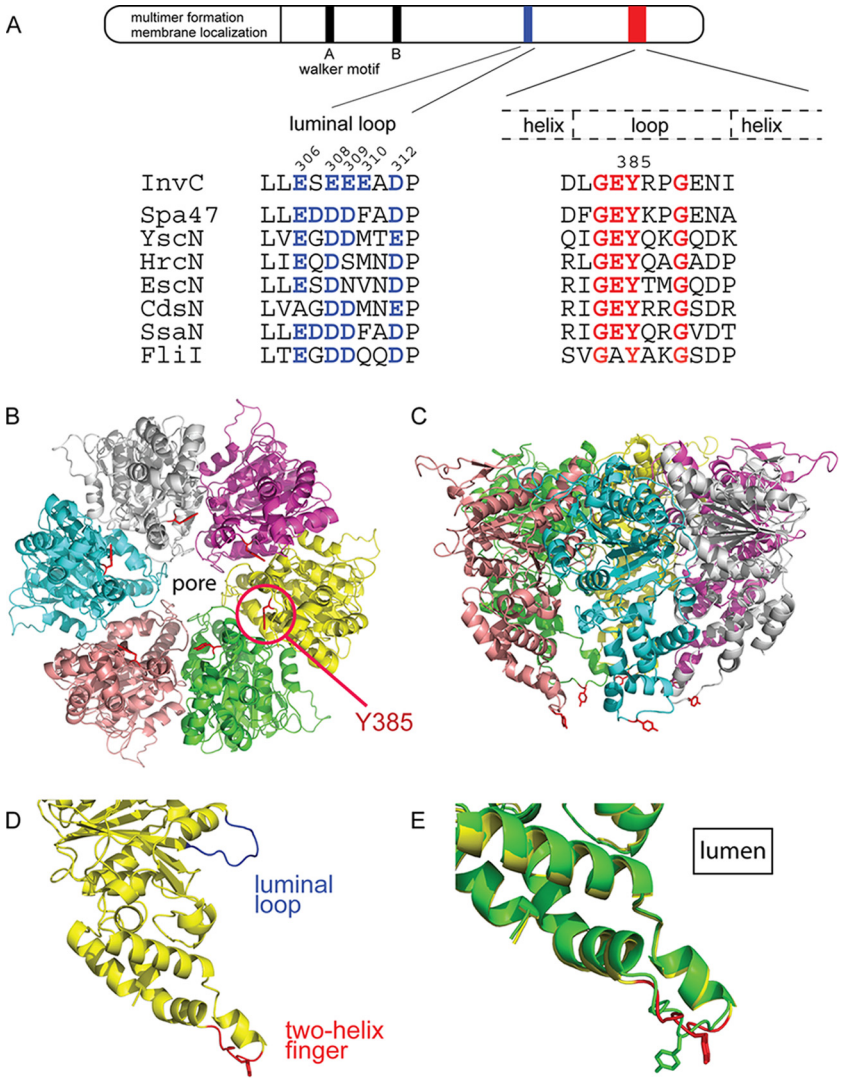

FIG 1 Modeling of the InvC hexamer reveals structural features reminiscent of ATP-driven protein translocases. (A) Schematic representation of InvC. Sequence alignments show the two-helix-finger motifs and the luminal loop domains of type III secretion- and flagellar export-associated ATPases. The sequences used in the alignment are as follows: InvC, SsaN, and FliI from *Salmonella enterica* serovar Typhimurium, Spa47 from *Shigella flexneri*, YscN from *Yersinia enterocolitica*, HrcN from *Pseudomonas syringae*, EscN from *Escherichia coli*, and CdsN from *Chlamydophila pneumoniae*. Conserved residues in the two-helix-finger motif and acidic residues within the luminal loop are shown in red and blue, respectively. (B) Hexamer model of InvC viewed from its C-terminal end. The Tyr385 side chain is highlighted in red. (C) Side view of the InvC hexamer model. (D) The two-helix-finger motif and the luminal loop domain are shown on an InvC monomer. (E) An apo structure of InvC (shown in green) is overlaid on the ATP-bound InvC shown in panel D. A change in angle around the axis is seen at the side chain of Tyr385.

ATP hydrolysis, although of much smaller magnitude. Therefore, based on the modeling, we hypothesize that the predicted two-helix-finger domain and luminal loop of InvC may be critical for its function.

Mutagenesis analyses of the two-helix-finger domain of InvC reveals its importance in type III secretion and needle complex assembly. Studies of other ATP-driven protein translocases have established that a tyrosine residue within the loop of the two-helix-finger motif is important for function (26, 27, 29, 34, 35). To ascertain the potential functional relevance of the two-helix-finger domain identified in InvC, we carried out mutagenesis analyses of the conserved tyrosine. We first changed tyrosine 385 to alanine, and to ensure wild-type levels of expression, we introduced the mutated allele into the *S.* Typhimurium chromosome. We evaluated the function of the InvC^{Y385A} mutant by examining the secretion of proteins in the various hierarchical classes in the type III secretion process. We monitored the secretion of the regulatory protein InvJ (early substrate), the translocases SipB, SipC, and SipD (middle substrate), and the effector proteins SptP and SopB (late substrates). No defects were observed in the secretion of both early and middle substrates (Fig. 2A). In contrast, the *S.* Typhimurium mutant strain expressing InvC^{Y385A} showed a marked defect in its ability to secrete the effector proteins SptP and SopB (Fig. 2A). In all cases, expression of the substrate proteins was unaffected by introduction of mutations in InvC. Proteins destined to travel the type III secretion pathway are bound by customized exclusive chaperones or by chaperones that bind several effector proteins. Chaperones that bind a single effector, such as those that

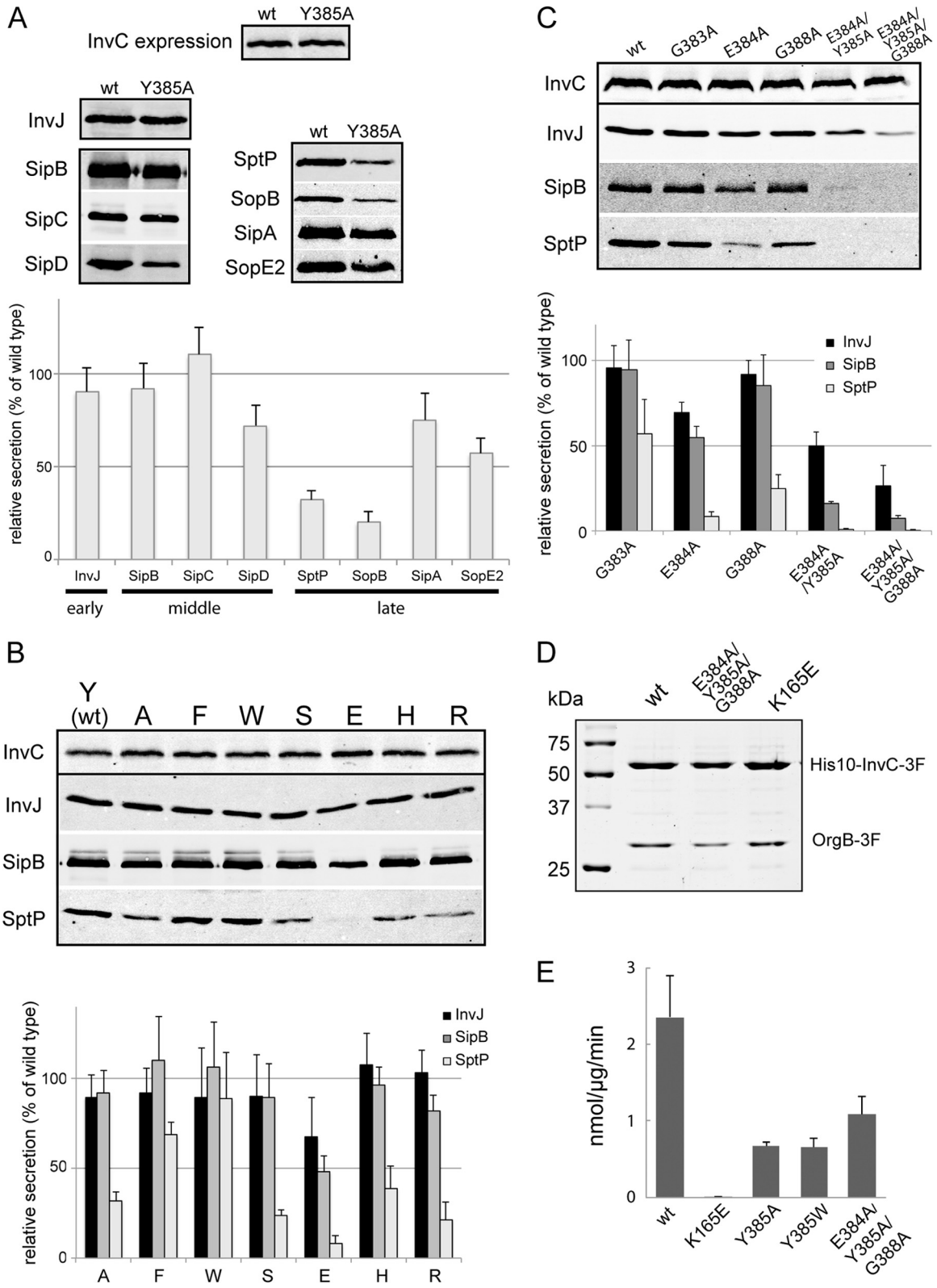

FIG 2 A two-helix-finger motif in InvC is essential for type III secretion. (A) Secretion profile of the Y385A mutant for the indicated T3SS substrates. InvC expression levels in the whole-cell lysates were visualized by Western immunoblotting. Levels of the T3SS substrates in the culture supernatants were also analyzed by Western immunoblotting and quantified with a LICOR-Odyssey system. Values in the lower panel are normalized to the wild type (wt) and represent the mean ± standard deviation from three independent observations. InvJ, early substrate; SipB, SipC, and SipD, middle substrates (translocators); SptP, SopB, SipA, and SopE2, late substrates (effectors). (B) Effect of replacing tyrosine 385 of InvC with the indicated amino acids on type III secretion. Analyses of the levels of InvC in whole-cell lysates and the indicated type III secretion substrates in culture supernatants were carried out as indicated for panel A. (C) Effect of replacing

chaperoned SopB—(i.e., SigE) (36) and SptP (i.e., SicP) (37)—form high-affinity complexes because they cover a large surface area of the chaperoned effectors (38). In contrast, multieffector chaperones exhibit a much less intimate interface and hence bind with lower affinity (39). To investigate whether the defect of InvC^{Y385A} mutant was specifically restricted to the secretion of effectors chaperoned by exclusive chaperones, we examined the secretion of the effector proteins SipA and SopE2, which are chaperoned by the multieffector chaperone InvB (40, 41). We found that the secretion of SipA and SopE2 was only marginally reduced in the *S.* Typhimurium strain expressing the InvC^{Y385A} mutant, suggesting that Tyr385 may be more important for the secretion of effectors that have a more intimate association with their chaperones (Fig. 2A). Previous mutagenesis studies of other two-helix-finger motifs have shown that the nature of the amino acid used to replace the critical tyrosine within the loop impacts the phenotype of the resulting mutant. We therefore replaced Tyr385 with different amino acids and examined the type III secretion phenotype of the mutants. We found that replacing Tyr385 with aromatic amino acids, such as phenylalanine or tryptophan, did not alter InvC function (Fig. 2B). This finding is consistent with previous observations made in other ATP-driven protein translocases (26, 27). In contrast, replacing Tyr385 with serine, histidine, or arginine showed a defect in the secretion of SptP but not in the secretion of early (InvJ) or middle (SipB) substrates (Fig. 2B). Replacing Tyr385 with negatively charged glutamic acid resulted in a stronger phenotype, almost completely abrogating the secretion of SptP and also affecting the secretion of early and middle substrates (Fig. 2B). In addition to the tyrosine residue at the center of the loop of the two-helix-finger motif of InvC, there are other highly conserved residues within the loop, such as G383, E384, and G388. We changed these residues to alanine and examined the resulting mutants for their ability to secrete early, middle, and late substrates of the T3SS (Fig. 2C). While mutations in each of the residues individually resulted in various levels of secretion defect, a combination of mutations in the loop residues resulted in a strain with a marked secretion defect for early, middle, and late substrates. To rule out that introduction of the mutations in the two-helix-finger domain of InvC could have resulted in gross changes in its overall conformation, we investigated additional InvC phenotypes that could detect such a possibility. We found no differences in the ability of InvC to form hexamers or to bind OrgB, an interacting protein that is required for its function (Fig. 2D). In addition, we compared the ATPase activities of the different InvC mutants. We found that some of the mutants exhibited slightly reduced levels of ATPase activity (Fig. 2E). However, the secretion defect observed in these mutants cannot be the result of this small reduction in ATP-hydrolyzing activity since a mutant strain expressing InvC^{Y385W}, which displayed an even greater reduction in its ATPase activity, exhibited a secretion profile identical to that of the wild type (Fig. 2E and B). These results showed that introduction of the different mutations in InvC did not alter its overall conformation and therefore indicate that the integrity of the loop of the two-helix-finger motif is essential for InvC function.

Type III protein secretion is essential for the assembly of the needle complex since assembly of the needle and inner rod substructures requires a competent type III secretion machine. Therefore, we examined needle complexes obtained from strains expressing different InvC mutants in the two-helix-finger motif. We observed a close correlation between the secretion phenotypes and needle complex assembly, and mutants that were affected in the secretion of early substrates showed defective needle complex assembly. For example needle complex preparations obtained from a strain expressing the InvC$^{E384A\ Y385A\ G388A}$ triple mutant showed an abundance of bases but very few complete structures (Fig. 3A). Consistent with this phenotype, analyses of these structures by Western blotting showed a marked reduction of the inner rod protein PrgJ (Fig. 3B). In contrast, needle complex preparations obtained from *S.* Typhimurium strains expressing InvC alleles that were not affected in early substrate secretion (InvC^{Y385A}) showed apparently normal structures containing wild-type levels of the inner rod protein PrgJ (Fig. 3B).

Taken together, these results indicate that the Tyr385 as well as other residues within the loop of the two-helix-finger motif are required for InvC function and highlight similarities between type III secretion ATPases and other ATP-driven protein translocases/unfoldases.

Residues predicted to form a loop within the pore of the InvC hexamer are required for function. Many ATP-driven translocases of the AAA+ family display loops that project into the lumen of their hexameric pores and are thought to propel substrates as they move through the central pore. Using structural modeling, we detected equivalently positioned loops within the lumen of the modeled InvC hexameric structure (Fig. 1A to D). Although there is no experimental evidence demonstrating that T3SS substrates move through the central pore of the type III secretion-associated ATPases, the presence of these predicted loops is intriguing. We therefore carried out a mutagenesis and functional analyses of the residues involved in forming this intraluminal loop. We found that introduction of mutations targeting this loop (i.e., InvC^{E306A}, InvC^{E308A}, InvC^{E309A}, or InvC^{E310A}) showed a significant defect in their ability to secrete a late substrate (SptP), although there were no defects in the secretion of early (InvJ) or middle (SipB) substrates (Fig. 4A). Introduction of these mutations did not affect their ability to multimerize or bind OrgB (Fig. 4B). In addition, these mutants exhibited levels of ATPase activity compatible with wild-type function, suggesting that the observed defects in secretion cannot be due to defective ATPase activity (Fig. 4C). However, one mutation in this loop (InvC^{D312A}) significantly affected the ATPase activity of InvC, and consequently the mutant was severely defective for the secretion of early, middle, and late substrates. This specific residue is located near the interface of the InvC protomers in the hexamer; thus, this mutation most likely affected the cooperative interaction of the protomers required for efficient ATP hydrolysis. Taken together, these results suggest that

conserved amino acids within the loop of the two-helix-finger motif, individually or in combination, with alanine. Analyses of the levels of InvC in whole-cell lysates and the indicated type III secretion substrates in culture supernatants were carried out as indicated for panel A. (D) Interaction of the indicated InvC mutants with OrgB. His-tagged InvC, coexpressed with OrgB in *E. coli*, was isolated by Ni affinity chromatography followed by size exclusion chromatography. The SDS-PAGE gel was stained with Coomassie brilliant blue. (E) ATPase activity of InvC wild-type and mutant proteins. ATPase activity was measured by a malachite green assay. Note that the Y385W mutation did not affect secretion, indicating that the level of ATPase activity of the Y385W mutant is sufficient for InvC function.

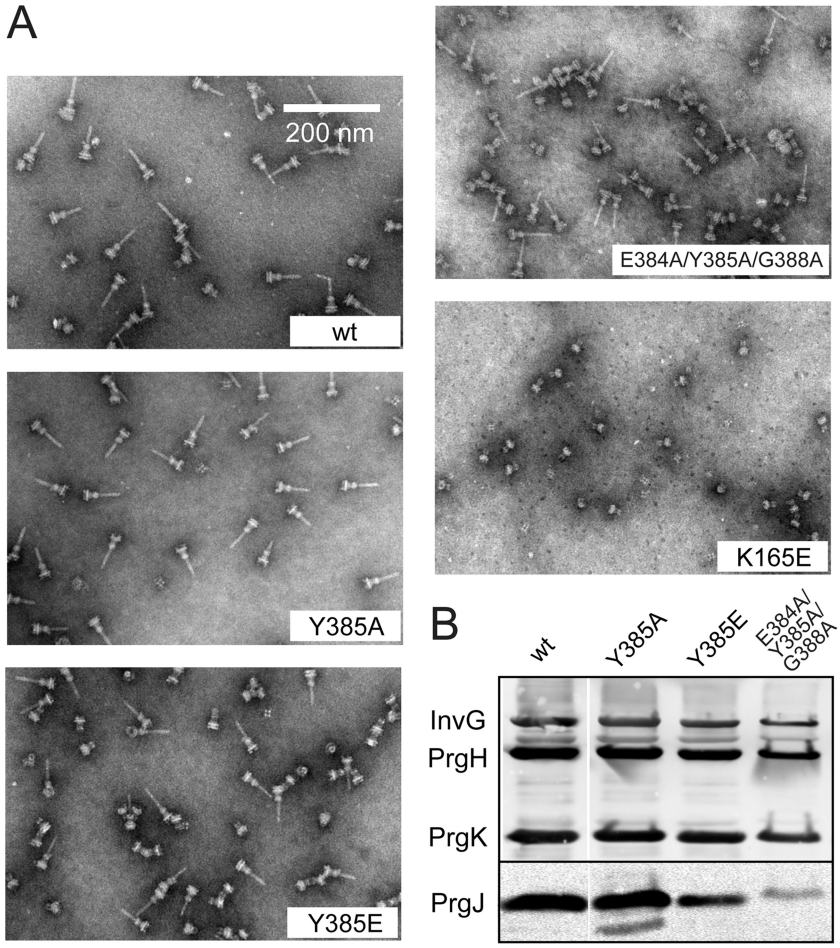

FIG 3 Effect of mutations on the two-helix-finger motif of InvC on needle complex assembly. (A) Electron micrographs of needle complexes prepared from *S.* Typhimurium strains expressing the indicated InvC mutants. Apparently normal needle complex structures were seen in strains expressing the InvC loop mutants, whereas only base structures were observed in strains expressing a catalytic mutant (the K165E mutant). (B) Efficiency of the inner rod protein PrgJ incorporation into needle complexes, a measure of the completion of their assembly, obtained from strains expressing the indicated InvC mutants. Fractions containing the needle complexes used for EM analyses were subjected to SDS-PAGE followed by Western immunoblotting. Sample loading was standardized by the amount of the base components (InvG, PrgH, and PrgK).

the internal loop within the predicted InvC pore is important for its function.

DISCUSSION

All type III secretion systems have a highly conserved hexameric ATPase, which is essential for their function. This ATPase is thought to be critical for substrate recognition, effector/chaperone complex disassembly, and effector unfolding prior to secretion (18). However, how these functions are specifically carried out is not understood. Other ATP-driven unfoldases are thought to use the energy of ATP hydrolysis to drive substrates through their hexameric pore, a process dependent on a series of highly coordinated conformational changes in specific loops located within the lumen and at the entrance of the central pore (28). These loops are thought to contact the unfolding substrates and propel their movement through the pore. To what extent, if any, the mechanisms of action of type III secretion-associated ATPases may resemble those of other ATP-driven protein translocases is not known. Using structural modeling and functional analyses, we have described here structural features in InvC, a T3SS-associated ATPase from *S.* Typhimurium, that are reminiscent of equivalent features in other ATP-driven protein translocases. One of these features is a two-helix-finger motif located at the entrance of the predicted InvC hexameric pore, which is similar to a motif present in other protein translocases, such as SecA. This motif undergoes conformational changes coupled to ATP hydrolysis that are thought to propel protein substrates to the translocation channel. Similarly, by comparing the modeled hexameric structures of InvC in its ATP-bound and nucleotide-free forms, we observed a significant conformational change on a critical tyrosine residue within the central loop of this motif. An equivalent tyrosine residue in SecA is also predicted to undergo a similar conformational change, which is critical for its function. Notably, this motif is highly conserved among T3SS-associated ATPases, including those associated with flagellar assembly. A detailed mutagenesis analyses indicated that this motif, including the critical tyrosine residue itself, is essential for InvC function. Additional biochemical and functional analyses indicated that introduction of the mutations did not

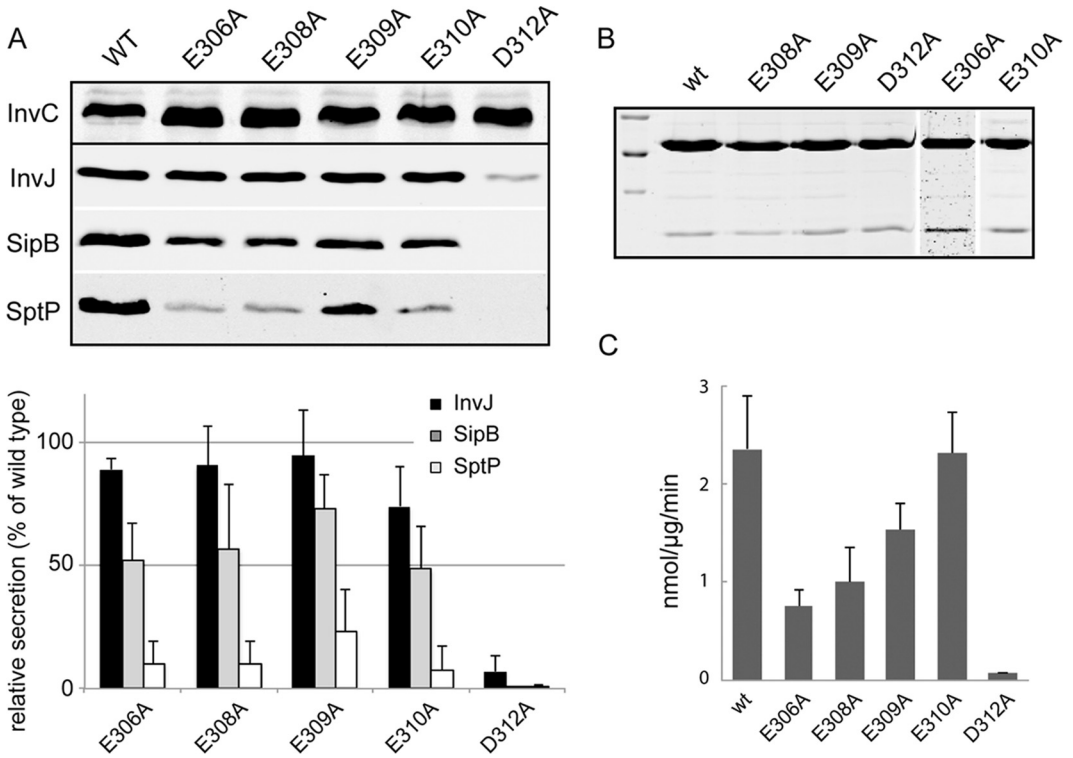

FIG 4 The luminal loop of the InvC hexameric pore is required for type III secretion. (A) Acidic residues of the luminal loop of InvC were individually replaced with alanine, and the impacts on type III secretion and InvC stability were analyzed as indicated in the Fig. 2 legend. (B and C) Effects of the indicated InvC mutations on its ability to bind OrgB (B) or its catalytic activity (C). These experiments were carried out as indicated in the Fig. 2 legend.

affect the overall conformation of InvC, further supporting the functional importance of this motif. It is unclear what the specific role of this motif in InvC function might be. InvC has been shown to be involved in dissociating the chaperone-effector complexes prior to secretion. It is therefore possible that this domain has a role in this process. Consistent with this hypothesis, the two-helix-finger domain in InvC is located in close proximity to its predicted site of interaction with the chaperone-effector complex. Furthermore, the phenotypes associated with mutations in this domain were much stronger for the secretion of effectors that exhibit much higher association affinity with their cognate specific chaperones. Indeed, the secretion of SptP and SopB, which have customized chaperones, was affected more significantly than the secretion of effectors that, like SipA and SopE2, are chaperoned by lower-affinity multieffector chaperones and thus may be less susceptible to alterations in this motif.

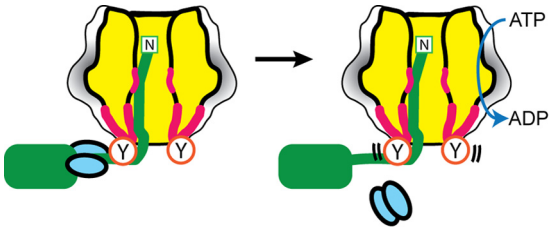

FIG 5 Model of the functional role of the InvC loops. The two-helix finger and the luminal loop (red) may help in engaging the T3SS chaperone/substrate complexes, possibly by interacting with the amino-terminal secretion signal (chaperone, blue; substrate, green). The side chain of Tyr385 is depicted in orange. The movement of the Tyr385 side chain by cycles of ATP binding, hydrolysis, and ADP release may provide mechanical force to release the chaperone from its substrate.

We also identified an additional loop located within the InvC central pore. This loop resembles an equivalent structural feature in other ATP-driven protein translocases/unfoldases that is required for substrate unfolding and mobilization through the central pore. We found that mutations in this loop selectively affected the secretion of SptP, without affecting the secretion of early and middle substrates. These observations suggest a specific role for this loop in the secretion of effector proteins that, like SptP, have a customized chaperone whose removal may require a much tighter gripping of the complex by the ATPase. It is possible that the amino-terminal secretion signal of the effector protein enters the lumen of the ATPase ring, thus providing a better grip on its substrate (Fig. 5). More experiments will be required to validate this hypothesis.

In summary, we have identified structural features in InvC that are reminiscent of similar motifs found in other ATP-driven protein translocases, thus suggesting potential parallels between the mechanisms of action of these evolutionarily related machines.

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