

The structure and mechanism of the bacterial type II secretion system

Souvik Naskar  | Michael Hohl  | Matteo Tassinari  | Harry H. Low 

Department of Infectious Disease, Imperial College, London, UK

Correspondence

Harry H. Low, Department of Infectious Disease, Imperial College, London, UK.
Email: h.low@imperial.ac.uk

Funding information

Wellcome Trust, Grant/Award Number: 215553/Z/19/Z; Wellcome Trust, Grant/Award Number: 215553 and 19

Abstract

The type II secretion system (T2SS) is a multi-protein complex used by many bacteria to move substrates across their cell membrane. Substrates released into the environment serve as local and long-range effectors that promote nutrient acquisition, biofilm formation, and pathogenicity. In both animals and plants, the T2SS is increasingly recognized as a key driver of virulence. The T2SS spans the bacterial cell envelope and extrudes substrates through an outer membrane secretin channel using a pseudopilus. An inner membrane assembly platform and a cytoplasmic motor controls pseudopilus assembly. This microreview focuses on the structure and mechanism of the T2SS. Advances in cryo-electron microscopy are enabling increasingly elaborate sub-complexes to be resolved. However, key questions remain regarding the mechanism of pseudopilus extension and retraction, and how this is coupled with the choreography of the substrate moving through the secretion system. The T2SS is part of an ancient type IV filament superfamily that may have been present within the last universal common ancestor (LUCA). Overall, mechanistic principles that underlie T2SS function have implication for other closely related systems such as the type IV and tight adherence pilus systems.

KEYWORDS

assembly platform, cryo-EM, secretin, structure, type II secretion system, type IV pilus system

1 | INTRODUCTION

The T2SS is a macromolecular complex that spans the cell envelope of many Gram-negative bacteria. It is part of a much larger superfamily of type IV filament containing systems all of which share homologous components and conserved mechanistic principles. These include the type 4a and 4b pilus (Craig et al., 2019), tight adherence (Tad) pilus (Tomich et al., 2007), mannose-sensitive hemagglutinin pilus (Marsh and Taylor, 1999), competence pilus

(Piepenbrink, 2019) and archaeal T4 pilus and flagellum (Makarova et al., 2016). These systems are ancient and fundamental to both bacterial and archaeal kingdoms with phylogeny analyses suggesting a lineage split from within the last universal common ancestor (LUCA) (Denise et al., 2019). The T2SS is particularly concentrated in the Alpha-, Beta-, Gamma- and Delta-proteobacteria, as well as the Bacteroidetes and Deferribacteres (Denise et al., 2019). It is a key virulence factor in many human pathogens including *Acinetobacter baumannii* (Elhosseiny and Attia, 2018), *Klebsiella*

Souvik Naskar and Michael Hohl are the authors contributed equally.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Molecular Microbiology* published by John Wiley & Sons Ltd

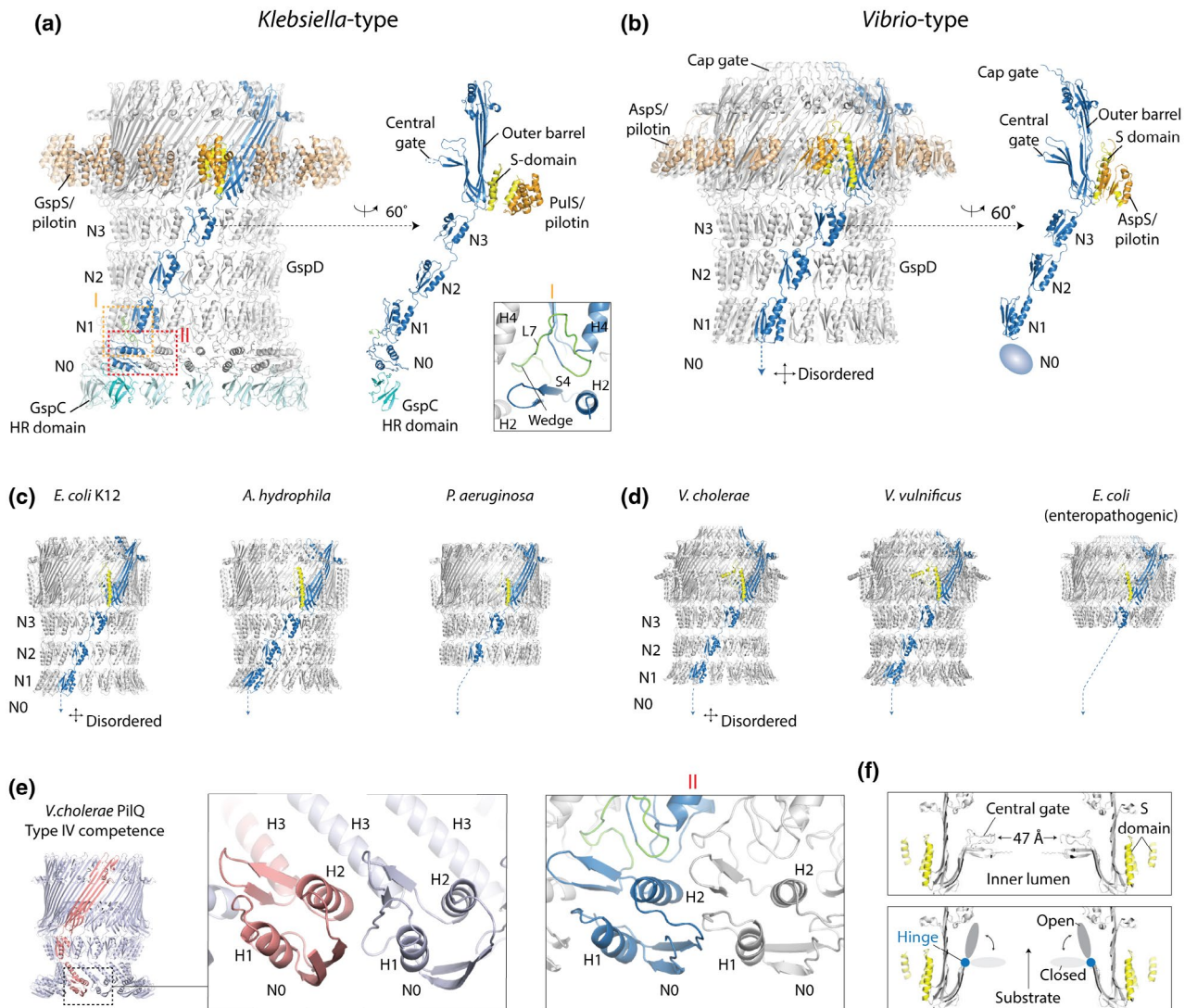


FIGURE 1 Type II secretion system outer membrane complex. (a) Structure of a *Klebsiella*-type outer membrane complex (PDB code 6HCG). The GspD/PulD secretin from *K. pneumoniae* is bound to the pilotin GspS/PulS. At the base of the secretin, the N0 domain is bound to the GspC/PulC HR domain. A single GspD, GspS, and GspC HR domain complex is extracted (right). Zoom box (I) shows region of the secretin outlined by an orange dotted rectangle (I). The Loop 7 (L7, green strand) stabilizing linker connecting N0 and N1 domains forms a wedge that promotes ordered N0-N1 domain ring formation. (b) Structure of a *Vibrio*-type outer membrane complex (PDB code 5ZDH). The GspD secretin from enterotoxigenic *Escherichia coli* (ETEC) is bound to the pilotin AspS. The N0 domain is disordered. Note the distinguishing secretin cap gate. A single GspD and AspS pilotin complex is extracted (right). (c) Gallery of *Klebsiella*-type secretins from *Escherichia coli* K12, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa* (PDB codes 5WQ7, 6I1X, 5WLN). N0 and N1 domains are either partially or fully disordered. (d) Gallery of *Vibrio*-type secretins from *Vibrio cholerae*, *Vibrio vulnificus*, and enteropathogenic *Escherichia coli* (PDB codes 5WQ8, 6I1Y, 5W68). N0 and N1 domains are either partially or fully disordered. (e) Comparison of N0 domains between the *V. cholerae* type IV competence pilus secretin PilQ and *K. pneumoniae* GspD as outlined by the red dotted rectangle in A (II). The N0 domains pack similarly to form rings with C14 or C15 symmetry, respectively. (f) Secretin gating mechanism. Top, central section through the *K. pneumoniae* GspD secretin shown in a. The section shows the inner lumen and central gate, which separates the periplasm from the outside milieu. Bottom, the central section has been adapted to show a gating mechanism based on the type III secretion system secretin

pneumoniae (Tomás et al., 2015), and *Pseudomonas aeruginosa* (Jyot et al., 2011). These are “ESKAPE” pathogens known for their ability to evolve anti-microbial resistance and are the leading cause of nosocomial infections (Santajit and Indrawattana, 2016). Many diverse effectors and toxins depend on the T2SS for secretion (Cianciotto, 2009; Shutinoski et al., 2010; Sikora et al., 2011). Such substrates are involved in adhesion, biofilm formation, nutrient acquisition, colonization, and invasion (Cianciotto and White, 2017).

Overall, the T2SS is of fundamental importance as it represents a key mechanism by which bacteria induce local and long-range modifications to their external environment with significant ramification for human disease and drug development (Waack et al., 2017). This microreview summarizes recent advances in T2SS structure and mechanism. It provides a snapshot of how the T2SS spans the cell envelope and examines how pseudopilus assembly may drive substrate secretion.

1.1 | Type 2 secretion system components

The canonical T2SS operon contains ~13 genes often arranged in a single operon, named *gspC* to *gspO* (Nivaskumar and Francetic, 2014; Pugsley, 1993). The T2SS may be subdivided into three sub-complexes (1) an outer membrane complex (OMC), which includes the secretin channel formed by GspD and the pilotin GspS; (2) an inner membrane complex called the assembly platform (AP), which includes GspC, GspF, GspL, and GspM (GspCFLM) plus the cytoplasmic ATPase GspE; (3) the pseudopilus, which constitutes the major pseudopilin GspG, and the minor pseudopilins GspH, GspI, GspJ, and GspK (GspGHIJK). GspO, the prepilin peptidase is embedded in the inner membrane and processes the pseudopilins GspGHIJK into a mature form ready for pseudopilus assembly (Dupuy and Pugsley, 1994; Francetic et al., 1998). In addition, a membrane protein called GspN is present in some T2SS and forms part of the AP within the inner membrane. Some bacteria additionally encode two inner membrane peptidoglycan-interacting proteins GspA and GspB, which are involved in locating GspD to the outer membrane (Howard et al., 2019; Strozen et al., 2011).

1.2 | Outer membrane complex ultrastructure and assembly

GspD forms a multi-subunit secretin channel with C15 symmetry that spans the outer membrane and protrudes into the periplasm (Chernyatina and Low, 2019; Hay et al., 2018; Hay et al., 2017; Howard et al., 2019; Yan et al., 2017; Yin et al., 2018). The secretin constitutes four N-terminal periplasmic domains called N0, N1, N2, and N3, a secretin core, and a C-terminal S-domain. Two types of secretin, the *Klebsiella*-type (Figure 1a,c) and the *Vibrio*-type (Figure 1b,d) are distinguished. Whereas the secretin core is highly ordered, flexibility increases toward the N-terminus with most cryo-electron microscopy (EM) secretin structures exhibiting partially disordered N2 or N1 domains and entirely absent densities for the N0 domain (Hay et al., 2018; Hay et al., 2017; Howard et al., 2019; Yan et al., 2017; Yin et al., 2018) (Figure 1b-d). While the fold of the N0 domain was known from N0-N2 domain crystal structures (Korotkov et al., 2011; Korotkov et al., 2009b; Van der Meeren et al., 2013), these structures did not fit the cryo-EM maps (Yan et al., 2017). Therefore, the position of N0 relative to the rest of the secretin was unknown. The purification of a ~2.4 MDa T2SS complex from *K. pneumoniae* comprising GspD with its pilotin GspS/PulS, plus GspE, GspL, GspM, GspN, and GspC (GspCDELMNS) yielded a complete secretin structure with ordered N0 domains (Chernyatina and Low, 2019) (Figure 1a). The secretin N0-N3 domains form pentadecameric stacked rings. N1-N3 domains are aligned with a ~36° angular offset to the channel axis. These ~8 kDa domains are connected to each other and the secretin core by short flexible linkers. At the level of the N3 domain, the internal lumen of the secretin is constricted with a disordered loop, which may function as a selectivity filter for exported substrates. The N0 domain consists of two helices flanked

on each side by β -sheets that pack laterally to form the secretin base (Figure 1a,e). Recently, the structure of the PilQ secretin from the *Vibrio cholerae* type IV competence pilus was determined by cryo-EM (Weaver et al., 2020). Although PilQ does not share N1-N3 domains with the T2SS, it does have an N0 domain. Interestingly, PilQ N0 domains pack similarly to T2SS N0 domains at the secretin base (Figure 1e). This packing arrangement may, therefore, be a conserved feature in other type IV filament system secretins, although it is not conserved in the T3SS where the secretin N0 domain helices pack laterally (Hu et al., 2018). In the *K. pneumoniae* T2SS, the N0 and N1 domains are connected by a substantial 26 amino acid linker called Loop 7, which forms a wedge that packs between neighboring N1 domains and stabilizes them. Wedge formation and stabilization of the *K. pneumoniae* secretin N-domains was likely promoted by additional components within the GspCDELMNS complex. The homology region domain of GspC (GspC_{HR}) was bound to GspD N0 domains (Figure 1a) as observed in the enterotoxigenic *Escherichia coli* (ETEC) GspD N0-N1 domain and GspC_{HR} co-crystal structure (Korotkov et al., 2011). Additionally, in the *K. pneumoniae* secretin cryo-EM map, an unidentified plug density was observed occluding the secretin lumen at the level of the N0-N1 domains (Chernyatina and Low, 2019). Given the position of the plug, it likely contributed to the stabilization of the secretin periplasmic domains. A similarly positioned plug density was observed in the cryo-electron tomography (cryo-ET) reconstruction of the *Legionella pneumophila* T2SS (Ghosal et al., 2019). Intriguingly, in the type 4 pilus system (T4PS), the N-terminal von Willebrand factor A domain of PilY1 constitutes a secretin plug while its C-terminus locates with the minor pilins at the pilus tip to form a priming complex (Treuner-Lange et al., 2020).

The secretin core spans the outer membrane and constitutes a barrel with an inner and outer layer of β -sheets (Yan et al., 2017). A central gate separates the external milieu from the inner lumen (Figure 1f) and must open to allow the substrate to pass through the channel. The gating in the T2SS might work similarly as for the type III secretion system (T3SS), where the central gate has been shown to hinge open (Hu et al., 2018; Yan et al., 2017) (Figure 1f). *Vibrio*-type secretins feature an additional cap gate at the top of the secretin (Figure 1b, d). The C-terminal S-domain forms a triple helix or helix-strand-helix motif located on the outside of the secretin core interacting with the pilotin (Figure 1a,b).

Pilotins are secretin-specific lipoprotein chaperones supporting the oligomerization, assembly, and correct insertion of GspD subunits into the outer membrane (Dunstan et al., 2013; Nickerson et al., 2011). They may be co-located with other T2SS genes in a single operon as for *yghG* in ETEC (Strozen et al., 2012) or separated and positioned elsewhere in the chromosome (Chernyatina and Low, 2019; Koo et al., 2012). Pilotins are sorted to the outer membrane via the Lol machinery after the cleavage of their signal sequences (Okuda and Tokuda, 2011). A conserved cysteine at the pilotin N-terminus undergoes lipidation, which serves as an anchor within the inner leaflet of the outer membrane (Zückert, 2014). There are two types of pilotins identified in the T2SS, the *Klebsiella*-type, and the *Vibrio*-type. The *Klebsiella*-type pilotins have a fold comprised

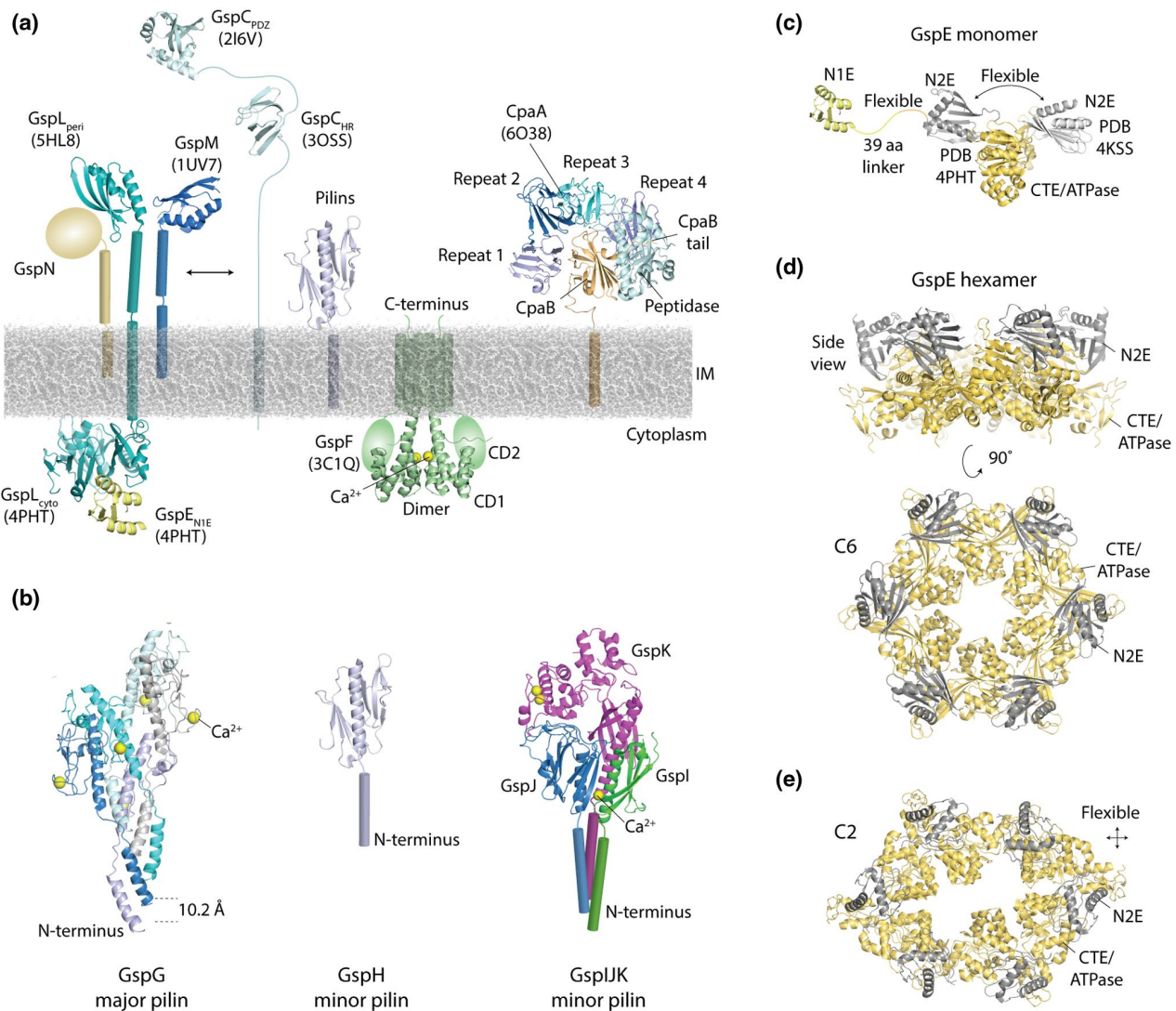


FIGURE 2 Gallery of T2SS inner membrane components. (a) Gallery of AP and inner membrane components including the substrate CpaA with associated chaperone CpaB. PDB codes are shown in parentheses. GspL, GspM, and GspN form an inner membrane complex. GspC also associates with GspL and GspM. Pseudopilins, as shown in b, are positioned within the inner membrane before pseudopilus assembly. The platform protein GspF may constitute a dimer. CD1 (amino acids 56–171) and CD2 correlate to cytoplasmic domains 1 and 2. The structure of CD2 and GspF transmembrane helices has not been determined. The *Acinetobacter baumannii* protease CpaA complexed with its membrane chaperone CpaB represents a novel chaperone-substrate interaction. (b) Gallery of minor and major pseudopilins that collectively form the pseudopilus. GspG (PDB code 5WDA) forms the bulk of the pseudopilus. GspH (PDB code 2QV8) and GspIJK (PDB code 3CI0) constitute the pseudopilus tip. (c) GspE is a highly flexible and dynamic ATPase. The monomer comprises N1E, N2E and C-terminal CTE domains all interconnected by flexible linkers. Superposition of the CTE domains from *V. vulnificus* and *V. cholerae* (PDB code 4PHT and 4KSS) highlights the flexibility of the N2E domain. (d, e) GspE forms a hexamer when crystallized as a Hcp1 fusion protein. GspE undergoes large scale conformational changes switching between C6 symmetry in d (PDB code 4KSS) and C2 symmetry in e (PDB code 4KSR). In both d and e, the Hcp1 fusion has been removed for clarity

of four α -helices (Gu et al., 2012; Korotkov and Hol, 2013; Rehman et al., 2013; Tosi et al., 2011) (Figure 1a). In contrast, the *Vibrio*-type pilotins constitute five stranded β -sheets flanked by four α -helices (Dunstan et al., 2013; Yin et al., 2018) (Figure 1b). Each pilotin has a groove into which the C-terminal helix of the S-domain inserts. The lack of a pilotin can lead to the mislocalization of the secretin to the inner membrane or its degradation (Hardie et al., 1996a; Hardie et al., 1996b; Viarre et al., 2009). T3SS and T4PS pilotins (Golovanov

et al., 2006; Lario et al., 2005) are not conserved with those of the T2SS so that overall, pilotins are secretion system specific and vary in both sequence and structure. Interestingly, some T2SS secretins assemble in the outer membrane without the requirement of a pilotin, for example, *Aeromonas hydrophila* (Howard et al., 2019; Strozen et al., 2011). In such cases, the accessory components GspA and GspB facilitate secretin assembly and membrane insertion through the binding and modulation of the peptidoglycan layer.

1.3 | The inner membrane components of the T2SS

The inner membrane module of the T2SS is composed of three components, a group of integral membrane proteins called the AP, a pseudopilus, and a cytoplasmic ATPase (Figure 2). The AP is composed of the monotopic membrane proteins GspM, the bitopic membrane proteins GspC (Bleves et al., 1999) and GspL, and the polytopic membrane protein GspF. In some T2SS, an additional possible monotopic membrane protein called GspN associates with GspL and GspM (Lee et al., 2001; Lee et al., 2000). Currently, the role of GspN is unclear given mutants in both *A. baumannii* and *Klebsiella oxytoca* do not affect substrate secretion (Possot et al., 2000; Waack et al., 2017).

GspC has multiple domains (Figure 2a) including a short N-terminal cytoplasmic stalk, a transmembrane helix followed by a long linker, the periplasmic GspC_{HR}, and a C-terminal PDZ domain (GspC_{PDZ}) (Gérard-Vincent et al., 2002; Korotkov et al., 2011; Korotkov et al., 2006). The transmembrane helix dimerises within the inner membrane (Login and Shevchik, 2006). Then through the binding of GspC_{HR} to the NO domains at the secretin base (Figure 1a), GspC likely forms a periplasmic cage-like structure that interconnects the AP with the OMC (Chernyatina and Low, 2019). While the crystal structure of GspC_{HR} revealed two stacks of three anti-parallel β -sheets (Korotkov et al., 2011), the GspC_{PDZ} crystal structure contained a typical bacteria-specific PDZ fold (Liao et al., 2000) comprising six β -sheets flanked by two helices (Korotkov et al., 2006). GspC_{PDZ} is not conserved and is sometimes either absent or replaced with a coiled-coil domain. Either of these domains is required for the recognition and secretion of particular substrates (Bleves et al., 1999; Bouley et al., 2001). For example, GspC_{PDZ} mutants were unable to secrete pectate lyases although they were still competent to secrete other substrates such as PemA or Cel5 at either normal or reduced levels (Bouley et al., 2001).

GspL and GspM contain a single membrane helix connected to a periplasmic domain (GspL_{peri} and GspM_{peri}). The crystal structures of *Vibrio parahaemolyticus* GspL_{peri} and GspM_{peri} both revealed a ferredoxin-like fold (Abendroth et al., 2009a; Abendroth et al., 2004b) (Figure 2a). Intriguingly, the equivalent fold was also observed in the T4PS proteins PilN and PilO (Karupiah et al., 2013; Sampaleanu et al., 2009), which suggested a common evolutionary ancestry and possible functional equivalence (Ayers et al., 2009). GspL_{peri} and GspM_{peri} formed dimers in the crystals although different interfaces were used for oligomerization. These interfaces may be present in vivo as cross-linking studies in *Dickeya dadantii* indicated GspL_{peri} and GspM_{peri} homodimer and heterodimer formation (Lallemant et al., 2013). GspL also includes a globular N-terminal cytoplasmic domain (GspL_{cyto}) (Figure 2a). The crystal structure of *Vibrio cholerae* GspL_{cyto} revealed three β -sheet-rich subdomains I, II, and III (Abendroth et al., 2004a). The subdomains I and III have an RNase H-like fold with five β -sheets flanked by α -helices and interestingly share structural homology with the 1A and 2A subdomains of the actin superfamily (Van den Abendroth et al., 2004a; Ent et al., 2001). GspL_{cyto} is unable to bind ATP though as it lacks an actin-like 2B subdomain. While GspL_{cyto} is a close structural ortholog of T4PS PilM,

they differ as PilM includes an actin-like 2B subdomain that binds ATP but cannot hydrolyze it (Karupiah and Derrick, 2011).

GspF is a polytopic membrane protein predicted to span the inner membrane three times and to contain two N-terminal cytoplasmic domains connected by a periplasmic loop. A short C-terminal tail is also accessible from the periplasm (Abendroth et al., 2009b; Arts et al., 2007; Thomas et al., 1997). This membrane topology was substantiated by alkaline phosphatase fusion assays (Arts et al., 2007). The crystal structure of the *V. cholerae* GspF (EpsF) first cytoplasmic domain showed a novel helical fold that dimerized and coordinated calcium through a conserved interface (Abendroth et al., 2009b) (Figure 2a). An equivalent fold was also predicted for the second cytoplasmic domain due to substantial shared sequence similarity (Peabody et al., 2003). A recent study in *P. aeruginosa* indicated that purified GspF (XcpS) adopts a dimeric structure (van Putte et al., 2018). Ultimately, the cellular oligomerization state of GspF within the T2SS is uncertain as the T4PS ortholog PilC has been isolated both as a dimer and tetramer in different systems (Bischof et al., 2016; Collins et al., 2007; Karupiah et al., 2010).

GspE is a cytoplasmic ATPase that acts as a motor to power pseudopilus assembly and substrate extrusion (Camberg et al., 2007; Camberg and Sandkvist, 2005; Patrick et al., 2011; Sandkvist et al., 1995). It is closely related to the T4PS ATPases PilB and PilT (Mancl et al., 2016; Planet et al., 2001; Satyshur et al., 2007) and is an essential component of the T2SS (Sandkvist et al., 1995). Sequence and structural analysis of GspE revealed three domains, the N1E, N2E, and C-terminal CTE connected by linkers (Lu et al., 2014; Lu et al., 2013; Robien et al., 2003) (Figure 2c). Superposition of the GspE structures from *Vibrio vulnificus* and *V. cholerae* highlighted the flexibility of the linker between the N2E and CTE domains (Lu et al., 2014; Lu et al., 2013) (Figure 2c). The CTE domain may be further segmented into three subdomains called C1E, C2E, and CME. The C1E subdomain contains the nucleotide-binding Walker A and B motifs as well as additional aspartate and histidine motifs. Importantly, a unique tetracysteine motif in the CME coordinates zinc, which is also conserved in T4PS assembly ATPases (Robien et al., 2003). Deletion of the tetracysteine motif or substitution of any of the cysteine residues abrogated secretion in *V. cholerae* (Rule et al., 2016). The GspE structure in the absence of the N1E domain was determined in two states including a crystallographic hexamer with C6 symmetry (Figure 2d) and a non-crystallographic hexamer with C2 symmetry (Figure 2e). However, crystallization required the assisting hexameric fusion protein Hcp1. ATPase activity was upregulated upon hexamer formation which, along with biochemical studies (Camberg et al., 2007; Shiue et al., 2006), supported the concept that a hexamer was the functional unit within an assembled T2SS (Lu et al., 2013). Ultimately though, the functional oligomeric state of GspE in vivo has not yet been conclusively shown. Large scale conformational changes were observed between the C6 and C2 symmetry states that were reminiscent of other related ATPases in both the T4PS and archaeal motors (Collins et al., 2018; McCallum et al., 2019; McCallum et al., 2017; Reindl et al., 2013; Satyshur et al., 2007). Overall, the dynamic nature of GspE and its inherent flexibility is suspected to be vital in coupling nucleotide-induced

conformational change in the mechanical loading of pseudopilins into the pseudopilus.

The pseudopilus functions to extrude substrates through the T2SS secretin channel. The tip of the pseudopilus is formed by a GspIJK trimeric complex that may bind one or two subunits of the minor pseudopilin GspH (Korotkov and Hol, 2008) (Figure 2b). The rest of the pseudopilus constitutes a helical filament formed by the major pseudopilin GspG (Campos et al., 2010; Köhler et al., 2004; López-Castilla et al., 2017). All the pseudopilins have a characteristic fold comprising an N-terminal α -helix, a short variable region, and a four-stranded C-terminal antiparallel β -sheet (López-Castilla et al., 2017; Raghunathan et al., 2014; Yanez et al., 2008). GspGHI has a conserved glutamic acid at amino acid position 5 (Glu5) within the mature sequence. In the case of GspG and GspH, Glu5 mediates both recruitment of these pseudopilins to the AP via direct contact with GspM, and stabilisation of neighbouring pseudopilins within the pseudopilus (Nivaskumar et al., 2014; Nivaskumar et al., 2016). GspG contains a conserved calcium-binding site, which is essential for subunit folding and stability (Korotkov et al., 2009a; López-Castilla et al., 2017). GspK also binds calcium ions and has a unique additional helical domain that likely caps the pseudopilus tip (Korotkov and Hol, 2008).

1.4 | Architecture of a type II secretion system

The T2SS is a multi-component complex that spans the cellular inner and outer membranes and periplasm. Other than the GspD secretin core which is a rigid and ordered structure, a high level of flexibility and dynamism underpins most T2SS components. This flexibility and the transient nature of the protein-protein interactions appear crucial for function. Recently, cryo-ET provided the first glimpse of a T2SS assembled in situ (Ghosal et al., 2019). Combined with cryo-ET on the T4PS (Chang et al., 2017; Chang et al., 2016; Gold et al., 2015; Treuner-Lange et al., 2020), we now have a topology map for the position of most components for both the T2SS and T4PS. In these systems, the secretin was observed in the outer membrane with its N-terminal domains protruding into the periplasm. Between the secretin base and the inner membrane, densities consistent with a ring-like structure were observed and assigned to the periplasmic domains of GspL and GspM in the T2SS, or PilN and PilO in the T4PS. A central stem density was positioned within the GspLM ring, which likely relates to the pseudopilus tip proteins GspHIJK or their equivalent in the T4PS (Chang et al., 2016; Treuner-Lange et al., 2020). Densities for the cytoplasmic domains of GspL or T4PS PilM were nestled on the inner membrane surface and were consistent with another ring-like structure. In the T2SS, the putative GspE hexamer was suspended ~5 nm below the inner membrane (Ghosal et al., 2019), presumably tethered in place by the long linker between the GspE N1E and N2E domains (Figure 2c). The N1E domain then connects GspE to GspL_{cyto} in a 1:1 stoichiometric complex (Abendroth et al., 2005; Lu et al., 2014). This contrasts with the T4PS ATPases PilB and PilT, which are positioned flush with the membrane (Chang et al., 2016). Based on this data, it was possible to generate a model showing T2SS architecture (Figure 3) using known structures (Figures 1 and 2).

Within the model, the stoichiometry for GspD and the associated pilotin was 1:1 with 15 copies of each per OMC (Chernyatina and Low, 2019; Yin et al., 2018). The *K. pneumoniae* GspCDELMNS complex yielded relative stoichiometries for the GspCELMN AP components (Chernyatina and Low, 2019). GspE:L:M:N was in 1:1:1:1 ratio based on protein gel densitometry measurements (Luo et al., 2006), which translated into a copy number of six for each protein given GspE likely functions as a hexamer in an assembled T2SS (Chernyatina and Low, 2019; Lu et al., 2013). Protein gel densitometry also indicated that there were at least two GspC subunits for each GspELMN subunits, which translated to a copy number of at least twelve GspC subunits per T2SS. As each GspC reaches across the periplasm from the inner membrane and binds to a single GspD N0 domain at the secretin base, this suggested a simple mechanism for overcoming the symmetry mismatch between the overall sixfold symmetry of the AP and the 15-fold symmetry of the OMC (Chernyatina and Low, 2019). It is possible that up to fifteen GspC subunits may be incorporated within each T2SS as each GspD N0 domain at the secretin base has the capacity to bind a single GspC_{HR} (Chernyatina and Low, 2019; Korotkov et al., 2011; Wang et al., 2012). Alternative mechanisms for overcoming symmetry mismatch have been suggested, which may not be mutually exclusive, including the formation of pseudo C6 symmetry within the secretin periplasmic domains (Douzi et al., 2017; Hay et al., 2017). Overall, GspC appears to represent the core mechanism for connecting the inner membrane to the OMC, which highlights the flexible and dynamic nature of the T2SS.

An outstanding question is how GspL, GspM, GspC, and GspN when present, are arranged within the inner membrane when the T2SS is assembled. Pull-down and yeast-two hybrid experiments in *D. dadantii* have shown direct interactions between the transmembrane regions of GspL, GspM and GspC, as well as the interaction between GspL and GspM periplasmic domains potentially through their ferredoxin-like domains (Lallemant et al., 2013). Studies in other systems such as *V. cholerae* (Johnson et al., 2007; Sandkvist et al., 1999; Sandkvist et al., 2000), *Erwinia chrysanthemi* (Py et al., 2001) and *P. aeruginosa* (Robert et al., 2005) also supported the interaction between GspL and GspM. In addition, GspN was shown to associate with GspL and GspM in both *Xanthomonas campestris* (Tsai et al., 2002) and in *K. pneumoniae* (Chernyatina and Low, 2019). A picture, therefore, emerges where GspC, GspL, GspM, and GspN form a complex within the inner membrane. In the *K. pneumoniae* GspCDELMNS complex, a modular and dynamic hexameric hub architecture was suggested for AP components where complexes of GspCELMN were flexibly arranged around the central GspE hexamer (Chernyatina and Low, 2019). Both GspL and GspM formed homodimers and heterodimers in vitro (Abendroth et al., 2009a; Douet et al., 2004; Lallemant et al., 2013; Py et al., 1999; Sandkvist et al., 1999) and it is possible that this arrangement is reproduced within an assembled T2SS. GspF is positioned at the center of the AP where it makes contact with GspL and GspE as shown in *P. aeruginosa* and *E. chrysanthemi* (Arts et al., 2007; Py et al., 2001). Bacterial two-hybrid analyses have also connected GspF with GspM as well as components of the pseudopilus including GspG and GspH (Nivaskumar et al., 2016). The pseudopilus is, therefore, located

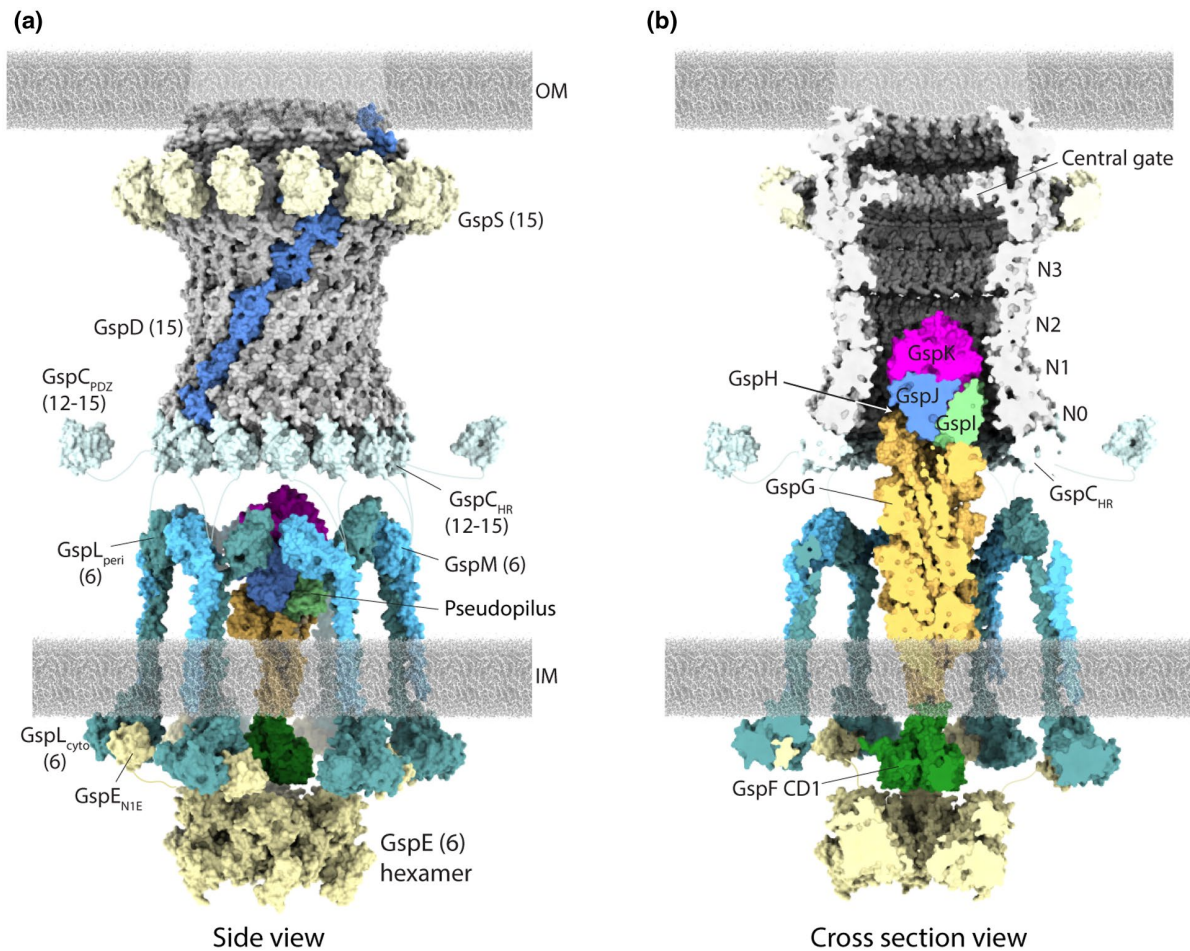


FIGURE 3 A model of the T2SS. (a) Side view of the T2SS. The positioning of the various T2SS components relative to the inner and outer membrane (IM and OM) was derived from the cryo-ET map of the *Legionella pneumophila* T2SS (EMD 20713). The outer membrane complex including the pilin GspS/PuS, GspD, and GspC_{HR} domain was derived from PDB code 6HCG. PDB codes for all other components are outlined in Figure 2. Inner membrane helices for GspL and GspM were generated using SWISS model (Waterhouse et al., 2018). The GspE C6 hexamer is shown (PDB code 4KSS). Stoichiometries are indicated in parentheses (Chernyatina and Low, 2019). GspLJK has 1 copy of each per pseudopilus and possibly one or two copies of GspH. Note that for clarity only the GspF cytoplasmic domain 1 (CD1) domain is shown here. How GspF interfaces with GspG pseudopilins within the membrane is unknown. (b) Cross-section view of the T2SS. The pseudopilus is shown extending into the secretin lumen

centrally associated with GspF where it is well positioned to extend across the periplasm into the secretin lumen.

1.5 | Substrate recruitment and secretion mechanism

T2SS substrates are initially transported across the inner membrane by the Sec or Tat systems (Pugsley et al., 1991; Voulhoux et al., 2001). From the periplasm, the substrate is then targeted for secretion through poorly understood and diverse mechanisms that appear specific to each substrate with no universal signal sequence. T2SS substrates include toxins, lipases, metalloproteases, and digestive enzymes (Korotkov and Sandkvist, 2019). Recently, the pathogen *Acinetobacter baumannii* was shown to secrete multiple T2SS substrates including the lipases LipA and LipH along with the protease CpaA (Tilley et al., 2014; Urusova et al., 2019) (Figure 2a). These

substrates are essential for *A. baumannii* virulence. Interestingly, both LipA and CpaA were paired with specific periplasmic chaperones called LipB (Frenken et al., 1993) and CpaB (Harding et al., 2016; Kinsella et al., 2017), which were anchored in the inner membrane and required for T2SS recruitment and secretion. Bioinformatics suggests that multiple T2SS effectors are actually paired in dimeric operons with putative membrane-bound chaperones, as identified in *V. cholerae*, *P. aeruginosa*, and *Burkholderia pseudomallei* (Harding et al., 2016).

Many studies have identified GspC as being critical for substrate recognition and recruitment into the T2SS (Bleves et al., 1999; Bouley et al., 2001; Douzi et al., 2011; Gérard-Vincent et al., 2002; Pineau et al., 2014) (Figure 4, steps 1 and 2). In some cases, direct interactions occur between the substrate and both GspC_{HR} and GspC_{PDZ} suggesting multiple contacts (Douzi et al., 2011; Pineau et al., 2014). In *P. aeruginosa*, the effector CbpD has recently been shown to be recruited first to GspM (XcpZ) and then to GspL (XcpY)

(Michel-Souzy et al., 2018). Substrate binding may stimulate GspL and GspM heterodimerization and transmembrane rotation so that allosteric conformational changes are induced on the cytoplasmic side. Such signal transduction may prime GspL_{cyto} and other components of the AP for pseudopilus assembly and active secretion (Lallemant et al., 2013; Michel-Souzy et al., 2018). Not all substrates may follow the same physical and temporal protein-protein pathway between GspC, GspL, and GspM during secretion. Ultimately substrates bind to the GspHIJK pseudopilus tip and make contact with the secretin N-domains (Douzi et al., 2011; Pineau et al., 2014; Reichow et al., 2011; Reichow et al., 2010; Shevchik, 1997) (Figure 4, step 3). This priming complex may be similar to the T4PS in its retracted state with PilY1 topologically analogous to T2SS substrate given it simultaneously contacts both minor pilins and plugs the secretin base (Treuner-Lange et al., 2020).

One of the outstanding questions in the T2SS field relates to how pseudopilus assembly occurs within the AP and how this

assembly is then coupled with substrate extrusion through the secretin channel. The inherent dynamism of the AP has hindered structural studies which might otherwise have provided a molecular snapshot of how the different components engage each other and undergo conformational changes. For example, current structures of GspE depended on the fusion protein Hcp1 to oligomerize and to capture a hexameric state (Lu et al., 2013). In contrast, how the T4PS ATPase motors extend and retract pili is better understood (Craig et al., 2019) and may provide insight into how the T2SS pseudopilus assembly works given evolutionary relatedness and the likelihood of shared mechanistic principles. In the T4PS a rotary mechanism has been suggested for pilus extension where ATP hydrolysis in the motor PilB is coupled with a clockwise sequence of conformational changes in the hexameric ring (Mancl et al., 2016; McCallum et al., 2019; McCallum et al., 2017; Solanki et al., 2018). These conformational changes may be directly transmitted to PilC (equivalent to GspF in the T2SS) so that it sequentially rotates and shifts 8–10 Å

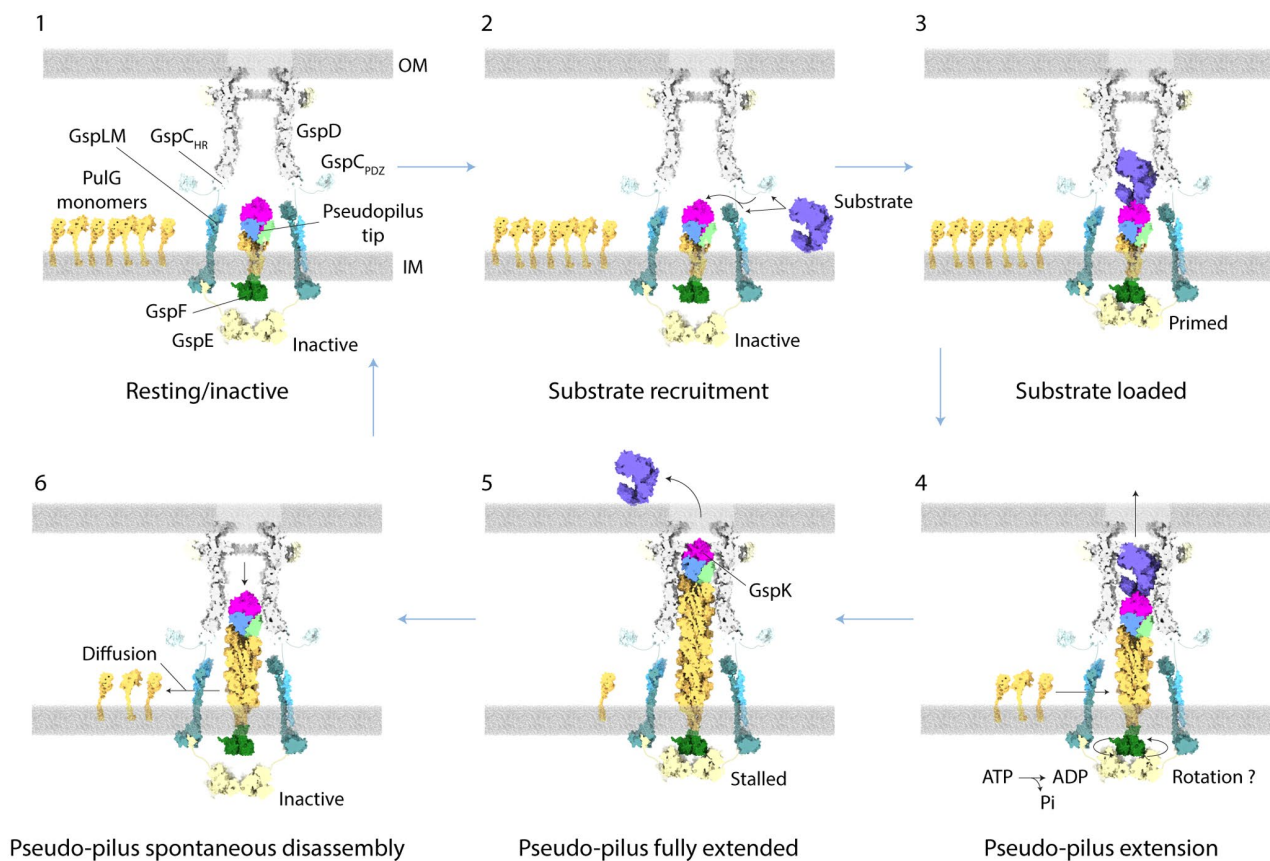


FIGURE 4 T2SS substrate secretion mechanism. 1, When the T2SS is inactive, the pseudopilus tip comprising GspHIJK resides in the inner membrane possibly associated with GspF. 2, Substrate is recruited into the secretion system through interactions with GspC_{PDZ}, GspM, and GspL and makes contact with the pseudopilus tip. 3, Substrate is loaded and the T2SS is primed for secretion. Given the dimensions of the pseudopilus tip and periplasm, loaded substrate is positioned within the secretin lumen contacting GspD N0 and N1 domains. The GspE hexamer has engaged GspF ready to initiate pseudopilus assembly. 4, GspE ATP nucleotide cycling drives pseudopilus assembly possibly through a GspF rotary mechanism. In such a model, the pseudopilus itself is not expected to rotate during assembly. GspG subunits are processed by the prepilin peptidase GspO (not shown), diffuse into the T2SS via GspM, and are spooled into the growing pseudopilus. 5, Substrate is extruded through the secretin and is secreted. It is estimated that ~24 copies of GspG are required for the pseudopilus tip to contact the secretin central gate, which may be a sufficient length for the substrate to be released. Pseudopilus assembly stalls GspE possibly through a GspK steric hindrance mechanism triggering retraction. 6, GspG diffusion from the base of the pseudopilus represents a passive mechanism for pseudopilus disassembly. GspE is disengaged from GspF. The T2SS returns to an inactive resting state

orthogonally from the membrane toward the periplasm. The effect is to spool incoming pilins, or pseudopilins in the case of the T2SS, from the inner membrane reservoir and to extrude them by this distance out of the membrane into the growing pilus or pseudopilus (Figure 4, step 4). Notably, this 8–10 Å translation correlates well with the 8–10 Å rise observed between pilin or pseudopilin subunits assembled in both the T4PS pilus (Kolappan et al., 2016; Li et al., 2012; Wang et al., 2017) and T2SS pseudopilus (López-Castilla et al., 2017; Nivaskumar et al., 2014) (Figure 2b). In this model, the rotary motion would be specific to PilC or GspF and does not include T4PS pilus or T2SS pseudopilus rotation. An alternative model where the pilin is extruded from the membrane by compression has also been proposed for the T4PS and may also be relevant for the T2SS (Craig et al., 2019). Based on the dimensions of the *L. pneumophila* T2SS (Ghosal et al., 2019), it is estimated that a pseudopilus incorporating ~24 GspG subunits is required for the GspHIJK pseudopilus tip to reach the secretin central gate, which may be sufficient for the substrate to then be extruded in a piston-like mechanism (Shevchik, 1997) (Figure 4, step 5). How the pseudopilus retracts or disassembles in the T2SS is another fundamental question that is not currently well understood. In the T4PS, retraction is actively driven through an ATPase such as PilT (Merz et al., 2000), which replaces PilB and likely processively reverses the assembly mechanism to unspool pilins from the pilus and to return them to the inner membrane reservoir. In the T2SS there is no known dedicated retraction ATPase. To account for this, one option is that the pseudopilus actually continually assembles at the base, while concurrently disassembling at the tip, thereby functioning similar to an Archimedes' screw (Nivaskumar and Francetic, 2014). The pseudopilus requires bound calcium ions for stability and in this type of model, pseudopilus disassembly may be coupled with a reduced calcium concentration in the external environment in comparison to the periplasm (López-Castilla et al., 2017). Alternatively, periplasmic proteolysis may simply control pseudopilus length. While the Archimedes' screw model is viable, the mechanistic principles upon which the T4PS functions, where the pilus cycles through extension and retraction cycles, supports a T2SS pseudopilus piston-like model. In this case and in the absence of a dedicated retraction ATPase, it is suggested that pseudopilus retraction occurs passively through spontaneous disassembly (Craig et al., 2019; Ng et al., 2016) (Figure 4, step 6). Stalling of the GspE motor may be induced by steric hindrance of GspK at the pseudopilus tip contacting the central gate. Indeed, GspK (XcpX) has been shown to control pseudopilus length in *P. aeruginosa* (Durand et al., 2005). Inactivation of the GspE motor would switch the equilibrium from pseudopilus assembly toward a disassembled resting state where GspG subunits diffuse out of the pseudopilus back into the inner membrane reservoir. The retraction of the GspHIJK pseudopilus tip to the inner membrane would mark one full secretion cycle where the secretion system is now competent to recruit further substrate (Figure 4, step 1). The limited length of a fully extended pseudopilus facilitates passive disassembly. Studies on various T4P systems support such a passive disassembly model. For

example, retraction of pili many microns in length have been described in three T4P systems carrying *pilT* mutations (Clausen et al., 2009; Ellison et al., 2018; Zöllner et al., 2019). Additionally, the pili of the *V. cholerae* toxin-coregulated pilus system, which is one of the simplest of T4P systems, are retractile even in the absence of a dedicated ATPase (Ng et al., 2016). While the Tad pilus system has no dedicated retraction ATPase, it may have compensated by evolving two PilC/GspF paralogs called TadB and TadC, whose role may be to deliver bi-directional pilus dynamics from a single motor (Ellison et al., 2019; Tomich et al., 2007).

2 | CONCLUSION

Since the discovery of the T2SS over two decades ago, significant progress has been made in understanding its structure and mechanism. A combination of X-ray crystallography and more recently cryo-EM means that most of the T2SS components have had their structure at least partially determined. Structural biology is important for dissecting the T2SS mechanism as it provides an essential means to understand the chemistry of individual proteins and protein complexes, while also providing a powerful framework from which to interpret and base biochemical and cell biology analyses. Arguably some of the most critical structures for showing how the T2SS functions remain outstanding. For example, the assembly platform represents the stator for the GspE motor and provides the scaffold to house GspF and the pseudopilus. Many of the assembly platform components have had their cytoplasmic and periplasmic components structurally determined but crucially in the absence of their membrane domains. It is known that a web of interactions occurs between GspL, GspM, GspF, and GspE, many of which occur within the membrane. The next frontier will be to resolve full-length complexes of these proteins. These should show snapshots of how the motor engages GspF and transitions power to pseudopilus assembly. Such complexes should also show how conformational change in the cytoplasmic side are allosterically coupled with movements on the periplasmic side, and vice versa, with implication for substrate recruitment and pseudopilus assembly control. Such structures, combined with higher resolution in situ cryo-ET, will ultimately yield a detailed molecular model and greatly enhanced the mechanistic understanding of the T2SS.

ACKNOWLEDGMENTS

This work was funded by a Wellcome Trust Senior Research Fellowship to HL (215553/Z/19/Z). The authors state that they have no conflict of interest.

ORCID

Souvik Naskar  <https://orcid.org/0000-0003-2896-1340>

Michael Hohl  <https://orcid.org/0000-0002-6046-4865>

Matteo Tassinari  <https://orcid.org/0000-0001-6423-4891>

Harry H. Low  <https://orcid.org/0000-0002-1226-3217>

REFERENCES

- Abendroth, J., Bagdasarian, M., Sandkvist, M. and Hol, W.G.J. (2004a) The structure of the cytoplasmic domain of EpsL, an inner membrane component of the type II secretion system of *Vibrio cholerae*: an unusual member of the actin-like ATPase superfamily. *Journal of Molecular Biology*, 344, 619–633.
- Abendroth, J., Kreger, A.C. and Hol, W.G.J. (2009a) The dimer formed by the periplasmic domain of EpsL from the type 2 secretion system of *Vibrio parahaemolyticus*. *Journal of Structural Biology*, 168, 313–322.
- Abendroth, J., Mitchell, D.D., Korotkov, K.V., Johnson, T.L., Kreger, A., Sandkvist, M. et al (2009b) The three-dimensional structure of the cytoplasmic domains of EpsF from the type 2 secretion system of *Vibrio cholerae*. *Journal of Structural Biology*, 166, 303–315.
- Abendroth, J., Murphy, P., Sandkvist, M., Bagdasarian, M. and Hol, W.G.J. (2005) The X-ray structure of the type II secretion system complex formed by the N-terminal domain of EpsE and the cytoplasmic domain of EpsL of *Vibrio cholerae*. *Journal of Molecular Biology*, 348, 845–855.
- Abendroth, J., Rice, A.E., McLuskey, K., Bagdasarian, M. and Hol, W.G.J. (2004b) The crystal structure of the periplasmic domain of the type II secretion system protein EpsM from *Vibrio cholerae*: the simplest version of the ferredoxin fold. *Journal of Molecular Biology*, 338, 585–596.
- Arts, J., de Groot, A., Ball, G., Durand, E., Khattabi, M.E., Filloux, A.F. et al (2007) Interaction domains in the *Pseudomonas aeruginosa* type II secretory apparatus component XcpS (GspF). *Microbiology*, 153, 1582–1592.
- Ayers, M., Sampaleanu, L.M., Tammam, S., Koo, J., Harvey, H., Howell, P.L. et al (2009) PilM/N/O/P Proteins Form an inner membrane complex that affects the stability of the *Pseudomonas aeruginosa* type IV Pilus Secretin. *Journal of Molecular Biology*, 394, 128–142.
- Bischof, L.F., Friedrich, C., Harms, A., Sogaard-Andersen, L. and Does, C.V. D. (2016) The type IV pilus assembly ATPase PilB of *Myxococcus xanthus* interacts with the inner membrane platform protein PilC and the nucleotide-binding protein PilM. *Journal of Biological Chemistry*, 291, 6946–6957.
- Bleves, S., Gérard-Vincent, M., Lazdunski, A.E. and Filloux, A. (1999) Structure-function analysis of XcpP, a component involved in general secretory pathway-dependent protein secretion in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, 181, 4012–4019.
- Bouley, J., Condemine, G. and Shevchik, V.E. (2001) The PDZ domain of OutC and the N-terminal region of OutD determine the secretion specificity of the type II Out pathway of *Erwinia chrysanthemi*. *Journal of Molecular Biology*, 308, 205–219.
- Camberg, J.L., Johnson, T.L., Patrick, M., Abendroth, J., Hol, W.G.J. and Sandkvist, M. (2007) Synergistic stimulation of EpsE ATP hydrolysis by EpsL and acidic phospholipids. *EMBO Journal*, 26, 19–27.
- Camberg, J.L. and Sandkvist, M. (2005) Molecular analysis of the *Vibrio cholerae* type II secretion ATPase EpsE. *Journal of Bacteriology*, 187, 249–256.
- Campos, M., Nilges, M., Cisneros, D.A. and Francetic, O. (2010) Detailed structural and assembly model of the type II secretion pilus from sparse data. *Proceedings of the National Academy of Sciences*, 107, 13081–13086.
- Chang, Y.W., Kjær, A., Ortega, D.R., Kovackova, G., Sutherland, J.A., Rettberg, L.A. et al (2017) Architecture of the *Vibrio cholerae* toxin-coregulated pilus machine revealed by electron cryotomography. *Nature Microbiology*, 2, 16269.
- Chang, Y.W., Rettberg, L.A., Treuner-Lange, A., Iwasa, J., Sogaard-Andersen, L. and Jensen, G.J. (2016) Architecture of the type IVa pilus machine. *Science*, 351, aad2001-1.
- Chernyatina, A.A. and Low, H.H. (2019) Core architecture of a bacterial type II secretion system. *Nature Communications*, 10, 5437.
- Cianciotto, N.P. (2009) Many substrates and functions of type II secretion: lessons learned from *Legionella pneumophila*. *Future Microbiology*, 4, 797–805.
- Cianciotto, N.P. and White, R.C. (2017) Expanding role of type II secretion in bacterial pathogenesis and beyond. *Infection and Immunity*, 85, e00014-17.
- Clausen, M., Jakovljevic, V., Sogaard-Andersen, L. and Maier, B. (2009) High-force generation is a conserved property of type IV pilus systems. *Journal of Bacteriology*, 191, 4633–4638.
- Collins, R., Karupiah, V., Siebert, C.A., Dajani, R., Thistlethwaite, A. and Derrick, J.P. (2018) Structural cycle of the *Thermus thermophilus* PilF ATPase: the powering of type IVa pilus assembly. *Scientific Reports*, 8, 14022.
- Collins, R.F., Saleem, M. and Derrick, J.P. (2007) Purification and three-dimensional electron microscopy structure of the *Neisseria meningitidis* Type IV Pilus biogenesis protein PilG. *Journal of Bacteriology*, 189, 6389–6396.
- Craig, L., Forest, K.T. and Maier, B. (2019) Type IV pili: dynamics, biophysics and functional consequences. *Nature Reviews Microbiology*, 17, 429–440.
- Denise, R., Abby, S.S. and Rocha, E.P.C.C. (2019) Diversification of the type IV filament superfamily into machines for adhesion, protein secretion, DNA uptake, and motility. *PLoS Biology*, 17, e3000390.
- Douet, V., Loiseau, L., Barras, F. and Py, B. (2004) Systematic analysis, by the yeast two-hybrid, of protein interaction between components of the type II secretory machinery of *Erwinia chrysanthemi*. *Research in Microbiology*, 155, 71–75.
- Douzi, B., Ball, G., Cambillau, C., Tegoni, M. and Voulhoux, R. (2011) Deciphering the Xcp *Pseudomonas aeruginosa* type II secretion machinery through multiple interactions with substrates. *Journal of Biological Chemistry*, 286, 40792–40801.
- Douzi, B., Trinh, N.T.T., Michel-Souzy, S., Desmyter, A., Ball, G., Barbier, P. et al (2017) Unraveling the self-assembly of the *Pseudomonas aeruginosa* XcpQ secretin periplasmic domain provides new molecular insights into type II secretion system secretin architecture and dynamics. *MBio*, 8, e01185–e1217.
- Dunstan, R.A., Heinz, E., Wijeyewickrema, L.C., Pike, R.N., Purcell, A.W., Evans, T.J. et al (2013) Assembly of the type II secretion system such as found in *Vibrio cholerae* depends on the novel pilotin AspS. *PLoS Pathogens*, 9, e1003117.
- Dupuy, B. and Pugsley, A.P. (1994) Type IV prepilin peptidase gene of *Neisseria gonorrhoeae* MS11: presence of a related gene in other pilated and nonpilated *Neisseria* strains. *Journal of Bacteriology*, 176, 1323–1331.
- Durand, É., Michel, G., Voulhoux, R., Kürner, J., Bernadac, A. and Filloux, A. (2005) XcpX controls biogenesis of the *Pseudomonas aeruginosa* XcpT-containing pseudopilus. *Journal of Biological Chemistry*, 280, 31378–31389.
- Elhosseini, N.M. and Attia, A.S. (2018) *Acinetobacter*: an emerging pathogen with a versatile secretome. *Emerging Microbes and Infections*, 7, 1–15.
- Ellison, C.K., Dalia, T.N., Vidal Ceballos, A., Wang, J.-C.-Y., Biais, N., Brun, Y.V. et al (2018) Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural transformation in *Vibrio cholerae*. *Nature Microbiology*, 3, 773–780.
- Ellison, C.K., Kan, J., Chlebek, J.L., Hummels, K.R., Panis, G., Viollier, P.H. et al (2019) A bifunctional ATPase drives tad pilus extension and retraction. *Science Advances*, 5, eaay2591.
- van den Ent, F., Amos, L.A. and Löwe, J. (2001) Prokaryotic origin of the actin cytoskeleton. *Nature*, 413, 39–44.
- Francetic, O., Lory, S. and Pugsley, A.P. (1998) A second prepilin peptidase gene in *Escherichia coli* K-12. *Molecular Microbiology*, 27, 763–775.
- Frenken, L.G.J., Groot, A., Tommassen, J. and Verrips, C.T. (1993) Role of the lipB gene product in the folding of the secreted lipase of *Pseudomonas glumae*. *Molecular Microbiology*, 9, 591–599.

- Gérard-Vincent, M., Robert, V., Ball, G., Bleves, S., Michel, G.P.F., Lazdunski, A. et al (2002) Identification of XcpP domains that confer functionality and specificity to the *Pseudomonas aeruginosa* type II secretion apparatus. *Molecular Microbiology*, 44, 1651–1665.
- Ghosal, D., Kim, K.W., Zheng, H., Kaplan, M., Truchan, H.K., Lopez, A.E. et al (2019) In vivo structure of the *Legionella* type II secretion system by electron cryotomography. *Nature Microbiology*, 4, 2101–2108.
- Gold, V.A.M., Salzer, R., Averbhoff, B. and Kühlbrandt, W. (2015) Structure of a type IV pilus machinery in the open and closed state. *Elife*, 4, e07380.
- Golovanov, A.P., Balasingham, S., Tzitzilonis, C., Goult, B.T., Lian, L.-Y., Homberset, H. et al (2006) The solution structure of a domain from the *Neisseria meningitidis* Lipoprotein PilP reveals a new β -sandwich fold. *Journal of Molecular Biology*, 364, 186–195.
- Gu, S., Rehman, S., Wang, X., Shevchik, V.E. and Pickersgill, R.W. (2012) Structural and functional insights into the pilotin-secretin complex of the type II secretion system. *PLoS Pathogens*, 8, e1002531.
- Hardie, K.R., Lory, S. and Pugsley, A.P. (1996a) Insertion of an outer membrane protein in *Escherichia coli* requires a chaperone-like protein. *EMBO Journal*, 15, 978–988.
- Hardie, K.R., Seydel, A., Guilvout, I. and Pugsley, A.P. (1996b) The secretin-specific, chaperone-like protein of the general pathway: Separation of proteolytic protection and piloting functions. *Molecular Microbiology*, 22, 967–976.
- Harding, C.M., Kinsella, R.L., Palmer, L.D., Skaar, E.P. and Feldman, M.F. (2016) Medically relevant acinetobacter species require a type II secretion system and specific membrane-associated chaperones for the export of multiple substrates and full virulence. *PLoS Pathogens*, 12, e1005391.
- Hay, I.D., Belousoff, M.J., Dunstan, R.A., Bamert, R.S. and Lithgow, T. (2018) Structure and membrane topography of the *Vibrio*-Type secretin complex from the type 2 secretion system of enteropathogenic *Escherichia coli*. *Journal of Bacteriology*, 200, e00521–e617.
- Hay, I.D., Belousoff, M.J. and Lithgow, T. (2017) Structural basis of type 2 secretion system engagement between the inner and outer bacterial membranes. *MBio*, 8, e01344–e1417.
- Howard, S.P., Estrozi, L.F., Bertrand, Q., Contreras-Martel, C., Strozen, T., Job, V. et al (2019) Structure and assembly of pilotin-dependent and -independent secretins of the type II secretion system. *PLoS Pathogens*, 15, e1007731–e1007825.
- Hu, J., Worrall, L.J., Hong, C., Vuckovic, M., Atkinson, C.E., Caveney, N. et al (2018) Cryo-EM analysis of the T3S injectisome reveals the structure of the needle and open secretin. *Nature Communications*, 9, 3840.
- Johnson, T.L., Scott, M.E. and Sandkvist, M. (2007) Mapping critical interactive sites within the periplasmic domain of the *Vibrio cholerae* type II secretion protein EpsM. *Journal of Bacteriology*, 189, 9082–9089.
- Jyot, J., Balloy, V., Jouvion, G., Verma, A., Touqui, L., Huerre, M. et al (2011) Type II secretion system of *pseudomonas aeruginosa*: In vivo evidence of a significant role in death due to lung infection. *Journal of Infectious Diseases*, 203, 1369–1377.
- Karuppiah, V., Collins, R.F., Thistlethwaite, A., Gao, Y. and Derrick, J.P. (2013) Structure and assembly of an inner membrane platform for initiation of type IV pilus biogenesis. *Proceedings of the National Academy of Sciences*, 110, 4638–4647.
- Karuppiah, V. and Derrick, J.P. (2011) Structure of the PilM-PilN inner membrane type IV pilus biogenesis complex from *Thermus thermophilus*. *Journal of Biological Chemistry*, 286, 24434–24442.
- Karuppiah, V., Hassan, D., Saleem, M. and Derrick, J.P. (2010) Structure and oligomerization of the PilC type IV pilus biogenesis protein from *Thermus thermophilus*. *Proteins: Structure, Function, and Bioinformatics*, 78, 2049–2057.
- Kinsella, R.L., Lopez, J., Palmer, L.D., Salinas, N.D., Skaar, E.P., Tolia, N.H. et al (2017) Defining the interaction of the protease CpaA with its type II secretion chaperone CpaB and its contribution to virulence in *Acinetobacter* species. *Journal of Biological Chemistry*, 292, 19628–19638.
- Köhler, R., Schäfer, K., Müller, S., Vignon, G., Diederichs, K., Philippsen, A. et al (2004) Structure and assembly of the pseudopilin PilG. *Molecular Microbiology*, 54, 647–664.
- Kolappan, S., Coureuil, M., Yu, X., Nassif, X., Egelman, E.H. and Craig, L. (2016) Structure of the *Neisseria meningitidis* type IV pilus. *Nature Communications*, 7, 13015.
- Koo, J., Burrows, L.L. and Lynne Howell, P. (2012) Decoding the roles of pilotins and accessory proteins in secretin escort services. *FEMS Microbiology Letters*, 328, 1–12.
- Korotkov, K.V., Gray, M.D., Kreger, A., Turley, S., Sandkvist, M. and Hol, W.G.J. (2009a) Calcium is essential for the major pseudopilin in the type 2 secretion system. *Journal of Biological Chemistry*, 284, 25466–25470.
- Korotkov, K.V. and Hol, W.G.J. (2008) Structure of the GspK-GspL-GspJ complex from the enterotoxigenic *Escherichia coli* type 2 secretion system. *Nature Structural and Molecular Biology*, 15, 462–468.
- Korotkov, K.V. and Hol, W.G.J. (2013) Crystal structure of the pilotin from the enterohemorrhagic *Escherichia coli* type II secretion system. *Journal of Structural Biology*, 182, 186–191.
- Korotkov, K.V., Johnson, T.L., Jobling, M.G., Pruneda, J., Pardon, E., Héroux, A. et al (2011) Structural and functional studies on the interaction of GspC and GspD in the type II secretion system. *PLoS Pathogens*, 7, e1002228.
- Korotkov, K.V., Krumm, B., Bagdasarian, M. and Hol, W.G.J. (2006) Structural and functional studies of EpsC, a crucial component of the type 2 secretion system from *Vibrio cholerae*. *Journal of Molecular Biology*, 363, 311–321.
- Korotkov, K.V., Pardon, E., Steyaert, J. and Hol, W.G.J. (2009b) Crystal structure of the N-terminal domain of the secretin GspD from ETEC determined with the assistance of a nanobody. *Structure*, 17, 255–265.
- Korotkov, K.V. & Sandkvist, M. (2019) Architecture, function, and substrates of the type II secretion system. *EcoSal Plus*. <https://doi.org/10.1128/ecosalplus.ESP-0034-2018>
- Lallemant, M., Login, F.H., Guschinskaya, N., Pineau, C., Effantin, G., Robert, X. et al (2013) Dynamic interplay between the periplasmic and transmembrane domains of GspL and GspM in the type II secretion system. *PLoS ONE*, 8, e79562.
- Lario, P.I., Pfuetzner, R.A., Frey, E.A., Creagh, L., Haynes, C., Maurelli, A.T. et al (2005) Structure and biochemical analysis of a secretin pilot protein. *EMBO Journal*, 24, 1111–1121.
- Lee, H.-M.-M., Tyan, S.-W.-W., Leu, W.-M.-M., Chen, L.-Y.-Y., Chen, D.C.C. and Hu, N.-T.-T. (2001) Involvement of the XpsN protein in formation of the XpsL-XpsM complex in *Xanthomonas campestris* pv. *campestris* type II secretion apparatus. *Journal of Bacteriology*, 183, 528–535.
- Lee, H.-M., Wang, K.-C., Liu, Y.-L., Yew, H.-Y., Chen, L.-Y., Leu, W.-M. et al (2000) Association of the cytoplasmic membrane protein XpsN with the outer membrane protein XpsD in the type II protein secretion apparatus of *Xanthomonas campestris* pv. *Campestris*. *Journal of Bacteriology*, 182, 1549–1557.
- Li, J., Egelman, E.H. and Craig, L. (2012) Structure of the *Vibrio cholerae* type IVb pilus and stability comparison with the *Neisseria gonorrhoeae* type IVa pilus. *Journal of Molecular Biology*, 418, 47–64.
- Liao, D.I., Qian, J., Chisholm, D.A., Jordan, D.B. and Diner, B.A. (2000) Crystal structures of the photosystem II D1 C-terminal processing protease. *Natural Structural Biology*, 7, 749–753.
- Login, F.H. and Shevchik, V.E. (2006) The single transmembrane segment drives self-assembly of OutC and the formation of a functional type II secretion system in *Erwinia chrysanthemi*. *Journal of Biological Chemistry*, 281, 33152–33162.
- López-Castilla, A., Thomassin, J.L., Bardiaux, B., Zheng, W., Nivaskumar, M., Yu, X. et al (2017) Structure of the calcium-dependent type 2 secretion pseudopilus. *Nature Microbiology*, 2, 1686–1695.

- Lu, C., Korotkov, K.V. and Hol, W.G.J. (2014) Crystal structure of the full-length ATPase GspE from the *Vibrio vulnificus* type II secretion system in complex with the cytoplasmic domain of GspL. *Journal of Structural Biology*, 187, 223–235.
- Lu, C., Turley, S., Marionni, S.T., Park, Y.J., Lee, K.K., Patrick, M. et al (2013) Hexamers of the type II secretion ATPase GspE from *Vibrio cholerae* with Increased ATPase activity. *Structure*, 21, 1707–1717.
- Luo, S., Wehr, N.B. and Levine, R.L. (2006) Quantitation of protein on gels and blots by infrared fluorescence of Coomassie blue and Fast Green. *Analytical Biochemistry*, 350, 233–238.
- Makarova, K.S., Koonin, E.V. and Albers, S.V. (2016) Diversity and evolution of type IV pili systems in Archaea. *Frontiers in Microbiology*, 7, 667.
- Mancl, J.M., Black, W.P., Robinson, H., Yang, Z. and Schubot, F.D. (2016) Crystal structure of a type IV Pilus assembly ATPase: insights into the molecular mechanism of PilB from *Thermus thermophilus*. *Structure*, 24, 1886–1897.
- Marsh, J.W. and Taylor, R.K. (1999) Genetic and transcriptional analyses of the *Vibrio cholerae* mannose-sensitive hemagglutinin type 4 pilus gene locus. *Journal of Bacteriology*, 181, 1110–1117.
- McCallum, M., Benlekhir, S., Nguyen, S., Tammam, S., Rubinstein, J.L., Burrows, L.L. and et al (2019) Multiple conformations facilitate PilT function in the type IV pilus. *Nature Communications*, 10, 5198.
- McCallum, M., Tammam, S., Khan, A., Burrows, L.L. and Howell, P.L. (2017) The molecular mechanism of the type IVa pilus motors. *Nature Communications*, 8, 15091.
- Van der Meer, R., Wen, Y., Van Gelder, P., Tommassen, J., Devreese, B. and Savvides, S.N. (2013) New Insights into the assembly of bacterial secretins. *Journal of Biological Chemistry*, 288, 1214–1225.
- Merz, A.J., So, M. and Sheetz, M.P. (2000) Pilus retraction powers bacterial twitching motility. *Nature*, 407, 98–102.
- Michel-Souzy, S., Douzi, B., Cadoret, F., Raynaud, C., Quinton, L., Ball, G. et al (2018) Direct interactions between the secreted effector and the T2SS components GspL and GspM reveal a new effector-sensing step during type 2 secretion. *Journal of Biological Chemistry*, 293, 19441–19450.
- Ng, D., Harn, T., Altindal, T., Kolappan, S., Marles, J.M., Lala, R. et al (2016) The *Vibrio cholerae* Minor Pilin TcpB initiates assembly and retraction of the toxin-coregulated pilus. *PLoS Pathogens*, 12, e1006109–e1006131.
- Nickerson, N.N., Tosi, T., Dessen, A., Baron, B., Raynal, B., England, P. et al (2011) Outer membrane targeting of secretin PulD protein relies on disordered domain recognition by a dedicated chaperone. *Journal of Biological Chemistry*, 286, 38833–38843.
- Nivaskumar, M., Bouvier, G., Campos, M., Nadeau, N., Yu, X., Egelman, E.H. et al (2014) Distinct docking and stabilization steps of the pseudopilus conformational transition path suggest rotational assembly of type IV pilus-like fibers. *Structure*, 22, 685–696.
- Nivaskumar, M. and Francetic, O. (2014) Type II secretion system: a magic beanstalk or a protein escalator. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1843, 1568–1577.
- Nivaskumar, M., Santos-Moreno, J., Malosse, C., Nadeau, N., Chamot-Rooke, J., Tran Van Nhieu, G. et al (2016) Pseudopilin residue E5 is essential for recruitment by the type 2 secretion system assembly platform. *Molecular Microbiology*, 101, 924–941.
- Okuda, S. and Tokuda, H. (2011) Lipoprotein sorting in bacteria. *Annual Review of Microbiology*, 65, 239–259.
- Patrick, M., Korotkov, K.V., Hol, W.G.J. and Sandkvist, M. (2011) Oligomerization of EpsE coordinates residues from multiple subunits to facilitate ATPase activity. *Journal of Biological Chemistry*, 286, 10378–10386.
- Peabody, C.R., Chung, Y.J., Yen, M.R., Vidal-Ingigliardi, D., Pugsley, A.P. and Saier, M.H. (2003) Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella. *Microbiology*, 149, 3051–3072.
- Piepenbrink, K.H. (2019) DNA uptake by type IV filaments. *Frontiers in Molecular Biosciences*, 6, 1441.
- Pineau, C., Guschinskaya, N., Robert, X., Gouet, P., Ballut, L. and Shevchik, V.E. (2014) Substrate recognition by the bacterial type II secretion system: more than a simple interaction. *Molecular Microbiology*, 94, 126–140.
- Planet, P.J., Kachlany, S.C., DeSalle, R. and Figurski, D.H. (2001) Phylogeny of genes for secretion NTPases: Identification of the widespread tadA subfamily and development of a diagnostic key for gene classification. *Proceedings of the National Academy of Sciences USA*, 98, 2503–2508.
- Possot, O.M., Vignon, G., Bomchil, N., Ebel, F. and Pugsley, A.P. (2000) Multiple interactions between pullulanase secretion components involved in stabilization and cytoplasmic membrane association of PulE. *Journal of Bacteriology*, 182, 2142–2152.
- Pugsley, A.P. (1993) The complete general secretory pathway in gram-negative bacteria. *Microbiological Reviews*, 57, 50–108.
- Pugsley, A.P., Kornacker, M.G. and Poquet, I. (1991) The general protein-export pathway is directly required for extracellular pullulanase secretion in *Escherichia coli* K12. *Molecular Microbiology*, 5, 343–352.
- van Putte, W., de Vos, T., van den Broeck, W., Stahlberg, H., Kudryashev, M. and Savvides, S. (2018) Inner-membrane GspF of the bacterial type II secretion system is a dimeric adaptor mediating pseudopilus biogenesis. *bioRxiv*. <https://doi.org/10.1101/435982>.
- Py, B., Loiseau, L. and Barras, F. (1999) Assembly of the type II secretion machinery of *Erwinia chrysanthemi*: direct interaction and associated conformational change between OutE, the putative ATP-binding component and the membrane protein OutL. *Journal of Molecular Biology*, 289, 659–670.
- Py, B., Loiseau, L. and Barras, F. (2001) An inner membrane platform in the type II secretion machinery of gram-negative bacteria. *EMBO Reports*, 2, 244.
- Raghunathan, K., Vago, F.S., Grindem, D., Ball, T., Wedemeyer, W.J., Bagdasarian, M. et al (2014) The 1.59 Å resolution structure of the minor pseudopilin EpsH of *Vibrio cholerae* reveals a long flexible loop. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1844, 406–415.
- Rehman, S., Gu, S., Shevchik, V.E. and Pickersgill, R.W. (2013) Anatomy of secretin binding to the Dickeya dadantii type II secretion system pilotin. *Acta Crystallographica, Section D: Biological Crystallography*, D69, 1381–1386.
- Reichow, S.L., Korotkov, K.V., Gonen, M., Sun, J., Delarosa, J.R., Hol, W.G.J. et al (2011) The binding of cholera toxin to the periplasmic vestibule of the type II secretion channel. *Channels*, 5, 215–218.
- Reichow, S.L., Korotkov, K.V., Hol, W.G.J. and Gonen, T. (2010) Structure of the cholera toxin secretion channel in its closed state. *Nature Structural and Molecular Biology*, 17, 1226–1232.
- Reindl, S., Ghosh, A., Williams, G.J., Lassak, K., Neiner, T., Henche, A.-L. et al (2013) Insights into flag functions in archaeal motor assembly and motility from structures, conformations, and genetics. *Molecular Cell*, 49, 1069–1082.
- Robert, V., Filloux, A. and Michel, G.P.F. (2005) Subcomplexes from the Xcp secretion system of *Pseudomonas aeruginosa*. *FEMS Microbiology Letters*, 252, 43–50.
- Robien, M.A., Krumm, B.E., Sandkvist, M. and Hol, W.G.J. (2003) Crystal structure of the extracellular protein secretion NTPase EpsE of *Vibrio cholerae*. *Journal of Molecular Biology*, 333, 657–674.
- Rule, C.S., Patrick, M., Camberg, J.L., Maricic, N., Hol, W.G. and Sandkvist, M. (2016) Zinc coordination is essential for the function and activity of the type II secretion ATPase EpsE. *Microbiologyopen*, 5, 870–882.
- Sampaleanu, L.M., Bonanno, J.B., Ayers, M., Koo, J., Tammam, S., Burley, S.K. et al (2009) Periplasmic domains of *Pseudomonas aeruginosa* PilN

- and PilO form a stable heterodimeric complex. *Journal of Molecular Biology*, 394, 143–159.
- Sandkvist, M., Bagdasarian, M., Howard, S.P. and DiRita, V.J. (1995) Interaction between the autokinase EpsE and EpsL in the cytoplasmic membrane is required for extracellular secretion in *Vibrio cholerae*. *EMBO Journal*, 14, 1664–1673.
- Sandkvist, M., Hough, L.P., Bagdasarian, M.M. and Bagdasarian, M. (1999) Direct Interaction of the EpsL and EpsM proteins of the general secretion apparatus in *Vibrio cholerae*. *Journal of Bacteriology*, 181, 3129–3135.
- Sandkvist, M., Keith, J.M., Bagdasarian, M. and Howard, S.P. (2000) Two Regions of EpsL involved in species-specific protein-protein interactions with EpsE and EpsM of the general secretion pathway in *Vibrio cholerae*. *Journal of Bacteriology*, 182, 742–748.
- Santajit, S. and Indrawattana, N. (2016) Mechanisms of antimicrobial resistance in ESKAPE Pathogens. *BioMed Research International*, 2016, 1–8.
- Satyshur, K.A., Worzalla, G.A., Meyer, L.S., Heiniger, E.K., Aukema, K.G., Misić, A.M. et al (2007) Crystal structures of the pilus retraction motor PilT suggest large domain movements and subunit cooperation drive motility. *Structure*, 15, 363–376.
- Shevchik, V.E. (1997) Specific interaction between OutD, an *Erwinia chrysanthemi* outer membrane protein of the general secretory pathway, and secreted proteins. *EMBO Journal*, 16, 3007–3016.
- Shiue, S.J., Kao, K.M., Leu, W.M., Chen, L.Y., Chan, N.L. and Hu, N.T. (2006) XpsE oligomerization triggered by ATP binding, not hydrolysis, leads to its association with XpsL. *EMBO Journal*, 25, 1426–1435.
- Shutinoski, B., Schmidt, M.A.A. and Heusipp, G. (2010) Transcriptional regulation of the Yts1 type II secretion system of *Yersinia enterocolitica* and identification of secretion substrates. *Molecular Microbiology*, 75, 676–691.
- Sikora, A.E., Zielke, R.A., Lawrence, D.A., Andrews, P.C. and Sandkvist, M. (2011) Proteomic analysis of the *Vibrio cholerae* type II secretome reveals new proteins, including three related serine proteases. *Journal of Biological Chemistry*, 286, 16555–16566.
- Solanki, V., Kapoor, S. and Thakur, K.G. (2018) Structural insights into the mechanism of Type IVa pilus extension and retraction ATPase motors. *FEBS Journal*, 285, 3402–3421.
- Stroten, T.G., Li, G. and Howard, S.P. (2012) YghG (GspS β) is a novel pilot protein required for localization of the gspS β type II secretion system secretin of enterotoxigenic *Escherichia coli*. *Infection and Immunity*, 80, 2608–2622.
- Stroten, T.G., Stanley, H., Gu, Y., Boyd, J., Bagdasarian, M., Sandkvist, M. et al (2011) Involvement of the GspAB complex in assembly of the type II secretion system secretin of *Aeromonas* and *Vibrio* species. *Journal of Bacteriology*, 193, 2322–2331.
- Thomas, J.D., Reeves, P.J. and Salmond, G.P.C. (1997) The general secretion pathway of *Erwinia carotovora* subsp. *carotovora*: analysis of the membrane topology of OutC and OutF. *Microbiology*, 143, 713–720.
- Tilley, D., Law, R., Warren, S., Samis, J.A. and Kumar, A. (2014) CpaA a novel protease from *Acinetobacter baumannii* clinical isolates deregulates blood coagulation. *FEMS Microbiology Letters*, 356, 53–61.
- Tomás, A., Lery, L., Regueiro, V., Pérez-Gutiérrez, C., Martínez, V., Moranta, D. et al (2015) Functional genomic screen identifies *Klebsiella pneumoniae* factors implicated in blocking nuclear factor κ B (NF- κ B) signaling. *Journal of Biological Chemistry*, 290, 16678–16697.
- Tomich, M., Planet, P.J. and Figurski, D.H. (2007) The tad locus: postcards from the widespread colonization island. *Nature Reviews Microbiology*, 5, 363–375.
- Tosi, T., Nickerson, N.N., Mollica, L., Jensen, M.R., Blackledge, M., Baron, B. et al (2011) Pilotin-secretin recognition in the type II secretion system of *Klebsiella oxytoca*. *Molecular Microbiology*, 82, 1422–1432.
- Treuner-Lange, A., Chang, Y.W., Glatter, T., Herfurth, M., Lindow, S., Chreifi, G. et al (2020) PilY1 and minor pilins form a complex priming the type IVa pilus in *Myxococcus xanthus*. *Nature Communications*, 11, 5054–5114.
- Tsai, R.-T., Leu, W.-M., Chen, L.-Y. and Hu, N.-T. (2002) A reversibly dissociable ternary complex formed by XpsL, XpsM and XpsN of the *Xanthomonas campestris* pv. *campestris* type II secretion apparatus. *The Biochemical Journal*, 367, 865–871.
- Urusova, D.V., Kinsella, R.L., Salinas, N.D., Haurat, M.F., Feldman, M.F. and Tolia, N.H. (2019) The structure of *Acinetobacter*-secreted protease CpaA complexed with its chaperone CpaB reveals a novel mode of a T2SS chaperone-substrate interaction. *Journal of Biological Chemistry*, 294, 13344–13354.
- Viarre, V., Cascales, E., Ball, G., Michel, G.P.F., Filloux, A. and Voulhoux, R. (2009) HxcQ liposecretin is self-piloted to the outer membrane by its N-terminal lipid anchor. *Journal of Biological Chemistry*, 284, 33815–33823.
- Voulhoux, R., Ball, G., Ize, B., Vasil, M.L., Lazdunski, A., Wu, L.F. et al (2001) Involvement of the twin-arginine translocation system in protein secretion via the type II pathway. *EMBO Journal*, 20, 6735–6741.
- Waack, U., Johnson, T.L., Chedid, K., Xi, C., Simmons, L.A., Mobley, H.L.T. and et al (2017) Targeting the type II secretion system: development, optimization, and validation of a high-throughput screen for the identification of small molecule inhibitors. *Frontiers in Cellular and Infection Microbiology*, 7, 380.
- Wang, F., Coureuil, M., Osinski, T., Orlova, A., Altindal, T., Gesbert, G. et al (2017) Cryoelectron microscopy reconstructions of the *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* type IV Pili at Sub-nanometer resolution. *Structure*, 25, 1423–1435.
- Wang, X., Pineau, C., Gu, S., Guschinskaya, N., Pickersgill, R.W. and Shevchik, V.E. (2012) Cysteine scanning mutagenesis and disulfide mapping analysis of arrangement of GspC and GspD protomers within the type 2 secretion system. *Journal of Biological Chemistry*, 287, 19082–19093.
- Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R. et al (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research*, 46, W296–W303.
- Weaver, S.J., Ortega, D.R., Sazinsky, M.H., Dalia, T.N., Dalia, A.B. and Jensen, G.J. (2020) CryoEM structure of the type IVa pilus secretin required for natural competence in *Vibrio cholerae*. *Nature Communications*, 11, 5080.
- Yan, Z., Yin, M., Xu, D., Zhu, Y. and Li, X. (2017) Structural insights into the secretin translocation channel in the type II secretion system. *Nature Structural and Molecular Biology*, 24, 177–183.
- Yanez, M.E., Korotkov, K.V., Abendroth, J. and Hol, W.G.J. (2008) Structure of the minor pseudopilin EpsH from the type 2 secretion system of *Vibrio cholerae*. *Journal of Molecular Biology*, 377, 91–103.
- Yin, M., Yan, Z. and Li, X. (2018) Structural insight into the assembly of the type II secretion system pilotin-secretin complex from enterotoxigenic *Escherichia coli*. *Nature Microbiology*, 3, 581–587.
- Zöllner, R., Cronenberg, T. and Maier, B. (2019) Motor properties of PilT-independent type 4 pilus retraction in *Gonococci*. *Journal of Bacteriology*, 201, e00778–18.
- Zückert, W.R. (2014) Secretion of bacterial lipoproteins: through the cytoplasmic membrane, the periplasm and beyond. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1843, 1509–1516.

How to cite this article: Naskar S, Hohl M, Tassinari M, Low HH. The structure and mechanism of the bacterial type II secretion system. *Mol Microbiol*. 2021;115:412–424. <https://doi.org/10.1111/mmi.14664>