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Mini Review

Structures and functions of autotransporter proteins in microbial pathogens

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ABSTRACT

Since their discovery more than 20 years ago the autotransporter protein superfamily has been growing continuously and currently represents the largest protein family in (pathogenic) Gram-negative bacteria. Autotransporter proteins (AT) adhere to a common structural principle and are composed of a C-terminal β-barrel-shaped 'translocator' domain and an N-terminal 'passenger' domain. The translocator is anchored in the outer membrane and is indispensable for the N-terminal passenger part to traverse the outer membrane. Most if not all AT harbor a chaperone segment that increases protein stability and may be located in the passenger or translocator domain. The passenger mediates the specific virulence function(s) of the particular AT. Accordingly, passenger domains of AT can be quite variable. Interestingly, AT have been identified as the first glycosylated proteins in Gram-negative bacteria. Despite the considerable efforts invested in the characterization of autotransporter biogenesis, various aspects such as the participation of accessory proteins, the fate of the translocator, or the translocation of glycosylated proteins still remain only poorly understood. In addition, recent evidence indicates that the prefix 'auto' might be slightly exaggerated. Here, we will selectively discuss novel insights at various stages of AT biogenesis.

Autotransporter proteins

Secretion systems in Gram-negative bacteria are numbered for convenience or lack of more creative terms type I–VII (Salmond and Reeves, 1993; Henderson et al., 2000, 2004; Economou et al., 2006; Van Ulsen and Tommassen, 2006). However, even the – at first glance – simplest protein secretion system V consists of several subtypes such as the classic autotransporter system (type Va or AT-1), the two-partner secretion system (Vb), and the Vc system (AT-2). At present, autotransporter proteins (AT) represent the largest protein family in pathogenic Gram-negative bacteria with more than 1000 identified family members (Kajava and Steven, 2006) and have been identified by sequence comparison in virtually all pathogenic Gram-negative bacteria. Although proteins secreted via the autotransporter pathways are similar in their overall organization and primary structure (Fig. 1) (Henderson et al., 2004), they are very heterogeneous concerning their specific functions. All classical AT share a common sequence organization: an often extended signal peptide followed by an N-terminal passenger domain (α-domain) ranging in size from less than 20 to more than 400 kDa and a C-terminal translocator domain (β-domain) of approximately 30 kDa. The functions of the passenger domains can be quite diverse and have frequently been associated with the pathogenesis of the specific bacterium. The C-terminal domain is essential for the translocation of the passenger domain to the bacterial surface and, therefore, translocator domains of different autotransporters share common features. During the process of translocation the passenger protein might be (autocatalytically) cleaved and secreted or might remain covalently bound to the translocator. In some cases, such as the 'adhesin-involved-in-diffuse-adherence (AIDA)' AT, the passenger is cleaved but remains non-covalently associated with the bacterial surface (Suhr et al., 1996; Charbonneau et al., 2009).

The role model for AT secretion is the IgA protease of *Neisseria gonorrhoeae* (Koomey et al., 1982; Halter et al., 1984). Pohlner et al. (1987) proposed a very elegant model of outer membrane (OM) translocation without a requirement for energy coupling or accessory factors. Hence proteins following this pathway of secretion were denoted 'autotransporter'. Since the introduction of this model, literally hundreds of further examples have been reported that share overall similarities in structural organization and mode of translocation across the OM. For a more general description of AT, please refer to recent excellent reviews (Henderson et al., 2004; Desvaux et al., 2004; Wells et al., 2007, 2010; Dautin and Bernstein, 2007; Yen et al., 2008).

In addition to classic autotransporter systems following as single-chain proteins the IgA protease model (such as AIDA-I, TibA, Ag43, BrkA, IcsA, NalP, and the group of SPATEs; see Table 1), trimeric autotransporter adhesins (TAA, AT-2, Vb) and two-partner secretion systems (TPSS, Vc) have been included also in the auto-

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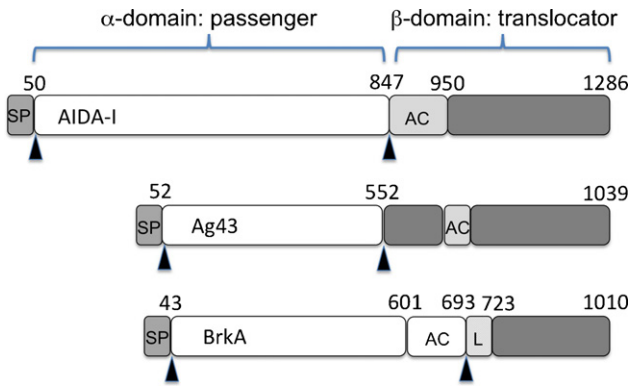

Fig. 1. Structural domains of processed classical autotransporter proteins of *E. coli* (AIDA-I, Ag43) and *B. pertussis* (BrkA). SP: signal peptide; AC: autochaperone domain essential for stability and folding; L: linker domain; arrowheads: processing sites.

transporter family. However, in this review TAA and TPSS will be touched only very briefly.

Trimeric autotransporters

Trimeric autotransporters (AT-2, Vb) are synthesized just like classic autotransporter molecules via a precursor molecule harboring domains representing passenger and translocator functions (for review see: Cotter et al., 2005; Linke et al., 2006). In AT-2 the domain resembling a translocator is rather short and consists of only 70–76 amino acids (Surana et al., 2004). As a consequence, it is not functional as a monomer. However, as a trimer where each AT pseudo-translocator domain donates four β-sheets, the minimum number of 12 β-sheets to constitute a β-barrel can be assembled for a functional translocator. Thus far the specific mechanism how three passengers might be translocated through a single 12 β-sheet barrel has remained elusive. Examples of trimeric autotransporters are YadA of *Yersinia* spp. (e.g. Heesemann et al., 2006; Skurnik, 2007), Hia (e.g. Meng et al., 2008) or HadA of *Haemophilus influenzae* (Serruto et al., 2009)

Two-partner secretion systems

In the two-partner secretion system (TPSS, Vc), the translocator and passenger functions are contributed by two separate proteins, which are encoded by structural genes frequently located in an operon (Mazar and Cotter, 2007). Fha (e.g. Delattre et al., 2010) and HMW1 (e.g. St Geme et al., 2009) belong to this autotransporter subgroup. Both proteins are transported through the inner membrane in a Sec-dependent way. The translocator consists of an N-terminal periplasmic region and a C-terminal β-barrel that is integrated in the OM (Clantin et al., 2007; Kim et al., 2007; Tommassen, 2007). The periplasmic part carries a polypeptide-transport-associated domain (POTRA), a sequence for interaction with passenger molecules. The passenger carries a corresponding TPSS domain for recognition of the translocator and for initiation of secretion. It appears that the TPSS might serve as a model for protein translocation across membranes giving insights into the function of distantly related eukaryotic proteins found in mitochondria and chloroplasts (Jacob-Dubuisson et al., 2009). Passengers transported via TPSS are as diverse as classical autotransporters. For more detailed information see Mazar and Cotter (2007) and St Geme et al. (2009).

Structural organization and biogenesis of classical autotransporter proteins

Signal peptide and transport through the inner membrane

The signal peptide of most AT consists of about ~20–30 residues to be cleaved by the signal peptidase subsequent to Sec-dependent translocation. Nevertheless, sequence homologies are not very high among AT signal peptides (Dautin and Bernstein, 2007). About 10% of the known AT show an unusual long signal peptide (e.g. SPATE, IcsA, Hbp, AIDA-I) of at least 40 residues that can be organized in five different domains. These domains are based on hydrophobic and charged residue distribution (N1, H1, N2, H2) and are followed by the C-(cleavage) region (Henderson et al., 2004). More recent studies propose a dual-domain organization (Hiss and Schneider, 2009). The C-terminal half of the signal peptide corresponds to a classic signal peptide and appears not to be particularly conserved while the N-terminal extension is conserved. Transport of the precursor proteins through the cytoplasmic membrane is Sec dependent for IcsA (Brandon et al., 2003), while for Hbp the signal-recognition particle (SRP) and SecB pathway is used (Sijbrandi et al., 2003). Recent evidence suggests that besides the requirements for a functional SRP pathway and the Sec translocon, at least for the AT examples EspC and Hbp, the signal peptide interacts with YidC early during biogenesis. YidC is in part associated with the SecYEG machinery acting downstream to facilitate lateral transfer (Jong

Table 1
Exemplary functions of autotransporter passenger domains in pathogenic bacteria.

Protein	Species	Function	Reference
Classical autotransporter proteins			
Ag43, antigen 43	*Escherichia coli*	Autoagglutinin, biofilm formation	Sherlock et al. (2006)
AIDA, adhesin involved in diffuse adherence	Enteropathogenic *E. coli*	Adhesin	Benz and Schmidt (1992)
BrkA	*Bordetella pertussis*	Serum resistance	Zhao et al. (2009)
Hbp, hemoglobin protease	Avian pathogenic *E. coli*	Heme binding protein	Otto et al. (2005)
IcsA	*Shigella flexneri*	Intercellular spread	Brandon and Goldberg (2001)
IgA protease	*Neisseria*	Immunoglobulin protease	Pohlner et al. (1987)
Pertactin	*B. pertussis*	Adhesin	Emsley et al. (1996)
SPATE, serine protease autotransporters of *Enterobacteriaceae*	*Enterobacteriaceae*	Protease	Yen et al. (2008)
SSP	*Serratia marcescens*	Protease	Shikata et al. (1993)
TibA	Enterotoxigenic *E. coli*	Adhesin/invasin	Lindenthal and Elsinghorst (1999)
VacA	*Helicobacter pylori*	Cytotoxin	Gangwer et al. (2007)
Trimeric autotransporter adhesins (TAA)			
Hia	*Haemophilus influenzae*	Adhesin	Yeo et al. (2004)
YadA	*Yersinia enterocolitica*	Serum resistance, adhesin	Nummelin et al. (2004)
Two-partner secretion systems (TPSS)			
Fha, filamentous hemagglutinin	*B. pertussis*	Hemagglutinin	Clantin et al. (2004)
HMW1, high molecular weight adhesin	*H. influenzae*	Adhesin	Grass et al. (2003)
ShlB	*S. marcescens*	Cytotoxin	Ondraczek et al. (1992)