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Biogenesis and function of the autotransporter adhesins YadA, intimin and invasin

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ABSTRACT

Bacteria often express numerous virulence factors. These virulence factors make them successful pathogens, by e.g. mediating attachment to host cells and thereby facilitating persistence or invasion, or by contributing to the evasion of the host immune system to allow proliferation and spread within the host and in the environment. The site of first contact of Gram negative bacteria with the host is the bacterial outer membrane (OM). Consisting of an asymmetrical lipid bilayer with phospholipids forming the inner, and lipopolysaccharides forming the outer leaflet, the OM harbors numerous integral membrane proteins that are almost exclusively β-barrel proteins. One distinct family of OM β-barrel proteins strongly linked to bacterial virulence are the autotransporter (AT) proteins. During the last years huge progress has been made to better understand the mechanisms underlying the insertion of AT proteins into the OM and also AT function for interaction with the host. This review shortly summarizes our current knowledge about outer membrane protein (OMP) and more specifically AT biogenesis and function. We focused on the AT proteins that we haved studied in most detail: i.e. the *Yersinia* adhesin A (YadA) and invasin of *Yersinia enterocolitica* (*Ye*) as well as its homolog intimin (Int) expressed by enteropathogenic *Escherichia coli.* In addition, this review provides a short outlook about how we could possibly use this knowledge to fight infection.

1. Introduction

The cell envelope of Gram negative bacteria is an excellent permeability barrier and as such one of the major obstacles for treatment of infections with Gram negative pathogens (Vaara, 1992; Nikaido, 2003; Delcour, 2009). OM permeability is mainly determined by the presence of lipopolysaccharide (LPS), and the abundance and repertoire of general and specific β-barrel porins allowing for active transport or diffusion of substrates (Delcour, 2003; Pages, James et al., 2008). Additionally, the OM harbors numerous OMPs that are tightly associated with virulence. Given the current limitations of antibiotic therapy of infections with Gram negative pathogens, and the emergence of more and more multidrug-resistant strains, it could thus be an attractive strategy to interfere with OMP biogenesis. By a targeted interference, Gram negative pathogens could possibly be rendered more accessible for antibiotic treatment and their virulence could be reduced at the same time. To be able to do so, however, it is inevitable to have a good understanding about the mechanisms of OMP biogenesis and also to precisely comprehend the role of dedicated virulence factors for the complex interplay with the host during infection. One family of OMPs are the AT proteins. They are fascinating because of their sophisticated biogenesis and their manifold contributions to virulence of Gram negative pathogens. Here, we will summarize our current knowledge about the virulence function and biogenesis of two representative AT proteins, i.e. YadA, intimin and its homolog invasin and how we could possibly exploit it to invent new means to fight infection.

2. Common themes and specific features of the AT adhesins YadA, Int and Inv

AT proteins are characterized by specific domains (Fig. 1A): (**I**) an N-terminal signal peptide (sp) facilitating the transport across the inner membrane via SecYEG, (**II**) a β-barrel domain that anchors the protein to the OM, and (**III**) an extracellular domain called passenger, that is

Abbreviations: AT, autotransporter; BAM, β-barrel assembly machinery; FH, factor H; IM, inner membrane; Inv, invasion; Int, intimin; OM, outer membrane; OMP, outer membrane protein; Skp, seventeen kilodalton protein; sp, signal peptide; SurA, survival factor A; T3SS, type three secretion system; TAA, trimeric autotransporter adhesion; TAM, translocation and assembly module; TCC, terminal complement complex; Vn, vitronectin; YadA, *Yersinia* adhesin A; *Ye*, *Yersinia enterocolitica*

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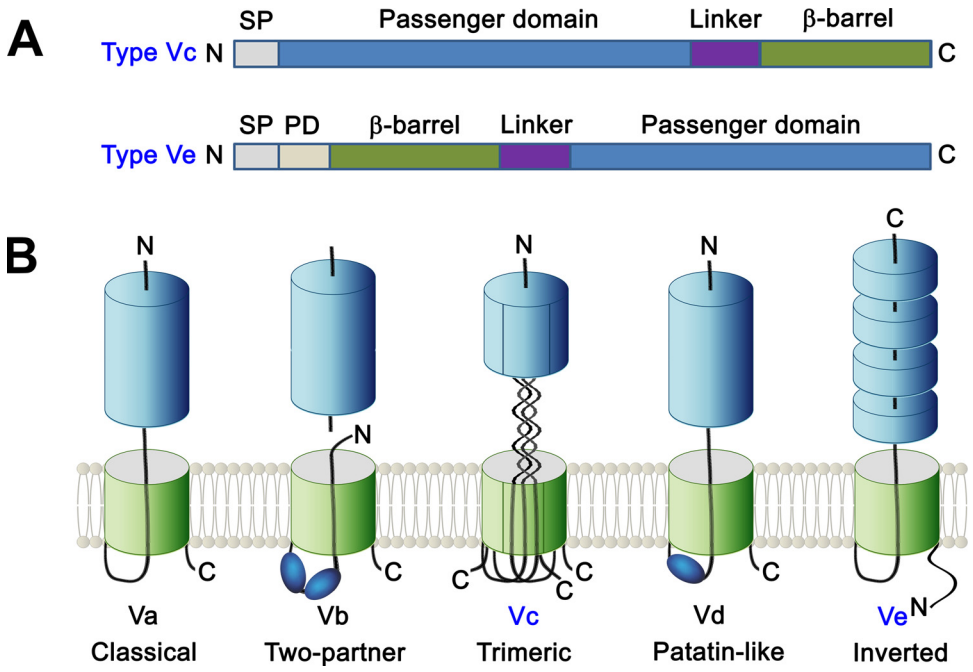

Fig. 1. Domain organization and topology models of type V secretion systems (A) Domain organization of a type Vc (e.g. YadA) and a type Ve (e.g. Int, Inv) AT. Both contain an N-terminal signal peptide (SP) that facilitates Sec-dependent transport across the inner membrane. In type Vc AT proteins, the SP is linked to a long passenger domain that is connected to the C-terminal β-barrel domain via a short linker. In type Ve AT proteins the domain order differs: the SP is connected to a periplasmic domain, followed by the β-barrel domain that is connected to the C-terminal passenger domain via a linker. **(B)** Classical, type Va AT proteins are monomeric. The C-terminal β-barrel domain (green) is embedded into the outer membrane. The N-terminal passenger (blue) is transported onto the bacterial surface and stays attached to the β-barrel. Type Vb secretion is also called two-partner secretion. Two separate polypeptide chains encode one translocated passenger protein (TpsA) and one membrane embedded β-barrel translocator domain (TpsB). TpsB proteins share homology to BamA, but contain only two POTRA domains instead of five. Type Vc secretion systems form homotrimers, where every monomer contributes 4 β-strands to form one trimeric 12-stranded β-barrel. Type Vd secretion systems comprise a patatin-like passenger domain which is fused to a TpsB-like C-terminal β-barrel domain. The type Ve secretion system includes inverted type AT proteins, such as Int and Inv. Inverted type Ve ATs carry their β-barrel domain at the N-terminus and are fused to the passenger at the C-terminus. Alternatively, the C-terminus carries a so-called plug domain thought to regulate the transport of a co-expressed passenger protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

transported to the surface and may mediate adhesion (as in the case of YadA, Inv and Int) or may be cleaved off and act e.g. as a protease (Henderson and Nataro, 2001; Linke et al., 2006; Leo et al., 2012). In addition, some AT proteins possess a short periplasmic domain (PD) that in the case of Int mediates dimerization and peptidoglycan binding (Leo et al., 2015a,2015b). All AT proteins belong to the type V secretion system, and harbor the domains mentioned above, however, the domain order may vary, and the type Vc AT proteins form trimers. YadA is the prototype of type Vc AT proteins, also called trimeric autotransporter adhesins (TAA), whereas Int and Inv belong to the family of inverse AT proteins, also called type Ve AT (Cotter et al., 2005; Linke et al., 2006; Leo et al., 2015a,2015b). Hallmark of type Ve AT proteins is a domain organization differing from all other type V systems (Fig. 1**A** and **B**): the β-barrel domain follows the N-terminal signal sequence and short periplasmic domain, whereas in all other type V secretion families (Va, b, c and d) the β-barrel is encoded by the C-terminus (Leo et al., 2015a,2015b, Albenne and Ieva, 2017).

3. Biogenesis of AT proteins

3.1. First step: translocation across the IM

All AT proteins and therefore also YadA, Inv and Int are translated in the cytoplasm with a cleavable N-terminal signal peptide (sp). In contrast to other OMPs, this sp seems to be extended at the N-terminus in many AT proteins. One proposed function of the extended sp is slowing down its cleavage. This prevents misfolding of the passenger by attaching it temporarily to the IM, before being released into the periplasm (Chevalier et al., 2004; Szabady et al., 2005; Peterson et al., 2006). The sp facilitates the transition of AT proteins across the IM via the general secretion pathway (Sec) (Tsirigotaki et al., 2017). Concomitantly with the transport through SecYEG, the N-terminal signal sequence is cleaved and the AT substrates reach the periplasm (van Ulsen et al., 2014; Tsirigotaki et al., 2017).

3.2. Within the periplasm: distinct roles of SurA & Skp for AT biogenesis?

One striking feature of AT proteins is that they contain a variable number of β-strands to build up the membrane integral β-barrel, thus they are very hydrophobic (Tamm et al., 2004). Moreover, the folding of the passenger is assumed to be rather slow (Junker et al., 2006). Both properties imply a high propensity to form aggregates within the aqueous periplasm. Therefore, once AT proteins have reached the periplasmic space, they interact with chaperones such as SurA, Skp and DegP (Strauch et al., 1989; Tormo et al., 1990; Chen and Henning, 1996; Lazar and Kolter, 1996; Sklar et al., 2007a,2007b). These chaperones secure that ATs are kept in an unfolded but folding-competent state while traveling the periplasm and thereby prevent their aggregation (Thoma et al., 2015). It has been shown that especially SurA plays a crucial role for the biogenesis of many type Va, as well as of type Ve AT proteins (Purdy et al., 2007; Sklar et al., 2007a,2007b, Bodelon et al., 2009; Ieva and Bernstein, 2009; Ruiz-Perez et al., 2009; Oberhettinger et al., 2015; Albenne and Ieva, 2017; Weirich et al., 2017; Klein et al., 2019). However, the importance of SurA for efficient OMP insertion may vary significantly also among representatives of the AT protein family. In one of our studies we found that whereas the OM abundance of Inv, comprising a monomeric 12-stranded β-barrel, was significantly reduced in a Δ*surA Ye* strain, the OM protein levels of YadA, being a trimeric 12-stranded β-barrel, were almost unaffected (Weirich et al., 2017). In the case of Inv, Skp was obviously not able to take over the role of SurA, and a stalled version of Int was associated with higher amounts of SurA compared to Skp, possibly indicating a more important role of SurA for Int biogenesis (Oberhettinger et al., 2015). Other studies found Skp to play a crucial role for biogenesis of some ATs (Wagner et al., 2009; Ulrich et al., 2014), and Skp seemed to be more important than SurA for biogenesis of PorA and PorB of *N. meningitidis* (Volokhina et al., 2011). Moreover, only Skp was able to promote insertion of YadA into mitochondrial membranes (Ulrich et al., 2014). However, the molecular basis for a specific preference of substrates for one or the other chaperone actually is not well understood. The most prevailing view is that SurA is the main periplasmic

chaperone and handles the majority of substrates, whereas Skp and the periplasmic chaperone-protease DegP operate an alternative pathway. Once substrates fall off the SurA pathway, e.g. because SurA is lacking, or under conditions of periplasmic stress, Skp and DegP become much more important. This is in good agreement with observations that a lack of SurA results in a profound rearrangements of the OM protein composition, whereas lack of Skp or DegP results in more subtle effects, at least in *E. coli*, Ye and *P. aeruginosa* (Sklar et al., 2007a,2007b, Vertommen et al., 2009; Weirich et al., 2017; Klein et al., 2019). Even so, more recent work suggested that SurA and Skp actually have rather distinct functional characteristics. In vitro analyses revealed that Skp was able to bind and resolve OmpC aggregates, whereas SurA could not. Moreover, SurA and Skp recognized different conformations of OmpC, and with a different stoichiometry. Therefore, the authors suggested the role of Skp not being redundant to that of SurA, but being more important under distinct conditions such as cellular stress (Li et al., 2018) which is in line with earlier studies (Sklar et al., 2007a,2007b; Denoncin et al., 2012). Still this does explain neither the molecular basis of substrate specificities, nor the regulation thereof. The presumed function of SurA is linked to its direct interaction with BamA via its POTRA1 domain (Bennion et al., 2010). This interaction presumably enables the hand-over of substrates from SurA to the BAM complex for the final assembly and insertion steps.

3.3. Almost done: passenger translocation by hairpin formation

After passage of the periplasm, the next steps in AT biogenesis are the folding and insertion of the AT β-barrel domain into the OM, and passenger translocation. Passenger translocation has been shown to take place by the formation of a hairpin, in classical, inverted and trimeric ATs (Junker et al., 2006; Ieva and Bernstein, 2009; Sauri et al., 2009; Peterson et al., 2010; Oberhettinger et al., 2015; Chauhan et al., 2019). As the periplasm is lacking ATP, the only source energizing the passenger translocation presumably is the free energy of protein folding. This has been shown by several studies demonstrating that it is the sequential folding of the passenger that drives the translocation (Junker et al., 2006; Peterson et al., 2010; Leo et al., 2016). Work from the Bernstein lab, however, showed that the formation of a stably folded, native structure actually is not essential for translocation of the passenger (Kang'ethe and Bernstein, 2013), but it is yet unclear how representative these findings are. In one of our studies we had shown that both Int and Inv export their C-terminus to the cell surface by an inverse mechanism compared to classical type Va AT proteins (Oberhettinger et al., 2012). At the time we initiated a follow-up to this study, the exact mechanism underlying the translocation of the extracellular passenger and experimental evidence for hairpin formation still was lacking. We generated a stalled intermediate of Int by the insertion of an affinity tag into the N-terminus of its passenger and could experimentally verify the proposed topology. Its β-barrel was folded and stably inserted into the OM, and we could clearly demonstrate a periplasmic localization of the passenger (Oberhettinger et al., 2015). Both findings supported the hypothesis of hairpin formation during transport of the passenger. Interestingly, we found that although the β-barrel of the stalled Int variant was already folded and stably inserted into the OM, it was still associated with huge amounts of BamA and SurA. This implicated a role of BamA not only for β-barrel formation, but also for the passenger transport, a model which is now widely accepted (Albenne and Ieva, 2017). Hairpin formation was proposed also for YadA (Shahid 2012) and actually proven recently (Chauhan 2019). Furthermore, a recent study has suggested that also the interaction with membrane lipids promotes passenger translocation (Peterson et al., 2018).

3.4. Finally: interactions with the BAM complex

The final step of AT biogenesis, i.e. the β-barrel insertion/folding, presumably takes place in concert with passenger translocation and is facilitated by an interaction with the BAM complex (Ieva and Bernstein, 2009; Sauri et al., 2009; Peterson et al., 2010; Pavlova et al., 2013; Peterson et al., 2018). The BAM is a multiprotein complex, composed of BamA, which is a β-barrel protein itself, and the four lipoproteins BamBCDE (Wu et al., 2005; Sklar et al., 2007a,2007b). The 16-stranded β-barrel domain of BamA is attached to five periplasmic polypeptide transport-associated (POTRA) repeats. They mediate the interaction of BamA with the other components of the BAM complex, as well as with nascent β-barrel OMPs and SurA (Kim et al., 2007; Gatzeva-Topalova et al., 2008; Bennion et al., 2010; Gatzeva-Topalova et al., 2010; Zhang et al., 2011; Albrecht et al., 2014).

Both BamA and BamD are essential for the viability of *E. coli* (Wu et al., 2005; Malinverni et al., 2006; Sklar et al., 2007a,2007b), *N. gonorrhoeae* (Sikora et al., 2018) and *N. meningitidis* (Volokhina et al., 2009), whereas BamB, C and E are dispensable, but necessary for full efficiency of BAM-mediated integration of β-barrel proteins into the OM. Deletion of BamB results in substantial membrane defects and increased susceptibility to antibiotics and bile salts in *E. coli* (Onufryk et al., 2005; Wu et al., 2005; Malinverni et al., 2006; Sklar et al., 2007a,2007b) and *Ye* (Weirich et al., 2017), whereas only minor effects were observed in cells lacking BamC or BamE. The function of BamB presumably is to serve as a platform that enhances the efficiency of assembly especially of OMPs having many β-strands (Heuck et al., 2011). Moreover, BamB together with BamA mediates interactions between different BAM complexes to form assembly precincts (Gunasinghe et al., 2018). The exact role of BamC and E for function of the BAM complex (Wu et al., 2005; Sklar et al., 2007a,2007b; Hagan et al., 2011) is not yet fully understood, but it has been shown that the deletion of BamE may modulate BamA conformation (Rigel et al., 2012, 2013).

The structure of the native *E. coli* BAM complex has been solved recently (Kim et al., 2011; Bakelar et al., 2016; Gu et al., 2016; Han et al., 2016; Iadanza et al., 2016; Bakelar et al., 2017). Within this ~ 200 kDa complex, BamA interacts with BamBCDE via its N-terminal POTRA domains that protrude into the periplasm. Together with BamBCDE, they form a periplasmic ring-shaped assembly assumed to serve as kind of a landing platform for nascent β-barrel proteins (Han et al., 2016). Strikingly, the BamA β-barrel can change between an open and a closed conformation, where the β-strands 1 and 16 are either partially paired or form a lateral gate between the BamA β-barrel lumen and the surrounding lipids (Noinaj et al., 2014; Gu et al., 2016; Iadanza et al., 2016; Lundquist et al., 2018). This feature is essential for BamA function, however, how exactly it contributes to β-barrel protein assembly is still unclear (Noinaj et al., 2017; Lundquist et al., 2018). One prevailing model implies that BamA in its open conformation serves as a lateral gate to release β-barrel OMPs into the OM. It does so by offering its β-strands for augmentation with β-strands of the nascent OMP. Thereby it promotes the consecutive arrangement of all β-strands of the nascent OMP into an open barrel fused to that of BamA at this stage. Later on the newly formed β-barrel buds off the BamA β-barrel and is laterally released into the OM (Konovalova et al., 2017). A striking asymmetry in the height of the BamA β-barrel is thought to be another key feature supporting the lateral release of substrates by thinning and destabilizing the lipid bilayer at BamA's β-barrel junction site (Noinaj et al., 2013, 2014, 2015).

During own studies we found a strong BamB-dependency of OM insertion for Inv -as it has been shown previously for the maltoporin LamB (Charlson et al., 2006)-, but not for YadA, supporting the hypothesis of different requirements of type V proteins for OM insertion and in line with previous findings regarding TolC (Mahoney et al., 2016) and FimD (Palomino et al., 2011). The dependence of Int insertion on BamA as well as the importance of SurA for periplasmic transport had been reported previously (Bodelon et al., 2009). Actually it seems that OMPs having larger β-barrels or more specifically: having more β-strands that need to be processed at the same time, exhibit a

stronger dependency on BamB compared to those having only few. This is exemplified by YadA, being a homotrimer where each monomer contributes only 4 β-strands to form the β-barrel. In contrast, Inv comprises a monomeric 12-stranded β-barrel, where 12 β-strands have to be arranged at one time which presumably requires the support of assembly by BamB. BamB-independence coincides with a strong BamA-dependency of YadA assembly (Lehr et al., 2010). Actually it could well be that this is the result of the necessity of several BAM complexes that have to work together at the same time for the assembly of multimers such as YadA or TolC (Mahoney et al., 2016; Albenne and Ieva, 2017; Weirich et al., 2017).

3.5. Need further help? The role of the translocation and assembly module (TAM) for AT biogenesis

AT biogenesis has been shown to depend not only on the function of the BAM complex. Also the translocation and assembly module (TAM) has been reported to contribute to biogenesis of some AT proteins such as Ag43, EhaA, p1121 and FdeC (Selkrig et al., 2012; Heinz et al., 2016). However, the TAM does not seem to play a role for biogenesis of other AT proteins, such as EspP and Hbp (Sauri et al., 2009; Kang'ethe and Bernstein, 2013). Heinz et al. showed that TAM may assist BamA for assembly of Int (2016). In contrast to the multipartite BAM complex, the TAM complex is formed by two subunits only, namely TamA and TamB (Selkrig et al., 2012). As BamA, TamA belongs to the Omp85 superfamily of proteins. TamA consists of an OM-located 16-stranded β-barrel able to form a lateral gate and is connected to 3 periplasmic POTRA domains, suggesting a similar function compared to BamA (Gruss et al., 2013; Bamert et al., 2017). TamB, the second subunit of the TAM, is a large protein (~ 1200 amino acids) located in the periplasm. TamB is tethered to the inner membrane via its N-terminus (Selkrig et al., 2012; Shen et al., 2014). Its C-terminal part interacts with the POTRA1 domain of TamA, thereby forming a bridge between the IM and the OM (Selkrig et al., 2015). Deletion of TAM is not lethal, but affects virulence of e.g. *Klebsiella*, *Salmonella* (Selkrig et al., 2012), *Proteus* (Burall et al., 2004) and *Citrobacter* (Kelly et al., 2006; Selkrig et al., 2012). A systematic analysis of which AT proteins (including Int, Inv and YadA) really benefit from interaction with the TAM, and under which environmental conditions this is the case (e.g. during infection), has not yet been performed. However, this would be highly desirable to better understand TAM relevance and regulation.

Several scenarios for TAM/BAM interactions during AT biogenesis have been suggested (Albenne and Ieva, 2017): TAM was hypothesized to possibly play a role during late steps of AT biogenesis, i.e. after interaction with the BAM, by supporting late assembly events and fostering completion of AT biogenesis. Furthermore, a cooperative and simultaneous interaction of TAM and BAM is conceivable, whereby the interaction enhances the assembly. Finally, the TAM could be activated only under distinct conditions demanding for enhanced AT assembly efficiency (e.g. during infection, or upon changes in the environment), or serve as a bypass or backup for the BAM. However, what qualifies an AT protein to be handled by the TAM, and whether and how the TAM and BAM complexes may really act together (also physically), and under which conditions they do so, remains to be elucidated.

4. Links between virulence and biogenesis of the AT adhesins YadA, Inv and Int

YadA is the predominant virulence factor of the Gram negative enteropathogen *Ye*. *Ye* lacking YadA or expressing low levels of YadA are significantly attenuated or even avirulent in mouse models of infection (Pepe et al., 1995; Di Genaro et al., 2003; Schütz et al., 2010). Therefore, many studies aimed to elucidate how YadA confers virulence to *Ye*. Actually, YadA contributes to virulence in many ways: YadA-mediated adhesion to host cells is essential prerequisite for the injection of effector proteins via a type three secretion system (T3SS) (Cornelis, 2002; Viboud and Bliska, 2005). YadA protects from complement attack via the binding of negative regulators of complement (Biedzka-Sarek et al., 2008a, b; Kirjavainen et al., 2008; Schindler et al., 2012; Mühlenkamp et al., 2017), and by binding distinct host proteins YadA may contribute to the selection of subsets of cells that are targeted by *Yersinia* (own unpublished observations, Maldonado-Arocho et al., 2013; Paczosa et al., 2014).

Int is a homolog of the *Ye* protein Inv. It is expressed by e.g. enteropathogenic and enterohemorrhagic *E. coli* strains and functions as an adhesin. The interaction of Int with host cells occurs via its dedicated receptor "translocated intimin receptor" (Tir). The interaction of Tir with Int results in the formation of attaching and effacing lesions, caused by a rearrangement of the host cell actin cytoskeleton (Jerse et al., 1990; Frankel et al., 1998). The *Ye* homolog to the *Ec* Int, Inv, also mediates attachment to host cells, but via direct interaction with β1-integrins. Inv is important during an early time point of infection by mediating attachment to and invasion of microfold cells within the small intestine (Isberg and Leong, 1990; Rankin et al., 1992; Leo and Skurnik, 2011).

Defects in biogenesis of virulence factors may directly translate into reduction or even the loss of AT-associated virulence functions. As described above, the passenger domain of YadA can interact with many different host cell proteins. Correct folding of the passenger and the abundance of passenger on the bacterial surface determine the efficacy and outcome of these interactions. Actually, both of these features can be strikingly impacted by disturbed folding and stability of the distant C-terminal β-barrel domain. In this context we identified one highly conserved residue (G389) within the β-barrel domain which has a crucial role for YadA trimer stability, efficiency of passenger transport and consequently for serum resistance and virulence of *Ye* (Grosskinsky et al., 2007; Schütz et al., 2010). Interestingly, molecular dynamics simulations with a monomer of the TAA Hia of *Haemophilus influenzae* have shown that G1064 (equivalent to YadA G389) is one key residue for forming interactions with the central α-helix, stabilizing the 4-stranded β-sheet (Holdbrook et al., 2013). Therefore, exchange of YadA G389 is likely to result in less stable interactions between β-sheet and passenger, impacting the stability of the YadA trimer and thereby explaining the phenotypic consequences. Furthermore, the structures of both the YadA and the Hia β-barrel domain disclosed the constraints within the β-barrel pore, only able to accommodate relatively small residues at this position (Meng et al., 2006; Shahid et al., 2015). Consequently, the exchange of G389 likely interferes with both β-barrel assembly and passenger transport. Of note, the exchange of the corresponding residue G260 in Inv also led to impaired passenger export (Oberhettinger et al., 2012), however, the effects were less pronounced compared to YadA. This makes perfectly sense, because in the assembled homotrimeric YadA the mutation is present trice, having more impact on both the interior constraints of the β-barrel as well as on the stabilizing interactions between the central α-helices and the interacting β-sheets. The general importance of the highly conserved glycine for AT biogenesis is further underlined by the fact that it is almost invariant in the entire Int/Inv family of proteins (Tsai et al., 2010; Fairman et al., 2012; Oberhettinger et al., 2012).

5. Identifying components required for OMP biogenesis as novel targets for antiinfective drugs

The OM contains numerous virulence factors and at the same time proteins that are closely linked to antibiotic resistance, e.g. as part of efflux systems (Phan et al., 2015; Puzari and Chetia, 2017). It is a highly efficient permeability barrier that may hinder antibiotics from entry, although they could in principle be active as their targets are present (Nikaido, 2003). Interference with the biogenesis of OMPs could thus be an attractive therapeutic option, as many OMPs contribute to virulence, are crucial to maintain the OM barrier, or confer antibiotic resistance. Given that most OMPs are inserted by the same biogenesis

pathway (Konovalova et al., 2017), we wondered whether we could define new therapeutic targets among the factors that are involved in OMP biogenesis. To this end, we generated knockouts for BamB, BamC, BamE, Skp, DegP and SurA and analysed the impact on *Ye* OM integrity, serum resistance, antibiotic susceptibility and virulence *in vivo* (Weirich et al., 2017). Two components turned out to be most interesting: SurA and BamB. Lack of either SurA or BamB severely impaired OM integrity, increased antibiotic susceptibility (even sensitized *Ye* to vancomycin), rendered *Ye* serum sensitive, and caused severe attenuation in a systemic mouse infection model (Weirich et al., 2017). Important roles for BamB and SurA also in other species have been described before (Sydenham et al., 2000; Rolhion et al., 2005; Justice et al., 2006; Fardini et al., 2007; Fardini et al., 2009; Obi et al., 2011; Namdari et al., 2012; Southern et al., 2016). We think that both SurA and BamB should be exploited to develop therapeutics that could be used in combination with antibiotics and enhance efficacy of treatment. In fact, it is even possible to re-sensitize a multi-resistant patient isolate of *Pseudomonas aeruginosa* to antibiotic treatment by depleting SurA (Klein et al., 2019). Thus it makes perfectly sense that the inhibition of β-barrel assembly has been recognized and is currently approached as potential target for novel sensitizers/antimicrobials (Mori et al., 2012; Urfer et al., 2016; Storek et al., 2018a, b; Robinson, 2019).

6. Conclusions & outlook

Our knowledge about AT biogenesis has advanced enormously during the last years and it is essential to understand this process in detail if we want to address AT and -more general- OMP biogenesis as potential point of attack for drug development. Although we know about the identity of factors aiding OMP biogenesis, and some interactions taking place, we still need to figure out how these interactions are orchestrated, and under which circumstances these interactions may change (e.g. by additional support by the TAM complex). How and when do BAM and TAM act together? We also do not know what defines substrate specificity of TAM and periplasmic chaperones. Is it a general principle that the passenger transport is energized apart from the energy derived from its folding as some studies suggested? Taken together, there are many open questions that need to be solved to get a complete picture about OMP and especially AT biogenesis. The same holds true for our understanding about the contribution of individual virulence factors for pathogenicity. If we want to target individual virulence factors to reduce the virulence of a given pathogen it is inevitable to understand how they contribute to host interaction, at which time point of infection this is relevant, and also how they function in the context of the numerous other virulence factors that also contribute to host interaction. Therefore, we need to get a picture as complete as possible to foresee the consequences of interference, and to be able to achieve desired effects.

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