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Assembly and targeting of secretins in the bacterial outer membrane

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ABSTRACT

In Gram-negative bacteria, secretion of toxins ensure the survival of the bacterium. Such toxins are secreted by sophisticated multiprotein systems. The most conserved part in some of these secretion systems are components, called secretins, which form the outer membrane ring in these systems. Recent structural studies shed some light on the oligomeric organization of secretins. However, the mechanisms by which these proteins are targeted to the outer membrane and assemble there into ring structures are still not fully understood. This review discusses the various species-specific targeting and assembly pathways that are taken by secretins in order to form their functional oligomers.

1. Secretion systems in bacteria

Constant sensing of the environment is critical for prokaryotes survival. One of the many mechanisms developed by bacteria to secrete molecules to their environment is via secretory systems. Bacteria have nine known types of secretion systems that are involved in transport and secretion of substrates through their outer membrane (Green and Mecsas, 2016; Kubori, 2016). The substrates of these secretion systems vary depending on the organism and the secretion system.

Among the secretion systems of bacteria, the type II, III and IV systems are involved in pathogenic pathways. These systems secrete toxins either into the exoplasm (Type II, T2SS) or directly into the cytoplasm of the host cells (Type III, IV pilli; T3SS, T4P) (Alvarez-Martinez and Christie, 2009; Galan and Wolf-Watz, 2006; Johnson et al., 2006; Korotkov et al., 2012). The secretion systems consist of a massive complex spanning both the inner and outer membranes. In all three secretion systems (T2SS, T3SS and T4P), the outer membrane complex is conserved and composed of proteins called secretins (Disconzi et al., 2014; Korotkov et al., 2011). Secretins form homo-oligomeric gated pores in the outer membrane with copy number that varies from 12 to 15 depending on the species. For example, the T2SS secretin, PulD from *klebsiella oxytosa* forms a channel consisting of 12 subunits whereas T3SS secretin from *Salmonella typhimurium* exists in the outer membrane as a 15-mer structure (Berry et al., 2012; Chami et al., 2005; Schraidt and Marlovits, 2011). This review will discuss the current knowledge on the targeting and assembly pathways that lead to the integration of these proteins into the outer membrane.

2. Type II secretion system

Gram-negative bacteria use the type II secretion system to secrete molecules like hydrolases, toxins, or lipases to the exoplasm (Korotkov et al., 2012). The secreted proteins include hydrolytic enzymes such as pullulanase and other toxins such as cholera toxin, which are involved in the pathogenic pathway of the bacterium (d'Enfert et al., 1989; Sandkvist et al., 1997). The cholera toxin secreted by *Vibrio cholera* and the enterotoxin secreted by enterotoxigenic *E. coli* (ETEC) are the most studied of these systems (Dunstan et al., 2013; Korotkov et al., 2009). The secretary apparatus is composed of four parts: the outer membrane gated channel, the inner membrane complex, an ATPase, and a pseudopilus (Johnson et al., 2006). The pseudopili are related to the family of the flagellar/pillus system, which is also driven by ATPases (Sauvonnet et al., 2000). Type II secretory systems translocate fully folded proteins across the outer membrane through a gated channel composed of homooligomeric secretins (Johnson et al., 2006; Kubori, 2016). The type II secretion is a two-step process (Campos et al., 2013). First, the substrate protein has to be translocated from the cytoplasm to the periplasm with the help of the Sec or Tat systems. Accordingly, the secreted protein must have a signal sequence that is recognized by one of these systems (Korotkov et al., 2012). The secreted protein is either already in the folded form in the periplasm or is folded there before secretion. Finally, the folded protein is secreted across the outer membrane.

Another system that contains secretins and is related to T2SS is the type IV pilus (T4P) system. These appendages are dynamic filaments that are rapidly polymerized and depolymerized from a pool of pilin

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subunits. Cycles of pilus extension, binding and retraction enable T4P to perform a phenomenally diverse array of functions, including twitching motility, DNA uptake, and microcolony formation (Craig et al., 2019).

3. Type III secretion system

The type III secretion system (T3SS) secretes toxins directly into the cytoplasm of the host cells (Galan and Wolf-Watz, 2006). Bacteria that use T3SS for their pathogenicity include *Salmonella*, *Shigella*, *Yersinia*, *Pseudomonas* and Enteropathogenic *E. coli* (EPEC) (Cornelis, 2010; Galan and Wolf-Watz, 2006; Login et al., 2010; Marlovits and Stebbins, 2010). The system is adapted to secrete either a specific protein in the case of T3SS of *Salmonella* or a multitude of toxins like in EPEC. The secreted proteins are either in their folded or unfolded state (Radics et al., 2014). T3SS is also known as injectisome or needle complex and is composed of three regions. One is the basal body consisting of secretins, which form the outer membrane ring, and of an inner membrane platform. The second region is a needle like filament extending across the two membranes until the plasma membrane of the host, and the third element harbours the cytoplasmic components (Marlovits et al., 2006, 2004; Schraidt and Marlovits, 2011). The best studied among the type III systems is the injectisome of *Salmonella typhimurium* SPI-1 (*Salmonella* Pathogenicity Island 1) (Kuhlen et al., 2018; Marlovits et al., 2004; Ochman et al., 1996; Schraidt and Marlovits, 2011). Interestingly, this bacterium has an additional needle complex expressed exclusively from the SPI-2 (*Salmonella* Pathogenicity Island 2). Activation of the genes in SPI-2 is induced upon engulfment of the bacterium by phagocytes and is essential for survival and replication of the bacterium inside these cells (Ochman et al., 1996; Rappl et al., 2003; Shea et al., 1996).

The factors involved in the recognition and transport of the 'secretion ready protein' to the needle apparatus are still less explored. The basal body of the needle complex has two consecutive inner membrane rings made of two proteins in a 24-mer stoichiometry (Worrall et al., 2016). In contrast, the secretins of this system have a 15-mer stoichiometry (Schraidt and Marlovits, 2011; Worrall et al., 2016). Recent studies reported that the three proteins in the export apparatus form a pseudo-hexamer in the import competent state (Kuhlen et al., 2018). The export apparatus provides the platform onto which the polymerisation of the helical filament is initiated (Hu et al., 2018; Kuhlen et al., 2018). The needle is hollow allowing the vectorial movement of secreted proteins (Demers et al., 2014; Roversi et al., 2006). The tip of the needle apparatus is involved in sensing the contact with host cells upon which the translocon initiates the pore formation in the host cell membrane (Goure et al., 2005, 2004; Rosqvist et al., 1994).

4. Secretins in the outer membrane

The common feature among the outer membrane structures in the aforementioned secretion systems are the secretins, which form homooligomeric rings in the outer membrane consisting of 12–15 copies (Berry et al., 2012; Chami et al., 2005; Schraidt and Marlovits, 2011). PulD (T2SS, Klebsiella *oxytoca*), GspD (T2SS, *Vibrio cholera*), PilQ (T4P, *Neisseria meningitides*), and MxiD (T3SS, *Shigella flexneri*) show a 12-fold symmetry, PilQ (T4P, *Pseudomonas aeroginosa*) has a 14-fold symmetry, whereas InvG (T3SS, *Salmonella typhimurium*) exhibits a 15-fold symmetry (Berry et al., 2012; Chami et al., 2005; Hodgkinson et al., 2009; Koo et al., 2016; Reichow et al., 2010; Schraidt and Marlovits, 2011). Secretins oligomers are highly stable and they are usually resistant against detergents, higher temperatures, and denaturing agent (Hardie et al., 1996; Linderoth et al., 1996; Nouwen et al., 2000).

The C-terminus of secretins is conserved across different secretion systems and has a high tendency to form membrane-embedded β-sheets (Genin and Boucher, 1994). Some secretins have a domain at their C-terminus called S domain, which is required for the interaction with their corresponding specific assembly factor, a lipoprotein called pilotins (Daefler et al., 1997; Koo et al., 2012). The most variable part of secretins is their N-terminal domain that is proposed to have a system- and specie-specific role rather than a universal function (Korotkov et al., 2006; Login et al., 2010; Reichow et al., 2010). The assembled secretin forms a gated pore in the outer membrane, which is open only for the translocation of proteins through the channel (Disconzi et al., 2014; Radics et al., 2014).

5. Structural studies on secretins

Cryo-EM (Cryogenic electron microscopy) and other structural studies have recently revealed remarkable insights into the structure of secretins (Hu et al., 2018; Korotkov et al., 2013, 2009; Spreter et al., 2009; Van der Meeren et al., 2013; Worrall et al., 2016; Yan et al., 2017). Cryo-EM of the *Salmonella* T3SS secretin, InvG revealed an unexpected double walled β-barrel architecture (Worrall et al., 2016). This study showed that each monomer of the secretin core has nine β-strands. The outer barrel consists of 60 anti-parallel β-strands with each monomer contributing four strands (number 1, 3, 8, and 9) to the outer barrel. The inner barrel is also made up of 60 β-strands where each monomer contributes four β-strands (number 4, 5, 6, and 7). β-strands four and five are parallel to the membrane while strands six and seven are kinked and are perpendicular to the membrane. These four strands from each monomer form together the periplasmic gate. This reported secretin structure suggests that InvG is the first known protein with strands parallel to the membrane and with massive double-walled β-barrel architecture (Fig. 1).

Another very intriguing structural feature of the secretin oligomer is

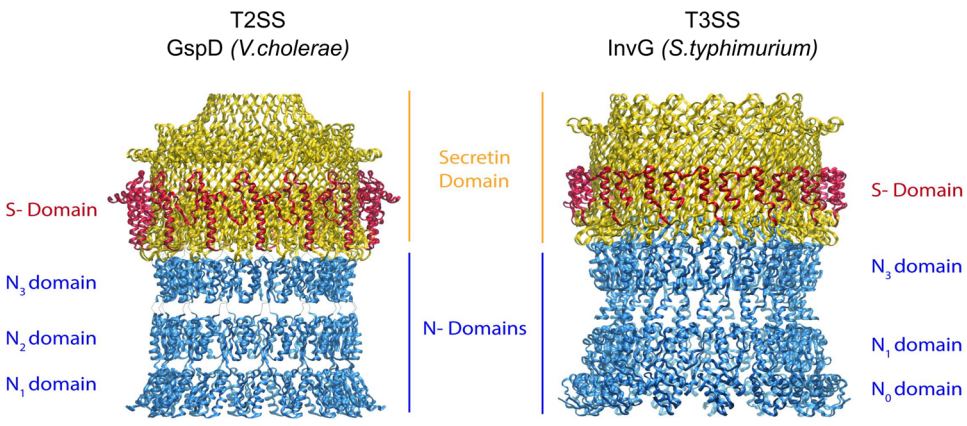

Fig. 1. Secretins structures. Comparison of type II secretion system secretin GspD in *Vibrio cholerae* (PDB accession number 5WQ8, Left) and the *Salmonella typhimurium* SPI-1 type III secretion injectisome secretin InvG (PDB accession number 6DV3, Right) complexes. The domain annotations of both secretins is colour coded for better comparison.

the observation that only small part of this massive structure is inserted into the membrane. Most of the protein is actually in the periplasm. The 'lip' of the complex consists of 60 β-strands with four strands from each monomer in the case of InvG (Hu et al., 2018; Worrall et al., 2016). In some secretin structures, as in the case of GspD (*V. cholera*), a cap gate has been observed which is formed by 30 β-strands, with each monomer contributing two β-strands (Yan et al., 2017). The `secretin lip´ is proximal to a highly amphipathic helical loop, which is structurally highly conserved. Mutation in this region interferes with membrane association of the secretin (Disconzi et al., 2014; Guilvout et al., 2011; Hu et al., 2018). The highly conserved nature of this part suggests a similar function across different secretion systems.

Structurally, the S domain of secretins has α helices, which protrude outside the barrel. The highly hydrophobic interface is critical for the stability of the fully assembled barrel, as any mutations in this region disrupt the stability of the oligomer (Fig. 1) (Guilvout et al., 2011).

The only common feature in the N-terminal region among all secretins, is the ring-binding motif (RBM) that is reported to initiate oligomerisation. The RBM motif has a characteristic structure with β-α-β-β-α motif (Yan et al., 2017). The oligomerization initiation leads to a membrane associated intermediate "prepore" that precedes the secretin assembly (Guilvout et al., 2017, 2014; Huysmans et al., 2015, 2013). This oligomerization in the N3 domain initiates the further interactions between adjacent protomers. Thus, S domain along with the N3 domain are the necessary factors required for the membrane insertion of the secretins. The rest of the N-terminal domain is variable, protrudes into the periplasm, and is proposed to serve a system-specific function (Hospenthal et al., 2017; Koo et al., 2012; Spreter et al., 2009; Tarry et al., 2011; Yan et al., 2017).

6. Proposed substrate secretion model

The fully assembled secretin is usually in its closed state where the periplasmic gate (˜ 15 Å) would restrict the pore for secretion of substrates (Hu et al., 2018; Worrall et al., 2016). Some substrates like the cholera toxin require a bigger pore size. In the case of T3SS, the substrate pushes through the flexible N3 domains to enter the pore's lumen. The push from the extending pseudopilus opens the periplasmic gate along with the cap gate (Yan et al., 2017). These conformational changes lead to free passage of the substrate into the cytosol of the host, thus initiating the infection.

The current hypothesis for the secretion of substrates via T2SS is the 'Piston model'. According to this model, the contact of secretins with the protein to be secreted promotes the activity of the ATPase, which leads to retraction of the pseudopilli, thereby leading to the secretion of the protein (Hobbs and Mattick, 1993; Sauvonnet et al., 2000). The contact of the secretion-ready protein with the secretins initiates a structural change thereby converting the closed conformation of secretin to an open one allowing secretion of the substrate (Korotkov et al., 2011; Reichow et al., 2010).

7. Secretins and their pilotins

It has been widely reported that several secretins have their own dedicated auxiliary protein, called pilotins (Koo et al., 2012). Pilotins are lipoproteins, which have a conserved motif that binds to lipids. The transport of pilotin via the Lol pathway potentially guides also the transport of the corresponding secretin from the inner to the outer membrane (Collin et al., 2011; Daefler et al., 1997; Nickerson et al., 2011). Pilotins from T2SS, identified so far, mostly belong to the PulS-OutS family of proteins. Such pilotins bind directly to the secretin via the S domain in the C-terminus of the latter. Structure analysis of the S-domain of PulD indicated that the domain is natively disordered (Nickerson et al., 2011; Worrall et al., 2016). Upon binding of the pilotin PulS to PulD, the S domain folds into a helical structure (Gu et al., 2012; Okon et al., 2008). The combined complex (PulS-PulD) is then transported to the outer membrane (Nickerson et al., 2011; Tosi et al., 2011). A well-structured α helix in the S-domain was captured along with its pilotin in the crystal structure of OutS (Gu et al., 2012). Experiments have shown that OutS proteins (homologues to PulS proteins) can be exchanged between different T2SSs but secretin and the substrates were not replaceable (Hardie et al., 1996; Possot and Pugsley, 1997; Pugsley, 1996).

A second family of pilotins, called ApsS (Alternate secretin pathway subunit S) was recently identified by a hidden Markov model analysis of the *Vibrio* secretins like GspD. The crystal structure of ApsS indicated no structural similarity between this lipoprotein and other known pilotins of T2SS. Nevertheless, studies on this lipoprotein suggested that it functions as pilotin and binds to the S-domain of *Vibrio* type secretins. These findings indicate a species specificity in pilotin-secretin interactions to accommodate a species-specific secretion of substrates (Dunstan et al., 2013).

8. General aspects of targeting and assembly of secretins in the outer membrane

The mechanism by which the complete secretins oligomer assemble in the outer membrane is still an enigma. The outer membrane secretins are synthesised in the cytoplasm and stabilized there by cytosolic factors (Bos et al., 2007; Ferbitz et al., 2004). The SecB chaperone ferries the protein to the Sec translocon (Bechtluft et al., 2010a, b; de Keyzer et al., 2003). Next, the energy obtained by hydrolysis of ATP by the ATPase SecA drives the transport of the newly synthesized secretin across the inner membrane and into the periplasm (de Keyzer et al., 2003; Zimmer et al., 2008). In the periplasm, the secretins are stabilized by the periplasmic chaperones SurA, Skp or by other factors (Patel et al., 2009; Sklar et al., 2007; Volokhina et al., 2011). The transport of the secretins from the periplasm to the outer membrane can follow four different pathways: Lol-dependent, BAM-dependent, unassisted one, or an accessory protein assisted process (Fig. 2). In the following sections, we discuss the various pathways by which secretins are targeted to and assembled into the outer membrane.

8.1. Lol-dependent membrane integration

It is anticipated that for secretins with known pilotin partners (like PulD and its pilotin PulS), the pilotin transport via the Lol pathway guides the secretin monomers from the periplasm to the outer membrane (Fig. 2a)(Collin et al., 2011; Daefler et al., 1997; Nickerson et al., 2011). The Lol pathway which is essential for sorting of lipoproteins, involves in *E. coli* the five proteins, Lol A–E, three compose the inner membrane receptor complex LolCDE, while the other two are the periplasmic LolA and outer membrane element LolB (Tokuda and Matsuyama, 2004). LolCDE, which harbours an ATP binding cassette transporter (ABC transporter), initiates the transfer of the outer membrane lipoprotein (pilotin) from the inner membrane to the periplasmic chaperone LolA. Next, LolA binds the lipoprotein in a 1:1 ratio and transfers the substrate to the outer membrane receptor LolB. The transfer of substrate from LolA to LolB is facilitated by the higher affinity of LolB to substrates (Matsuyama et al., 1995; Tokuda and Matsuyama, 2004; Yokota et al., 1999).

In the case of PulD, the pilotin PulS assists the initial assembly process of the secretin by the transport of the monomer units to the outer membrane. After the transfer, the secretin monomers form a prepore in a process that is independent of PulS (Daefler et al., 1997; Gu et al., 2012; Guilvout et al., 2006, 2011; Huysmans et al., 2013; Nouwen et al., 2000). The pre-pore then inserts into the membrane in a manner that was suggested to be unassisted (Guilvout et al., 2017). The only critical region of the protein identified so far for formation of the prepore is the N3 domain, which is just upstream to the secretin barrel domain (Huysmans et al., 2013). Without their respective pilotins, such secretins tend to be mistargeted to the inner membrane where they

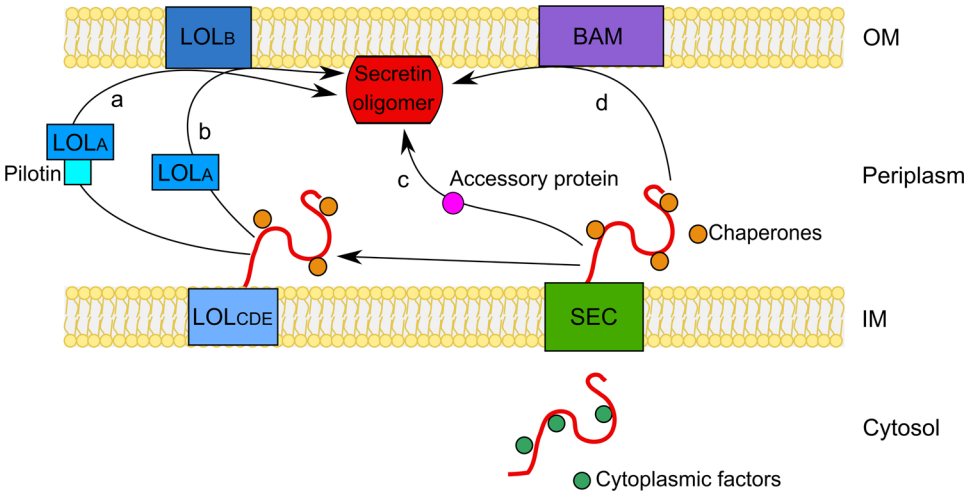

Fig. 2. Mechanism of secretins targeting to the OM. The secretin monomers is translated in the cytosol and enters the periplasm via the SEC translocon. In the periplasm, secretins can follow various species-specific pathways. The various assembly processes are: (a) Secretin monomers bound to their specific pilotin and use the Lol pathway for transport to the outer membrane. (b) Secretin monomers can be lipoproteins themselves and use the Lol pathway for transport to the outer membrane. (c) Secretin monomers require accessory proteins for either localization and/or assembly in the outer membrane. (d) Secretin monomers are integrated into the outer membrane via the BAM machinery.

oligomerize thereby inducing a phage shock response (Burghout et al., 2004; Crago and Koronakis, 1998; Guilvout et al., 2006). Similarly, PilQ from *Pseudomonas aeruginosa* is targeted to the inner membrane in the absence of its pilotin PilF. Moreover, a mutation in PilF, which affects its lipidation site, results in the assembly of the secretin PilQ in both the inner and the outer membrane (Koo et al., 2008).

Even though the majority of secretins have been identified to have specific lipoprotein to help with their localization, there are also secretins, which were identified to be lipoproteins by themselves. The HxcQ secretin from *P. aeruginosa* is an example of a protein that can target itself to the outer membrane with the help of the Lol pathway, without assistance of any pilotin (Fig. 2b) (Viarre et al., 2009).

8.2. Accessory proteins-assisted integration and/or assembly

Another class of secretins can assemble without help from other factors but require accessory proteins to facilitate their targeting to the outer membrane. The absence of such accessory proteins cause reduction in the amounts of the corresponding secretins. For example, the ability of the GspD secretin from *Vibrio* to form multimers is reduced by the absence of the accessory protein GspA (Strozen et al., 2011). Similarly, the multimerization of the secretin ExeD from *A. hydrophilia* was severely affected upon mutating the accessory protein *exeA/B* (Ast et al., 2002). An even more complex example is provided by the secretin MxiD. This secretin from *S. fexneri* requires for optimal assembly not only the pilotin MxiM but also its accessory protein MxiJ. (Schuch and Maurelli, 2001) (Fig. 2c).

Some secretins are reported to be able to localize to the OM in an unassisted manner. However, in the absence of their accessory proteins, the monomeric secretins cannot oligomerise. A prominent example is PilQ from *N. meningitis,* which accumulates as monomers in the OM in the absence of the accessory protein PilW and cannot support activity of T4P (Carbonnelle et al., 2005). Along the same line, BfpB secretin from *E. coli* T4P cannot form multimers in the absence of its accessory protein, BfpG (Bose and Taylor, 2005). Furthermore, the T4P secretin TcpC from *V. cholerae* is mutually stabilizing its accessory protein TcpQ. The absence of either one results in degradation of the other partner and additionally a defective T4P assembly (Balasingham et al., 2007; Carbonnelle et al., 2006, 2005; Drake et al., 1997) (Fig. 2c).

8.3. BAM-dependent membrane integration

Membrane assembly of β-barrel outer membrane proteins by the β-barrel assembly machinery (BAM complex) has been extensively studied in the last years. The BAM complex in *E. coli* consists of a central β-barrel protein, BamA which harbours five periplasmic polypeptide associated transport (POTRA) domains and is associated with four lipoproteins BamB-E (de Keyzer et al., 2003; Hagan et al., 2011; Paetzel, 2014; Voulhoux et al., 2003; Wu et al., 2005). The most conserved components of the complex among all Gram-negative bacteria are BamA and BamD, both are essential for cell viability (Malinverni et al., 2006; Webb et al., 2012; Wu et al., 2005). Structural studies on the BAM complex revealed that the substrate β-barrel proteins are released laterally into the membrane via the substrate exit part of the BamA barrel (Hagan et al., 2011; Han et al., 2016; Zeth, 2015).

The type IV secretin PilQ from *N. meningitis* was reported to assemble in a BAM-dependent manner (Voulhoux et al., 2003) (Fig. 2d). In contrast, PilQ from *P. aerogenosa* appears to use the Lol pathway for membrane targeting (Koo et al., 2008). In addition, even though these two PilQ secretins perform similar functions, their sequence homology is rather low. Thus, this variability suggest that PilQ's follow species-specific assembly pathways.

9. Concluding remarks

Secretins are homo-oligomeric proteins present in type II, III secretion systems and type IV pili. The assembly of the secretin in the OM is a species-specific process. Structural studies on the assembled secretin oligomer have revealed remarkable similarities between secretins from different secretion systems and species. Moreover, it has become apparent that the transfer of DNA via the T4P systems contributes to increase of drug resistant bacterial strains. In addition, since the absence of secretins oligomer in the outer membrane disrupts completely the secretion system, these components are potential drug targets.

Localization and/or assembly studies have demonstrated that most secretins require pilotins or accessory proteins to guide them towards their final destination and for their correct conformation. These proteins bind to the secretin protein aiding not only in the transport but also prevent the premature formation of oligomers at the inner membrane. Additionally, it has been shown that only in very rare cases the accessory proteins are interchangeable among different secretion systems. The species and system specificity seem to be a trait evolved to cope with the variety of the substrates present in the different systems and to assure infection of other species.

Despite the recent progress in our understanding of secretins biogenesis, many questions are still open. For example: (i) How do the individual components communicate with each other to promote assembly? (ii) Are the assembly process of the outer and inner membrane components coordinated? or (iii) Do interactions of secretins with other components of the secretion systems occur only after assembly of the separate units? Future studies will shed light into these fascinating processes.

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References

Ast, V.M., Schoenhofen, I.C., Langen, G.R., Stratilo, C.W., Chamberlain, M.D., Howard, S.P., 2002. Expression of the ExeAB complex of *Aeromonas hydrophila* is required for the localization and assembly of the ExeD secretion port multimer. Mol. Microbiol. 44, 217–231.

Balasingham, S.V., Collins, R.F., Assalkhou, R., Homberset, H., Frye, S.A., Derrick, J.P., Tonjum, T., 2007. Interactions between the lipoprotein PilP and the secretin PilQ in Neisseria meningitidis. J. Bacteriol. 189, 5716–5727.

Bechtluft, P., Kedrov, A., Slotboom, D.J., Nouwen, N., Tans, S.J., Driessen, A.J., 2010a. Tight hydrophobic contacts with the SecB chaperone prevent folding of substrate proteins. Biochemistry 49, 2380–2388.

Bechtluft, P., Nouwen, N., Tans, S.J., Driessen, A.J., 2010b. SecB–a chaperone dedicated to protein translocation. Mol. Biosyst. 6, 620–627.

Berry, J.L., Phelan, M.M., Collins, R.F., Adomavicius, T., Tonjum, T., Frye, S.A., Bird, L., Owens, R., Ford, R.C., Lian, L.Y., Derrick, J.P., 2012. Structure and assembly of a trans-periplasmic channel for type IV pili in Neisseria meningitidis. PLoS Pathog. 8, e1002923.

Bos, M.P., Robert, V., Tommassen, J., 2007. Biogenesis of the gram-negative bacterial outer membrane. Annu. Rev. Microbiol. 61, 191–214.

Bose, N., Taylor, R.K., 2005. Identification of a TcpC-TcpQ outer membrane complex involved in the biogenesis of the toxin-coregulated pilus of Vibrio cholerae. J. Bacteriol. 187, 2225–2232.

Burghout, P., Beckers, F., de Wit, E., van Boxtel, R., Cornelis, G.R., Tommassen, J., Koster, M., 2004. Role of the pilot protein YscW in the biogenesis of the YscC secretin in Yersinia enterocolitica. J. Bacteriol. 186, 5366–5375.

Campos, M., Cisneros, D.A., Nivaskumar, M., Francetic, O., 2013. The type II secretion system - a dynamic fiber assembly nanomachine. Res. Microbiol. 164, 545–555.

Carbonnelle, E., Helaine, S., Nassif, X., Pelicic, V., 2006. A systematic genetic analysis in *Neisseria meningitidis* defines the Pil proteins required for assembly, functionality, stabilization and export of type IV pili. Mol. Microbiol. 61, 1510–1522.

Carbonnelle, E., Helaine, S., Prouvensier, L., Nassif, X., Pelicic, V., 2005. Type IV pilus biogenesis in *Neisseria meningitidis*: PilW is involved in a step occurring after pilus assembly, essential for fibre stability and function. Mol. Microbiol. 55, 54–64.

Chami, M., Guilvout, I., Gregorini, M., Remigy, H.W., Muller, S.A., Valerio, M., Engel, A., Pugsley, A.P., Bayan, N., 2005. Structural insights into the secretin PulD and its trypsin-resistant core. J. Biol. Chem. 280, 37732–37741.

Collin, S., Guilvout, I., Nickerson, N.N., Pugsley, A.P., 2011. Sorting of an integral outer membrane protein via the lipoprotein-specific Lol pathway and a dedicated lipoprotein pilotin. Mol. Microbiol. 80, 655–665.

Cornelis, G.R., 2010. The type III secretion injectisome, a complex nanomachine for intracellular' toxin' delivery. Biol. Chem. 391, 745–751.

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

Craig, L., Forest, K.T., Maier, B., 2019. Type IV pili: dynamics, biophysics and functional consequences. Nature Rev Microbiol. https://doi.org/10.1038/s41579-019-0195-4. in press.

d'Enfert, C., Reyss, I., Wandersman, C., Pugsley, A.P., 1989. Protein secretion by gram-negative bacteria. Characterization of two membrane proteins required for pullulanase secretion by *Escherichia coli* K-12. J. Biol. Chem. 264, 17462–17468.

Daefler, S., Guilvout, I., Hardie, K.R., Pugsley, A.P., Russel, M., 1997. The C-terminal domain of the secretin PulD contains the binding site for its cognate chaperone, PulS, and confers PulS dependence on pIVf1 function. Mol. Microbiol. 24, 465–475.

de Keyzer, J., van der Does, C., Driessen, A.J., 2003. The bacterial translocase: a dynamic protein channel complex. Cell. Mol. Life Sci. 60, 2034–2052.

Demers, J.P., Habenstein, B., Loquet, A., Kumar Vasa, S., Giller, K., Becker, S., Baker, D., Lange, A., Sgourakis, N.G., 2014. High-resolution structure of the *Shigella* type-III secretion needle by solid-state NMR and cryo-electron microscopy. Nat. Commun. 5, 4976.

Disconzi, E., Guilvout, I., Chami, M., Masi, M., Huysmans, G.H., Pugsley, A.P., Bayan, N., 2014. Bacterial secretins form constitutively open pores akin to general porins. J. Bacteriol. 196, 121–128.

Drake, S.L., Sandstedt, S.A., Koomey, M., 1997. PilP, a pilus biogenesis lipoprotein in *Neisseria gonorrhoeae,* affects expression of PilQ as a high-molecular-mass multimer. Mol. Microbiol. 23, 657–668.

Dunstan, R.A., Heinz, E., Wijeyewickrema, L.C., Pike, R.N., Purcell, A.W., Evans, T.J., Praszkier, J., Robins-Browne, R.M., Strugnell, R.A., Korotkov, K.V., Lithgow, T., 2013. Assembly of the type II secretion system such as found in Vibrio cholerae depends on the novel Pilotin AspS. PLoS Pathog. 9, e1003117.

Ferbitz, L., Maier, T., Patzelt, H., Bukau, B., Deuerling, E., Ban, N., 2004. Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. Nature 431, 590–596.

Galan, J.E., Wolf-Watz, H., 2006. Protein delivery into eukaryotic cells by type III secretion machines. Nature 444, 567–573.

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

Goure, J., Broz, P., Attree, O., Cornelis, G.R., Attree, I., 2005. Protective anti-V antibodies inhibit *Pseudomonas* and *Yersinia* translocon assembly within host membranes. J. Infect. Dis. 192, 218–225.

Goure, J., Pastor, A., Faudry, E., Chabert, J., Dessen, A., Attree, I., 2004. The V antigen of *Pseudomonas aeruginosa* is required for assembly of the functional PopB/PopD translocation pore in host cell membranes. Infect. Immun. 72, 4741–4750.

Green, E.R., Mecsas, J., 2016. Bacterial secretion systems: an overview. Microbiol. Spectr. 4.

Gu, S., Rehman, S., Wang, X., Shevchik, V.E., Pickersgill, R.W., 2012. Structural and functional insights into the pilotin-secretin complex of the type II secretion system. PLoS Pathog. 8, e1002531.

Guilvout, I., Brier, S., Chami, M., Hourdel, V., Francetic, O., Pugsley, A.P., Chamot-Rooke, J., Huysmans, G.H., 2017. Prepore stability controls productive folding of the BAM-independent multimeric outer membrane secretin PulD. J. Biol. Chem. 292, 328–338.

Guilvout, I., Chami, M., Disconzi, E., Bayan, N., Pugsley, A.P., Huysmans, G.H., 2014. Independent domain assembly in a trapped folding intermediate of multimeric outer membrane secretins. Structure 22, 582–589.

Guilvout, I., Chami, M., Engel, A., Pugsley, A.P., Bayan, N., 2006. Bacterial outer membrane secretin PulD assembles and inserts into the inner membrane in the absence of its pilotin. EMBO J. 25, 5241–5249.

Guilvout, I., Nickerson, N.N., Chami, M., Pugsley, A.P., 2011. Multimerization-defective variants of dodecameric secretin PulD. Res. Microbiol. 162, 180–190.

Hagan, C.L., Silhavy, T.J., Kahne, D., 2011. Beta-Barrel membrane protein assembly by the bam complex. Annu. Rev. Biochem. 80, 189–210.

Han, L., Zheng, J., Wang, Y., Yang, X., Liu, Y., Sun, C., Cao, B., Zhou, H., Ni, D., Lou, J., Zhao, Y., Huang, Y., 2016. Structure of the BAM complex and its implications for biogenesis of outer-membrane proteins. Nat. Struct. Mol. Biol. 23, 192–196.

Hardie, K.R., Lory, S., Pugsley, A.P., 1996. Insertion of an outer membrane protein in *Escherichia coli* requires a chaperone-like protein. EMBO J. 15, 978–988.

Hodgkinson, J.L., Horsley, A., Stabat, D., Simon, M., Johnson, S., da Fonseca, P.C., Morris, E.P., Wall, J.S., Lea, S.M., Blocker, A.J., 2009. Three-dimensional reconstruction of the *Shigella* T3SS transmembrane regions reveals 12-fold symmetry and novel features throughout. Nat. Struct. Mol. Biol. 16, 477–485.

Hospenthal, M.K., Costa, T.R.D., Waksman, G., 2017. A comprehensive guide to pilus biogenesis in gram-negative bacteria. Nat. Rev. Microbiol. 15, 365–379.

Hu, J., Worrall, L.J., Hong, C., Vuckovic, M., Atkinson, C.E., Caveney, N., Yu, Z., Strynadka, N.C.J., 2018. Cryo-EM analysis of the T3S injectisome reveals the structure of the needle and open secretin. Nat. Commun. 9, 3840.

Huysmans, G.H., Guilvout, I., Chami, M., Nickerson, N.N., Pugsley, A.P., 2015. Lipids assist the membrane insertion of a BAM-independent outer membrane protein. Sci. Rep. 5, 15068.

Huysmans, G.H., Guilvout, I., Pugsley, A.P., 2013. Sequential steps in the assembly of the multimeric outer membrane secretin PulD. J. Biol. Chem. 288, 30700–30707.

Johnson, T.L., Abendroth, J., Hol, W.G., Sandkvist, M., 2006. Type II secretion: from structure to function. FEMS Microbiol. Lett. 255, 175–186.

Koo, J., Burrows, L.L., Howell, P.L., 2012. Decoding the roles of pilotins and accessory proteins in secretin escort services. FEMS Microbiol. Lett. 328, 1–12.

Koo, J., Lamers, R.P., Rubinstein, J.L., Burrows, L.L., Howell, P.L., 2016. Structure of the *Pseudomonas aeruginosa* type IVa pilus secretin at 7.4 a. Structure 24, 1778–1787.

Koo, J., Tammam, S., Ku, S.Y., Sampaleanu, L.M., Burrows, L.L., Howell, P.L., 2008. PilF is an outer membrane lipoprotein required for multimerization and localization of the *Pseudomonas aeruginosa* Type IV pilus secretin. J. Bacteriol. 190, 6961–6969.

Korotkov, K.V., Delarosa, J.R., Hol, W.G.J., 2013. A dodecameric ring-like structure of the N0 domain of the type II secretin from enterotoxigenic Escherichia coli. J. Struct. Biol. 183, 354–362.

Korotkov, K.V., Gonen, T., Hol, W.G., 2011. Secretins: dynamic channels for protein transport across membranes. Trends Biochem. Sci. 36, 433–443.

Korotkov, K.V., Krumm, B., Bagdasarian, M., Hol, W.G., 2006. Structural and functional studies of EpsC, a crucial component of the type 2 secretion system from Vibrio cholerae. J. Mol. Biol. 363, 311–321.

Korotkov, K.V., Pardon, E., Steyaert, J., Hol, W.G., 2009. 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., Hol, W.G., 2012. The type II secretion system: biogenesis, molecular architecture and mechanism. Nat. Rev. Microbiol. 10, 336–351.

Kubori, T., 2016. Life with bacterial secretion systems. PLoS Pathog. 12, e1005562.

Kuhlen, L., Abrusci, P., Johnson, S., Gault, J., Deme, J., Caesar, J., Dietsche, T., Mebrhatu, M.T., Ganief, T., Macek, B., Wagner, S., Robinson, C.V., Lea, S.M., 2018. Structure of the core of the type III secretion system export apparatus. Nat. Struct. Mol. Biol. 25, 583–590.

Linderoth, N.A., Model, P., Russel, M., 1996. Essential role of a sodium dodecyl sulfate-resistant protein IV multimer in assembly-export of filamentous phage. J. Bacteriol. 178, 1962–1970.

Login, F.H., Fries, M., Wang, X., Pickersgill, R.W., Shevchik, V.E., 2010. A 20-residue peptide of the inner membrane protein OutC mediates interaction with two distinct sites of the outer membrane secretin OutD and is essential for the functional type II secretion system in Erwinia chrysanthemi. Mol. Microbiol. 76, 944–955.

Malinverni, J.C., Werner, J., Kim, S., Sklar, J.G., Kahne, D., Misra, R., Silhavy, T.J., 2006. YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in Escherichia coli. Mol. Microbiol. 61, 151–164.

Marlovits, T.C., Kubori, T., Lara-Tejero, M., Thomas, D., Unger, V.M., Galan, J.E., 2006. Assembly of the inner rod determines needle length in the type III secretion injectisome. Nature 441, 637–640.

Marlovits, T.C., Kubori, T., Sukhan, A., Thomas, D.R., Galan, J.E., Unger, V.M., 2004. Structural insights into the assembly of the type III secretion needle complex. Science 306, 1040–1042.

Marlovits, T.C., Stebbins, C.E., 2010. Type III secretion systems shape up as they ship out.

Curr. Opin. Microbiol. 13, 47–52.
Matsuyama, S., Tajima, T., Tokuda, H., 1995. A novel periplasmic carrier protein involved in the sorting and transport of *Escherichia coli* lipoproteins destined for the outer membrane. EMBO J. 14, 3365–3372.
Nickerson, N.N., Tosi, T., Dessen, A., Baron, B., Raynal, B., England, P., Pugsley, A.P., 2011. Outer membrane targeting of secretin PulD protein relies on disordered domain recognition by a dedicated chaperone. J. Biol. Chem. 286, 38833–38843.
Nouwen, N., Stahlberg, H., Pugsley, A.P., Engel, A., 2000. Domain structure of secretin PulD revealed by limited proteolysis and electron microscopy. EMBO J. 19, 2229–2236.
Ochman, H., Soncini, F.C., Solomon, F., Groisman, E.A., 1996. Identification of a pathogenicity island required for *Salmonella* survival in host cells. Proc. Natl. Acad. Sci. U. S. A. 93, 7800–7804.
Okon, M., Moraes, T.F., Lario, P.I., Creagh, A.L., Haynes, C.A., Strynadka, N.C., McIntosh, L.P., 2008. Structural characterization of the type-III pilot-secretin complex from Shigella flexneri. Structure 16, 1544–1554.
Paetzel, M., 2014. Structure and mechanism of *Escherichia coli* type I signal peptidase. Biochim. Biophys. Acta 1843, 1497–1508.
Patel, G.J., Behrens-Kneip, S., Holst, O., Kleinschmidt, J.H., 2009. The periplasmic chaperone Skp facilitates targeting, insertion, and folding of OmpA into lipid membranes with a negative membrane surface potential. Biochemistry 48, 10235–10245.
Possot, O.M., Pugsley, A.P., 1997. The conserved tetracysteine motif in the general secretory pathway component PulE is required for efficient pullulanase secretion. Gene 192, 45–50.
Pugsley, A.P., 1996. Multimers of the precursor of a type IV pilin-like component of the general secretory pathway are unrelated to pili. Mol. Microbiol. 20, 1235–1245.
Radics, J., Konigsmaier, L., Marlovits, T.C., 2014. Structure of a pathogenic type 3 secretion system in action. Nat. Struct. Mol. Biol. 21, 82–87.
Rappl, C., Deiwick, J., Hensel, M., 2003. Acidic pH is required for the functional assembly of the type III secretion system encoded by *Salmonella* pathogenicity island 2. FEMS Microbiol. Lett. 226, 363–372.
Reichow, S.L., Korotkov, K.V., Hol, W.G., Gonen, T., 2010. Structure of the cholera toxin secretion channel in its closed state. Nat. Struct. Mol. Biol. 17, 1226–1232.
Rosqvist, R., Magnusson, K.E., Wolf-Watz, H., 1994. Target cell contact triggers expression and polarized transfer of *Yersinia* YopE cytotoxin into mammalian cells. EMBO J. 13, 964–972.
Roversi, P., Johnson, S., Field, T., Deane, J.E., Galyov, E.E., Lea, S.M., 2006. Expression, purification, crystallization and preliminary crystallographic analysis of BipD, a component of the *Burkholderia pseudomallei* type III secretion system. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 62, 861–864.
Sandkvist, M., Michel, L.O., Hough, L.P., Morales, V.M., Bagdasarian, M., Koomey, M., DiRita, V.J., Bagdasarian, M., 1997. General secretion pathway (eps) genes required for toxin secretion and outer membrane biogenesis in Vibrio cholerae. J. Bacteriol. 179, 6994–7003.
Sauvonnet, N., Vignon, G., Pugsley, A.P., Gounon, P., 2000. Pilus formation and protein secretion by the same machinery in Escherichia coli. EMBO J. 19, 2221–2228.
Schraidt, O., Marlovits, T.C., 2011. Three-dimensional model of Salmonella's needle complex at subnanometer resolution. Science 331, 1192–1195.
Schuch, R., Maurelli, A.T., 2001. MxiM and MxiJ, base elements of the Mxi-Spa type III secretion system of *Shigella*, interact with and stabilize the MxiD secretin in the cell envelope. J. Bacteriol. 183, 6991–6998.
Shea, J.E., Hensel, M., Gleeson, C., Holden, D.W., 1996. Identification of a virulence locus encoding a second type III secretion system in Salmonella typhimurium. Proc. Natl. Acad. Sci. U. S. A. 93, 2593–2597.
Sklar, J.G., Wu, T., Kahne, D., Silhavy, T.J., 2007. Defining the roles of the periplasmic chaperones SurA, Skp, and DegP in Escherichia coli. Genes Dev. 21, 2473–2484.
Spreter, T., Yip, C.K., Sanowar, S., Andre, I., Kimbrough, T.G., Vuckovic, M., Pfuetzner, R.A., Deng, W., Yu, A.C., Finlay, B.B., Baker, D., Miller, S.I., Strynadka, N.C., 2009. A conserved structural motif mediates formation of the periplasmic rings in the type III secretion system. Nat. Struct. Mol. Biol. 16, 468–476.
Strozen, T.G., Stanley, H., Gu, Y., Boyd, J., Bagdasarian, M., Sandkvist, M., Howard, S.P., 2011. Involvement of the GspAB complex in assembly of the type II secretion system secretin of *Aeromonas* and *Vibrio* species. J. Bacteriol. 193, 2322–2331.
Tarry, M., Jaaskelainen, M., Paino, A., Tuominen, H., Ihalin, R., Hogbom, M., 2011. The extra-membranous domains of the competence protein HofQ show DNA binding, flexibility and a shared fold with type I KH domains. J. Mol. Biol. 409, 642–653.
Tokuda, H., Matsuyama, S., 2004. Sorting of lipoproteins to the outer membrane in *E. Coli*. Biochim. Biophys. Acta 1694 IN1-9.
Tosi, T., Nickerson, N.N., Mollica, L., Jensen, M.R., Blackledge, M., Baron, B., England, P., Pugsley, A.P., Dessen, A., 2011. Pilotin-secretin recognition in the type II secretion system of Klebsiella oxytoca. Mol. Microbiol. 82, 1422–1432.
Van der Meeren, R., Wen, Y., Van Gelder, P., Tommassen, J., Devreese, B., Savvides, S.N., 2013. New insights into the assembly of bacterial secretins: structural studies of the periplasmic domain of XcpQ from Pseudomonas aeruginosa. J. Biol. Chem. 288, 1214–1225.
Viarre, V., Cascales, E., Ball, G., Michel, G.P., Filloux, A., Voulhoux, R., 2009. HxcQ liposecretin is self-piloted to the outer membrane by its N-terminal lipid anchor. J. Biol. Chem. 284, 33815–33823.
Volokhina, E.B., Grijpstra, J., Stork, M., Schilders, I., Tommassen, J., Bos, M.P., 2011. Role of the periplasmic chaperones Skp, SurA, and DegQ in outer membrane protein biogenesis in Neisseria meningitidis. J. Bacteriol. 193, 1612–1621.
Voulhoux, R., Bos, M.P., Geurtsen, J., Mols, M., Tommassen, J., 2003. Role of a highly conserved bacterial protein in outer membrane protein assembly. Science 299, 262–265.
Webb, C.T., Heinz, E., Lithgow, T., 2012. Evolution of the beta-barrel assembly machinery. Trends Microbiol. 20, 612–620.
Worrall, L.J., Hong, C., Vuckovic, M., Deng, W., Bergeron, J.R., Majewski, D.D., Huang, R.K., Spreter, T., Finlay, B.B., Yu, Z., Strynadka, N.C., 2016. Near-atomic-resolution cryo-EM analysis of the *Salmonella* T3S injectisome basal body. Nature.
Wu, T., Malinverni, J., Ruiz, N., Kim, S., Silhavy, T.J., Kahne, D., 2005. Identification of a multicomponent complex required for outer membrane biogenesis in Escherichia coli. Cell 121, 235–245.
Yan, Z., Yin, M., Xu, D., Zhu, Y., Li, X., 2017. Structural insights into the secretin translocation channel in the type II secretion system. Nat. Struct. Mol. Biol. 24, 177–183.
Yokota, N., Kuroda, T., Matsuyama, S., Tokuda, H., 1999. Characterization of the LolA-LolB system as the general lipoprotein localization mechanism of Escherichia coli. J. Biol. Chem. 274, 30995–30999.
Zeth, K., 2015. Structure determination of the BAM complex accessory lipoproteins BamB-E. Methods Mol. Biol. 1329, 189–202.
Zimmer, J., Nam, Y., Rapoport, T.A., 2008. Structure of a complex of the ATPase SecA and the protein-translocation channel. Nature 455, 936–943.