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Autotransporter Proteins

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Abstract
Autotransporter proteins, which are examined under five groups on the basis of their secretory systems, constitute the largest protein family found in Gram-negative pathogenic bacteria. The determination that all autotransporter proteins are associated with pathogenicity and virulence in these bacteria has made them the focus of molecular pathogenicity studies. Although the structural organization of autotransporter proteins and the base sequences of the genes encoding them are highly similar, their functions in the strains they are found in show a high diversity. This indicates that pathogen-host adaptation may also result from differences in protein processing and secretion system, and that new and effective approaches can be developed in the fight against pathogens. In this review article, the contemporary literature of this important protein family has been examined, and it has been tried to be interpreted as a basis for new scientific studies.

Key Words
"Autotransporter proteins, genetic and biochemical structure, secretion systems"

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1. Introduction

Autotransporter proteins, which are the proteins with the highest number of members in all pathogenic Gram-negative bacteria, have been identified to date, more than 1000 members. The general organization and primary structures of autotransporter proteins, which are closely related to the evolution of pathogenicity in these bacteria, are quite similar. However, when the specific functions of autotransporter proteins in bacteria are examined, a high level of diversity is observed. In general organizations; a signal peptide is followed by an N-terminal passenger domain (α domain) of 20-400 kDa, depending on the species, followed by a C-terminal translocator (β-domain) domain with an average of 30 kDa. In the first studies conducted with these proteins, it was determined that the passenger domain (N-domain) was associated with pathogenicity functions in Gram-negative bacteria, and the C-domain (translocator domain) was associated with protein transport (Veiga et al. 2004; Fulcher et al. 2006). Although these initial determinations are still valid, the initial knowledge on autotransporter proteins has been considerably elaborated in parallel with the intensification of research.

The first autotransporter protein identified in Gram-negative pathogens was the immunoglobulin A protease (IgAP) produced by *Neisseria gonorrhoeae*. The protein in question serves to protect the pathogen from the host immune system by breaking down antibodies. Since there is no information about the secretory systems of these proteins in this process, researchers evaluated the limited data based on protein structure analyzes and predicted that these proteins could carry out their own transport and named them as autotransporter proteins (Van Ulsen et al. 2003, Bhullar et al. 2015, Abreu et al. 2016). However, after this first determination, as the information on the relationship between autotransporter proteins and virulence increased, the secretory mechanisms began to be investigated in detail. As a result of these studies, it has been determined that today, contrary to the first belief, autotransporter proteins are transported using Type V secretion systems. Type V secretion system is a common secretion system found in petogenous bacteria and compared to other secretion systems, it is a secretory system whose existence has only recently been understood. As researches on this secretory system have intensified, it has been determined that different autotransports are transported through different subclasses of Type V secretion system (Chauhan et al. 2016, Da Mata Madeira et al. 2016, Chauhan et al. 2019).

Despite the structural characteristics conserved in evolutionary processes and known similar secretion mechanisms, the specific functions of these proteins have very different effects on pathogenicity and virulence, which can be expressed as the main characteristic that distinguishes the autotransporter protein family from other large protein families. In bacteria where the autotransporter proteins studied so far are found; It has been determined that it has functions such as suppression of immune response, toxin, adhesion and colonization factor (adhesin), host cell invasion factor (invasin), protease, lipase, serum resistance factor, intercellular spreading factor, iron binding protein, autoagglutinin and biofilm factor (Chauhan et al. 2016, Chauhan et al. 2019). In particular, the determination of a close relationship between biofilm forms formed in different pathogens to provide resistance to adverse environmental conditions and autotransporter proteins has given a new dimension and impetus to research on the functions of autotransporter proteins.

2. Diversity of Autotransporter Proteins

As previously stated, over 1000 different autotransporter proteins produced by Gram-negative bacteria have been identified. *Escherichia coli* was the richest species in terms of diversity of autotransporter proteins. Major autotransporter proteins isolated from Escherichia coli and identified according to their structural and functional differences; Eha (EHEC, adhesin, biofilm factor), Upa (UPEC biofilm factor), Ag43 (autoagglutinin and biofilm factor), YfaL (biofilm factor), AIDA (adhesin), TibA (adhesin, invasin), YgcV (biofilm factor), EspP (proteolytic toxin), Pet (proteolytic toxin), Sat (proteolytic toxin), Tsh (hemagglutinin) and Pic (mucinase).These are autotransporter proteins that have been studied in detail. In *Bordetella* spp. pertactin (adhesin), BrkA (serum resistance factor), TcfA (adhesin) and VagB (adhesin); BprV, BprB, AprV2 and BprX, all of which have elastase activity in *Dichleobacter nodosus*; Apart from the IgA1 protease identified in *Haemophilus influenzae*, Adhesin Hap, Hia and Hsf; VacA(toxin) and BabA(adexzin) identified in *Helicobacter pylori*, MisL(adhesin, invazin), ShdA(adhesin) and ApeE (esterase) identified in *Salmonella* constitute the other most well-known autotransporter family members (Guyer et al. 2002, Jain et al. Goldberg 2007, Wells et al. 2007, Brockmeyer et al. 2009, Nava-Acosta and Navarro-Garcia 2013, Habouria et al. 2019).

As studies on the diversity of autotransporter proteins increase, the functional diversity of these proteins also increases, and the fact that all of these functional diversity is associated with bacterial pathogenicity and virulence is the main reason why isolation and diagnosis studies carried out on this protein family are kept up-to-date. (Habouria et al. 2019) (**Table 1**).

3. General Structure and Secretion Mechanisms of Autotransporter Proteins

Studies on the genetic and biochemical basis of autotransporter proteins have shown that they are highly conserved proteins in the evolutionary process. The conserved structure in all of these proteins is characteristic, with a passenger domain (located at the N-terminus of the polypeptide, α domain) directing translocation from the inner membrane to the cell surface, and a translocator domain (β-domain) directing secretion from outer membrane to external space of the cell by a secretory pore. Autochaperones and protein binding domains may also be present in the translocator regions, which differ according to the type of autotransporter protein. (**Figure**

1a). Despite all these similar structural features, there is a high diversity in the functions of autotransporter proteins. Their classification is generally based on their functions and secretion systems (Otto et al. 2005, Tajima et al. 2010).

The transport of autotransporter proteins from the cytoplasm is a process that starts with the transportation from the inner membrane systems to the periplasm and after reaching the outer membrane systems from the periplasm, they are either secreted in contact with the cell surface or are completely secreted into the extracellular environment. The passage of autotransporters through barriers such as the inner cell membrane, periplasm and outer cell membrane takes place by energy-dependent and highly complex reactions. There are 6 different secretion systems (protein secretion pathways) used by Gram-negative bacteria in autotransporter protein transport. Type V secretion pathway, which is classified according to the elements they use and the analogy in the biological processes that take place, is also known as the autotransporter secretion pathway (Rutherford and Mourez, 2006, Ruiz-Perez et al. 2009).

Type V secretion system or autotransporter secretion pathway is based on the similarities and differences in the biological processes it uses; It is divided into five subcategories as Type Va, Type Vb, Type Vc, Type Vd and Type Ve (Tian et al. 2010, Casasanta et al. 2017). The point common to all the five categories we mentioned here is the transport of all secreted proteins across the membranes (Translocation) through a transmembrane pore formed by the β-barrel structure (Desvaux et al., 2005, Casasanta et al. 2017, Coppens et al. 2018) (**Figure 1b**).

Table 1. Major adhesins produced by gram-negative bacteria

Protein	**Producer**	**Function**	**Refrence**
Pertaktin	*Bordetella*	Adezin	Junker et al. 2006
Ag43, Antigen 43	*Escherichia coli*	Autoaglutinin, biyofilm formation	Navarro-Garcia vd. 2014
BrkA	*Bordetella pertusis*	Serum resistance	Braud et al. 2014
EspP	*Escherichia coli*	Proteolytic toxin	Deibel et al. 1998
Ig1A protease	*Haemophilus influenzae*	Ig1A cleavage	Bhullar et al. 2015
IcsA	*Shigella flexneri*	Intracellular invasion	Otto et al. 2005
MisL	*Salmonella* Typhimurium	Adhesin, biyofilm formation, invasin	Tükel et al. 2007
ShdA	*Salmonella* Typhimurium	Adhesin, invasin	Kingsley et al. 2002
VacA	*Helicobacter pylori*	Toxin	El Tahir and Skurnik 2001
YdaA	*Yersinia Enterocolitica*	Adezin, Serum Direnci	El Tahir and Skurnik 2001
BapA	*Yersinia Enterocolitica*	Adhesin	El Tahir and Skurnik 2001
AIDA-I	*Escherichia coli*	Adhesin	El Tahir and Skurnik 2001
Bpr 5	*Dichleobacter nodosus*	Elastase	Suhr et al. 1996
ShlB	*Serratia marcesens*	Protease	Wells et al. 2007
			Wells et al. 2007

Their secretions strategies can be classified as classical monomeric autotransporters in Type Va secretion system, two partner autotransporters in Type 5b secretion system, trimeric autotransporter adhesins (TAA) in Type 5c secretion system, hybrid autotransporters in Type 5d secretion system, and inverted autotransporters in Type 5e secretion system (Thanassi et al. 2005, Berne et al. 2015) (Thanassi et al. 2005, Berne et al. 2015). In all subclasses of the type V secretion system mentioned above; a signal sequence, the passenger domain of the secreted protein, and a β-barrel translocation domain are necessarily involved. However, the exact composition, order and size of these areas differ between subclasses (Schimid et al. 2003, Desvaux et al. 2005, Junker et al. 2006, Junker et al. 2009, Leo and Linke 2018) (**Figure 1a**).

3.1.Type Va Secretion System (Classic Monomeric Autotransporters)

In this system, the secretion of monomeric autotransporter proteins takes place. autotransporters using the Type Va secretion pathway are also called classical autotransporters. The most known members of this class are IgA protease from *Neisseria meningitidis*, EstA, a lipase from *Pseudomonas aeruginosa* and AIDA-I from *Escherichia coli*. This class of autotransporters can also be subclassed into serine proteases (SPATE), serine protease-like (SPATE-like), and serine non-prosthetic (non-SPATE) based on their protease activity (Hendrixson and St Geme 1998, Heimer et al. 2004, Junker et al. 2009, Navarro-Garcia et al. 2014). In some cases, the type Va passenger domain may separate after secretion. Passenger domains with enzymatic activity such as SPATE proteases have also been identified in some members of adhesins such as AIDA-I. Other examples of this subclass are SAATs (self-aggregating autotransporters) such as the *E. coli* autotransporter Ag43 (Westendorf et al. 2005, Navarro-Garcia et al. 2014).

The most studied subclass in the Type 5 secretion pathway is Type Va. Monomeric autotransporter proteins contain a 12-sheet β-barrel domain that functions as a C-terminal anchor on the outer membrane and is required for transport of the N-terminal passenger domain into the extracellular environment. Passenger domains usually have a repeating β-helix folding outward from the bacterial cell surface, as determined in the passenger domain of pertactin. These proteins can also contain the α-helix passenger domain, as has been detected for EstA. The passenger domain serves the specific function of the protein, which is related to virulence.

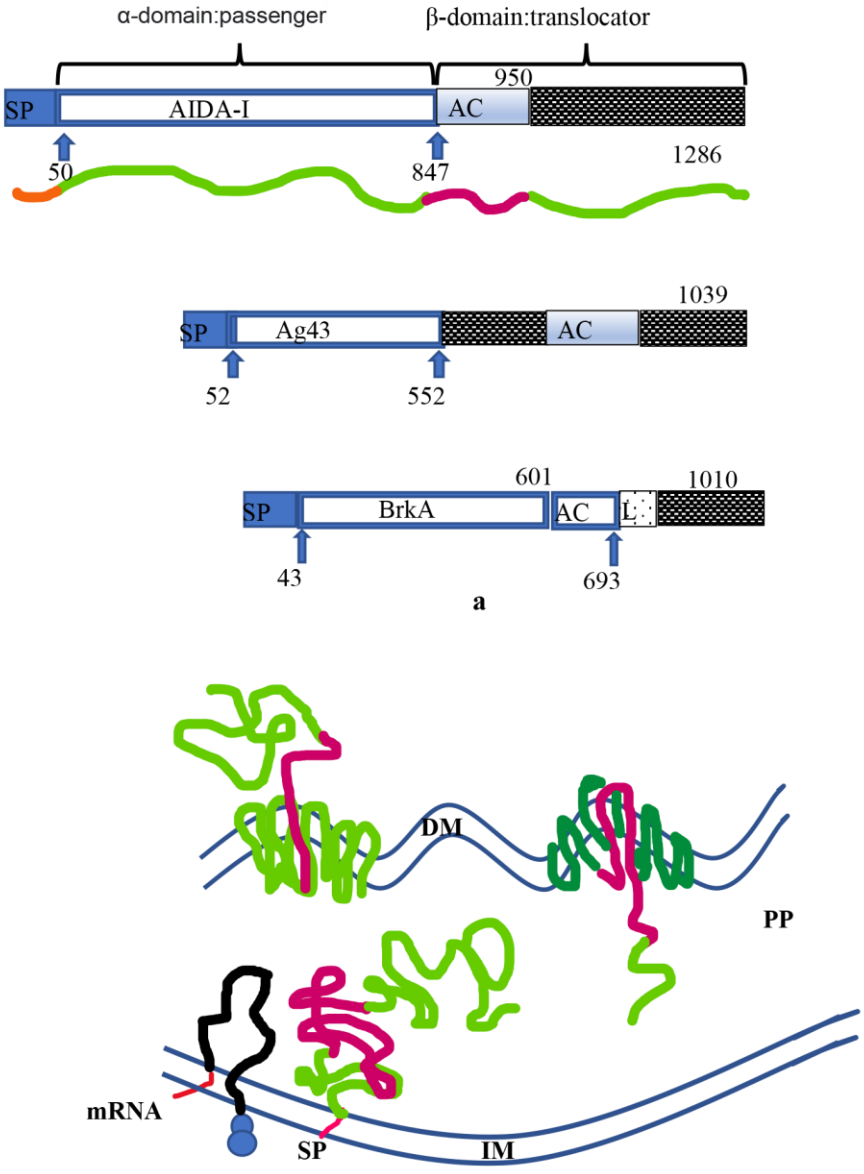

Figure 1. Structural domains (a) and secretion (b) of engineered classical autotransporter proteins of E. coli (AIDA-I, Ag43) and B. pertussis (BrkA). SP: signal peptide; AC: autochaperone area required for stability and folding; L: linker domain; arrows: binding and processing sites, IM: inner membrane, SP: signal peptide, PP: periplasm, DM: outer membrane.

In this system, autotransporter protein; It contains a signal sequence, a passenger domain, and a β-barrel translocation domain of approximately 300 amino acids. The signal sequence directs translocation (displacement) across the cell membrane in a process mediated by the SecYEG translocon (Desvaux et al. 2005, Meuskens et al. 2015). After the autotransporter protein enters the periplasm, which is located between the outer and inner membranes, the signal sequence is enzymatically removed from the peptide structure. After this step has taken place, specific periplasmic chaperones (SurA, Skp, and DegP) help deliver the peptide to the outer membrane β-barrel assembly machinery (Bam) complex (Serruto et al. 2010, Braud et al. 2014, Bdenanti et al. 2015). This complex facilitates insertion of the C-terminal translocation domain into the outer membrane as a monomeric β-barrel. The β-barrel domain of the Type Va secretory system initiates displacement of the passenger domain to the cell surface and acquires a folded conformation when this process is complete (**Figure 1b**). Although the precise mechanism of passenger domain-directed translocation has not yet been elucidated, it has been determined that the Bam complex is an integral part of this process (Bdenanti et al. 2015, Meuskens et al. 2015). Bam complex in *Escherichia coli*; It has been determined that it consists of five proteins, namely BamA, Bamb, BamC, BamD and BamE (Maroncle et al. 2006, Jain and Goldberg, 2007). BamA is an outer membrane protein that has a critical role in the formation of this complex. BamA; BamB, BamC, BamD, and BamE bind to them by non-covalent interactions to form the lipoprotein complex. It has been determined that BamA and BamD, which are included in this final complex, also have critical roles in cell viability, unlike other components of the complex (Manrocle et al. 2006). However, all components of the Bam complex are known to function in the integrity of the outer membrane and in the biogenesis of some outer membrane proteins. Recent studies evaluating the contribution of individual Bam complex proteins to the biogenesis of autotransporters have shown that BamA and BamD are required for secretion of classical autotransporters such as AIDA-1 from *E. coli*. Similar functions of the Bam complex have been described in the secretion of *S.* Typhimurium MisL and ShdA proteins, which are determined to be members of AIDA-1 (Kingsley et al. 2002, Kingsley et al. 2004). Although some studies have found evidence that BamB, BamC, and BamE proteins are not essential for the secretion of classical autotransporter proteins, evidence that they contribute to the functionality of the Bam complex has been identified as a result of functional analyzes of mutants for these genes (Zhao et al. 2009, Pokharel et al. 2020).). Detection that the translocation assembly machine (Tam) complex is an essential secretory element in addition to the Bam complex in the Type Va secretion system has provided the explanation of the basic mechanism of this secretion system at the molecular level. Its complete complex consists of TamA, an outer membrane protein, and TamB, an inner membrane protein. TamB and BamA contain a high level of structural similarity. Further studies, Full complex; demonstrated that it facilitated the efficient assembly of both Type I fimbriae and Type 5e autotransporters. However, the precise contribution of the Tam complex to the biogenesis of all types of the Type V secretory system has not yet been elucidated (Zhao et al. 2009, Wouter et al. 2010, Pokharel et al. 2020).

3.2.Type Vb Secretion System (Two Partner Secretion System)

The typical characteristic of two partner autotransporter proteins is that they consist of two polypeptides, one with a passenger function and the other with a translocation function. Filamentous hemagglutinin (FHA) produced from *Bordetella* is the best described member of this subclass (Jacob-Dubuisson et al. 2013) Of the two polypeptide chains defined in FHA, it was determined that TspA was responsible for the passenger function and TspB2 was responsible for the translocator function. It has been determined that these two protein genes are located in the same operon. As a result of detailed molecular analysis, it was determined that TspB is a β-barrel protein in the outer membrane and contains two POTRA (polypeptide transport-associated domain). Because these autotransporters are composed of two polypeptides, they do not require a proteolytic cleavage reaction during the secretion of the passenger domain into the external environment. After the transport, the passenger domain can go through different processes. Some TpsB proteins remain non-covalently attached to the outer membrane, as exemplified in HMW1 and HMW2, the autotransporter proteins of *Haemophilus influenza*e, while others, such as *the Serratia marcescens* protein ShlA, can be secreted outside the cell (St. Geme 1994, Jacob-Dubuisson et al. 2013, Braud et al. 2014, Casasanta et al., 2017).

3.3.Type Vc Secretion System (Trimeric Autotransporter Adhesins (TAA's)

Trimeric autotransporters generally have adhesin functions and with these properties, they play a critical role in the pathogenicity of Gram-negative bacteria. These characteristics clearly distinguish them from TipVa autotransporters. Since these proteins do not contain enzymatic properties, they are not secreted from the cell surface by an autoproteolytic process. These autotransporter proteins, which are characterized by highly nested trimeric structures, are called TAAs for short. YadA (*Yersinia* adhesin) is a typical example of this autotransporter class. These proteins are composed of three identical polypeptide chains and, in their final folded form, contain a C-terminal β-barrel structure (the β-barrel structure has 12 sheets, with 4 β-strands per monomer) (El Tahir and Skurnik 2001).

Type 5c secretion system and proteins belonging to the family of trimeric autotransporter adhesins (TAA's) secreted by this secretory system have important differences when compared to Type Va secretion system members. TAAs contain a short C-terminal translocation domain of 70-100 amino acids, and therefore, the protein must form a homotrimer to form a complete β-barrel (Ishikawa et al. 2012). Ultimately, unlike the passenger domains of the Type V5 secretion system, which is usually defined by a monomeric β-helix structure, TAA passenger domains form a trimeric structure composed of oligomeric helix-coil regions interspersed with distinct head and neck motifs. Considering this situation, those with the simplest secretion system among the subclasses accepted in the Type 5 secretion system; It can be said that there are Type Va, Type Vb (two-element system) and Type 5c (AT-2 system) mechanisms (Olvera et al. 2011).

3.4.Type Vd Secretion System (Hybrid Secretion System)

It is possible to define the Type Vd secretion system as a mixture of Type Va and Type Vb secretion systems. Type members of the type Vd secretion system include PlpD (palatin-like protein) isolated from *Pseudomonas aeruginosa*, FplA isolated from *Fusobacterium nucleatum* and PlpD homologs from *Aeromonas hydrophila* (a fish pathogen), *Burkholderia pseudomallei* (a human pathogen) and *Ralstonia solanacearum* (a plant pathogen). The passenger domain of Type 5d autotransporter proteins has lipase activity and when secreted by the Type Vd system, this region is released as a result of autocatalytic activity. This autocatalytic activity of the N passenger domain (α-domain) of Type Vd autotransporters is the main difference that distinguishes them from Type Va autotransporters. The β-barrel domain located in the C-terminal domain of Type Vd autotransporter proteins is highly similar to the β-barrel domain of Type Vb autotransporters. However, while Type Vb autotransporter proteins require two PORTRA domains for their functionality, a single PORTRA domain is sufficient for the functionality of Type Vb proteins. In type Vd proteins, the PORTRA is an intrinsic domain (Da Mata Madeira et al., 2016; Casasanta et al., 2017; Leo and Linkle 2018). The inclusion of these proteins, which have characteristic lipase and esterase activities in their passenger domains, in a different subsecretory class is still controversial, due to their high similarity to the Va and Vb-type autotransporters, apart from the minor differences mentioned above (Pokharel, et al. 2020).

3.5Type Ve (Reverse Autotransporters)

The most typical member of this class of autotransporter proteins is intimin, which was isolated from *Escherichia coli*. In addition, *Yersinia* adhesins (for example YadA isolated from *Yersinia enterocolitica*) are the most extensively studied members of this group. YadA is known to contain an immunoglenic (Ig) domain associated with the bacterial outer membrane. On the other hand, intimin from *Escherichia coli* plays critical roles in the attachment of this bacterium to host cells and in the surface colonization. As a result of molecular studies on the structural features of the Type Ve autotransporters, it was determined that they contain an N-terminal domain in the periplasm and a transmembrane-terminal on the outer membrane of the cell. Unlike other autotransporters, the order of the passenger and translocator domains of Type Ve autotransporter proteins is reversed. Because of this inverse relationship, the extracellular domain is C-terminal in these proteins *(*Leo and Linke 2018) . This has led to these proteins being termed "reverse autotransporters". The passenger domain of reverse autotransporters typically contains domains with Ig- or lectin-like folds. In some samples, long and repetitive Ig-like domains covered with a lectin-like domain were detected. Some other types and autotransporter proteins have been found to contain an additional periplasmic domain not found in all members. This periplasmic domain aids in dimerization as well as interactions with peptidoglycan. This function; probably by contributing to the stabilization of the peptidoglycan layer and assisting the receptor interactions needed at the stage of host invasion to the pathogen (Yeo et al. 2004, Meuskens et al. 2015, Leo and Linke 2018).

4. Conclusion

Determining that autotransporter proteins, which are defined only in bacteria in the living world, are important pathogen-associated molecular elements (PAMP) that govern the pathogenicity and virulence of Gram-negative bacteria, accelerated the studies on the molecular nature of these proteins. The identification of new autotransporters diversifies studies on the genetic and biochemical basis of these proteins and details information on their evolutionary origins. The unexpectedly high diversity of these proteins, which are genetically and biochemically conserved, indicates that this is due to the diversity in their processing, folding and secretion systems. For this reason, research on autotransporter proteins has been mostly shifted to this field. In summary, although the genetic structure and regulation properties of the genes encoding autotransporter proteins identified from different bacteria and the structural and functional domains of the peptides encoded by these genes have been well defined, the information about the changes they undergo during their transport through the membranes is not sufficient.

The highly diverse functions of autotransporters associated with pathogenicity in Gram-negative bacteria indicate that they can be used as new molecular targets in the fight against pathogens. Therefore, the information detailed on these proteins has a high potential to be the harbinger of new vaccines and drug agents.

References

Abreu, A. G., Abe, C. M., Nunes, K. O., Moraes, C. T. P., Chavez-Dueñas, L., Navarro-Garcia, F. (2016). The serine protease Pic as a virulence factor of atypical enteropathogenic Escherichia coli. Gut Microbes 7, 115–125. doi: 10.1080/19490976.2015.1136775.

Baud, C., Guérin, J., Petit, E., Lesne, E., Dupré, E., Locht, C. (2014). Translocation path of a substrate protein through its Omp85 transporter. Nat. Commun. 5:5271-5275. doi: 10.1038/ncomms6271.

Benanti, E. L., Nguyen, C. M., and Welch, M. D. (2015). Virulent burkholderiaspecies mimic host actin polymerases to drive actin-based motility. Cell 161, 348–360. doi: 10.1016/j.cell.2015.02.044.

Berne, C., Ducret, A., Hardy, G. G., and Brun, Y. V. (2015). Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. Microbiol. Spectr. 3,1–45. doi: 10.1128/microbiolspec.MB-0018-2015.

Bhullar, K.; Zarepour, M.; Yu, H.; Yang, H.; Croxen, M.; Stahl, M.; Finlay, B.B.; Turvey, S.E.; Vallance, B.A. (2015). The Serine Protease Autotransporter Pic Modulates Citrobacter Rodentium Pathogenesis and Its Innate Recognition by the Host. Infect. Immun. 83, 2636–2650.

Brockmeyer, J.; Spelten, S.; Kuczius, T.; Bielaszewska, M.; Karch, H. (2009). Structure and Function Relationship of the Autotransport and Proteolytic Activity of Espp from Shiga Toxin-Producing *Escherichia coli*. PLoS ONE, 4, e6100.

Casasanta, M. A., Yoo, C. C., Smith, H. B., Duncan, A. J., Cochrane, K., Varano, A. C. (2017). A chemical and biological toolbox for Type Vd secretion: characterization of the phospholipase A1 autotransporter FplA from *Fusobacterium nucleatum*. J. Biol. Chem. 292, 20240–20254. doi: 10.1074/jbc. M117.819144.

Chauhan, N., Wrobel, A., Skurnik, M., and Leo, J. C. (2016). Yersinia adhesins: an arsenal for infection. Proteomics Clin. Appl. 10, 949–963. doi: 10.1002/prca. 201600012.

Chauhan, N., Hatlem, D., Orwick-Rydmark, M., Schneider, K., Floetenmeyer, M., van Rossum, B. (2019). Insights into the autotransport process of a trimeric autotransporter, Yersinia Adhesin A (YadA). Mol. Microbiol. 111,844–862. doi: 10.1111/mmi.14195.

Coppens, F., Castaldo, G., Debraekeleer, A., Subedi, S., Moonens, K., Lo, A. (2018). Hop-family Helicobacter outer membrane adhesins form a novel class of Type 5-like secretion proteins with an interrupted b-barrel domain. Mol. Microbiol. 110, 33–46. doi: 10.1111/mmi.14075.

Da Mata Madeira, P. V., Zouhir, S., Basso, P., Neves, D., Laubier, A., Salacha, R. (2016). Structural basis of lipid targeting and destruction by the type v secretion system of Pseudomonas aeruginosa. J. Mol. Biol. 428, 1790–1803. doi: 10.1016/j.jmb.2016.03.012.

Deibel, C., Krämer, S., Chakraborty, T., Ebel, F. (1998). EspE, a novel secreted protein of attaching and effacing bacteria, is directly translocated into infected host cells, where it appears as a tyrosine-phosphorylated 90 kDa protein. Mol. Microbiol. 28, 463–474. doi: 10.1046/j.1365-2958.1998.00 798.

Desvaux, M., Khan, A., Beatson, S. A., Scott-Tucker, A., and Henderson, I. R. (2005). Protein secretion systems in Fusobacterium nucleatum: Genomic identification of Type 4 piliation and complete Type V pathways brings new insight into mechanisms of pathogenesis. Biochim. Biophys. Acta 1713, 92–112. doi: 10.1016/j.bbamem.2005.05.002.

El Tahir, Y., Skurnik, M. (2001). YadA, the multifaceted Yersinia adhesin. Int. J. Med. Microbiol. 291, 209–218. doi: 10.1078/1438-4221- 00119.

Fulcher, R. A., Cole, L. E., Janowicz, D. M., Toffer, K. L., Fortney, K. R., Katz, B. P. (2006). Expression of Haemophilus ducreyi collagen binding outer membrane protein NcaA is required for virulence in swine and human challenge models of chancroid. Infect. Immun. 74, 2651–2658. doi: 10.1128/iai. 74.5.2651-2658.2006.

Guyer, D.M.; Radulovic, S.; Jones, F.-E.; Mobley, H.L.T. (2002). Sat, the Secreted Autotransporter Toxin of Uropathogenic Escherichia coli, Is a Vacuolating Cytotoxin for Bladder and Kidney Epithelial Cells. Infect. Immun. 70, 4539–4546.

Habouria, H., Pokharel, P., Maris, S., Garénaux, A., Bessaiah, H., Houle, S., Veyrier, F.J., Guyomard-Rabenirina, S., Talarmin, A., Dozois, C.M. (2019). Three New Serine-Protease Autotransporters of Enterobacteriaceae (SPATEs) from Extra-Intestinal Pathogenic Escherichia coli and Combined Role of Spates for Cytotoxicity and Colonization of the Mouse Kidney. Virulence. 10, 568–587.

Heimer, S.R., Rasko, D.A., Lockatell, C.V., Johnson, D.E., Mobley, H.L.T. (2004). Autotransporter Genes Pic and Tsh Are Associated with Escherichia coli Strains That Cause Acute Pyelonephritis and Are Expressed During Urinary Tract Infection. Infect. Immun. 72, 593–597.

Hendrixson, D.R.; St Geme, J.W. (1998). The Haemophilus Influenzae Hap Serine Protease Promotes Adherence and Microcolony Formation, Potentiated by a Soluble Host Protein. Mol. Cell. 2, 841–850.

Ishikawa, M., Nakatani, H., Hori, K., Hori, K., Matsumoto, S., Soto, G. (2012). AtaA, a new member of the Trimeric Autotransporter Adhesins from Acinetobacter sp. Tol 5 mediating high adhesiveness to various abiotic surfaces.PLoS One 7:e48830. doi: 10.1371/journal.pone.0048830.

Jacob-Dubuisson, F., Guérin, J., Baelen, S., Clantin, B. (2013). Two-partnerv secretion: As simple as it sounds? Res. Microbiol. 164, 583–595. doi: 10.1016/j.resmic.2013.03.009.

Jain, S., Goldberg, M. B. (2007). Requirement for YaeT in the outer membrane assembly of autotransporter proteins. J. Bacteriol. 189, 5393–5398. doi: 10.1128/ jb.00228-07.

Junker, M., Schuster, C. C., McDonnell, A. V., Sorg, K. A., Finn, M. C., Berger, B. (2006). Pertactin beta-helix folding mechanism suggests common themes for the secretion and folding of autotransporter proteins.

Junker, M., Besingi, R. N., Clark, P. L. (2009). Vectorial transport and folding of an autotransporter virulence protein during outer membrane secretion. Mol.Microbiol. 71, 1323–1332. doi: 10.1111/j.1365-2958.2009.06607.

Kingsley, R. A., Santos, R. L., Keestra, A. M., Adams, L. G., and Bäumler, A. J. (2002). Salmonella enterica serotype Typhimurium ShdA is an outer membrane fibronectin-binding protein that is expressed in the intestine. Mol.Microbiol. 43, 895–905. doi: 10.1046/j.1365-2958.2002.02805.

Kingsley, R. A., Ghanem, D. A., Puebla-Osorio, N., Keestra, A. M., Berghman, L., Bäumler, A. J. (2004). Fibronectin binding to the Salmonella enterica serotype typhimurium ShdA autotransporter protein is inhibited by amonoclonal antibody recognizing the A3 repeat. J. Bacteriol. 186, 4931–4939. doi: 10.1128/jb.186.15.4931-4939.2004.

Leo, C. J., Linke, D. (2018). A unified model for BAM function that takes into account type Vc secretion and species differences in BAM composition. AIMS Microbiol. 4, 455–468. doi: 10.3934/microbiol.2018.3.455.

Maroncle, N.M., Sivick, K.E., Brady, R., Stokes, F.-E., Mobley, H.L.T. (2006). Protease Activity, Secretion, Cell Entry, Cytotoxicity, and Cellular Targets of SecretedAutotransporter Toxin ofUropathogenic *Escherichia coli*. Infect. Immun. 74, 6124–6134.

Meuskens, I., Saragliadis, A., Leo, J.C., Linke, D. (2015). Type V Secretion Systems: An Overview of Passenger Domain Functions. Frontiers in Microbiology, doi: 10.3389/fmicb.2019.01163.

Nava-Acosta, R., Navarro-Garcia, F. (2013). Cytokeratin 8 Is an Epithelial Cell Receptor for Pet, a Cytotoxic Serine Protease Autotransporter of Enterobacteriaceae. MBio. 4, e00838-13.

Navarro-Garcia, F., Serapio-Palacios, A., Vidal, J.E., Salazar, M.I., Tapia-Pastrana, G. (2014). Espc Promotes Epithelial Cell Detachment by Enteropathogenic *Escherichia coli* Via Sequential Cleavages of a Cytoskeletal Protein and Then Focal Adhesion Proteins. Infect. Immun. 2014, 82, 2255–2265.

Olvera, A., Pina, S., Pérez-Simó, M., Aragón, V., Segalés, J., Bensaid, A. (2011). Immunogenicity and protection against *Haemophilus parasuis* infection after vaccination with recombinant virulence associated trimeric autotransporters (VtaA). Vaccine 29, 2797–2802. doi: 10.1016/j.vaccine.2011.01.105.

Otto, B. R., Sijbrandi, R., Luirink, J., Oudega, B., Heddle, J. G., Mizutani, K. (2005). Crystal structure of hemoglobin protease, a heme binding autotransporter protein from pathogenic Escherichia coli. J. Biol. Chem. 280, do17339–17345. doi: 10.1074/jbc.m412885200.

Pokharel, P., Habouria, H., Bessaiah, H., Dozois, C.M. (2019). Serine Protease Autotransporters of the Enterobacteriaceae (SPATEs): Out and About and Chopping It Up. Microorganisms. 7, 594-600.

Pokharel, P., Díaz, J.M,, Bessaiah, H., Houle, S., Guerrero-Barrera, A., Dozois., C.M. (2020). The Serine Protease Autotransporters TagB, TagC, and Sha from Extraintestinal Pathogenic Escherichia coliAre Internalized by Human Bladder Epithelial Cells and Cause Actin Cytoskeletal Disruption Int. J. Mol. Sci. 2020, 21, 3047-3052.

Ruiz-Perez, F., Henderson, I.R., Leyton, D.L., Rossiter, A.E., Zhang, Y., Nataro, J.P. (2009). Roles of periplasmic chaperone proteins in the biogenesis of serine protease autotransporters of Enterobacteriaceae. J. Bacteriol. 191, 6571–6583.

Rutherford, N., Mourez, M. (2006). Surface display of proteins by Gram-negative bacterial autotransporters. Microb. Cell Factories 5, 22.

Schmidt, M.A., Riley, L.W., Benz, I. (2003). Sweet new world: glycoproteins in bacterial pathogens. Trends Microbiol. 11, 554–561.

Serruto, D., Spadafina, T., Scarselli, M., Bambini, S., Comanducci, M., Höhle, S., Kilian, Soprova, Z., Sauri, A., van Ulsen, P., Tame, J.R., den Blaauwen, T., Jong, W.S., Luirink, J. (2010). A conserved aromatic residue in the autochaperone domain of the autotransporter Hbp is critical for initiation of outer membrane translocation. J. Biol. Chem. 285, 38224–38233.

St Geme III, J.W., Yeo, H.J. (2009). A prototype two-partner secretion pathway: the Haemophilus influenza HMW1 and HMW2 adhesin systems. Trends Microbiol.17, 355–360.

Suhr, M., Benz, I., Schmidt, M.A. (1996). Processing of the AIDA-I precursor: removal of AIDAc and evidence for the outer membrane anchoring as a -barrel structure. Mol. Microbiol. 22, 31–42.

Tajima, N., Kawai, F., Park, S.Y., Tame, J.R.H. (2010). A novel intein-like autoproteolytic mechanism in autotransporter proteins. J. Mol. Biol. 402, 645–656.

Thanassi, D.G., Stathopoulos, C., Karkal, A., Li, H. (2005). Protein secretion in the absence of ATP: the autotransporter, two-partner secretion and chaperon/ usher pathways of Gram-negative bacteria. Mol. Membr. Biol. 22,63–72.

Tian, P., Bernstein, H.D. (2010). Molecular basis for the structural stability on an enclosed-barrel loop. J. Mol. Biol. 402, 475–489.

Van Ulsen, P., van Alphen, L., ten Hove, J., Fransen, F., van der Ley, P., Tommassen, J. (2003). A neisserial autotransporter NalP modulating the processing of other autotransporters. Mol. Microbiol. 50, 1017–1030.

Veiga, E., de Lorenzo, V., Fernández, L.A. (2004). Structural tolerance of bacterial autotransporters for folded passenger protein domains. Mol. Microbiol. 52,1069–1080.

Wells, T.J., Tree, J.J., Ulett, G.C., Schembri, M.A. (2007). Autotransporter proteins: novel targets at the bacterial cell surface. FEMS Microbiol. Lett. 274, 163–172.

Westendorf, A.M., Gunzer, F., Deppenmeier, S., Tapadar, D., Hunger, J.K., Schmidt, M.A., Buer, J., Bruder, D. (2005). Intestinal immunity of *Escherichia coli* NISSLE 1917: a safe carrier for therapeutic molecules. FEMS Immunol. Med. Microbiol. 43, 373–384.

Wouter, S.P.J., Sauri, A., Luirink, J. (2010). Extracellular production of recombinant proteins using bacterial autotransporters. Curr. Opin. Biotechnol. 21, 646–652.

Yeo, H.J., Cotter, S.E., Laarmann, S., Juehne, T., St Geme III, J.W., Waksman, G. (2004). Structural basis for host recognition by the *Haemophilus influenzae* Hia autotransporter. EMBO J. 23, 1245–1256.

Zhao, L., Nguyen, N.T., Fernandez, R.C., Murphy, M.E. (2009). Crystallographic characterization of the passenger domain of the *Bordetella* autotransporter BrkA. ActaCrystallogr. Sect. F Struct. Biol. Cryst. Comm. 65, 608–611.