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Type V Secretion in Gram-Negative Bacteria

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SUMMARY

Type V or “autotransporter” secretion is a term used to refer to several simple protein export pathways that are found in a wide range of Gram-negative bacteria. Autotransporters are generally single polypeptides that consist of an extracellular (“passenger”) domain and a β barrel domain that anchors the protein to the outer membrane (OM). Although it was originally proposed that the passenger domain is secreted through a channel formed solely by the covalently linked β barrel domain, experiments performed primarily on the type Va or “classical” autotransporter pathway have challenged this hypothesis. Several lines of evidence strongly suggest that both the secretion of the passenger domain and the membrane integration of the β barrel domain are catalyzed by the barrel assembly machinery (Bam) complex, a conserved heterooligomer that plays an essential role in the assembly of most integral OM proteins. The secretion reaction appears to be driven at least in part by the folding of the passenger domain in the extracellular space. Although many aspects of autotransporter biogenesis remain to be elucidated, it will be especially interesting to determine whether the different classes of proteins that fall under the type V rubric—most of which have not been examined in detail--are assembled by the same basic mechanism as classical autotransporters.

INTRODUCTION

“Type V” or “autotransporter” secretion is an umbrella term that is often used to refer to a group of distinct but conceptually related protein export pathways that are widely distributed in Gram-negative bacteria. Autotransporters are generally single polypeptides that contain a signal peptide that promotes translocation across the inner membrane (IM) via the Sec pathway, an extracellular (“passenger”) domain, and a domain that anchors the protein to the outer membrane (OM). Passenger domains have a wide variety of functions, but often promote virulence (1). In the archetypical or “classical” (type Va) autotransporter pathway that was discovered in 1987, the passenger domain is located at the N terminus of the protein adjacent to the signal peptide (2). Although passenger domains range in size from ~20–300 kD and are highly diverse in sequence (3), X-ray crystallographic and in silico studies predict that they usually fold into a repetitive structure known as a β helix (4–8) (Fig. 1). The membrane anchor domains are ~30 kD in size and are also highly diverse in sequence but contain short conserved sequence motifs (3, 9). Like most membrane spanning segments associated with OM proteins (OMPs), these domains fold into a closed, amphipathic β sheet

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or “β barrel” structure. The C- terminal domains that have been crystallized to date all form nearly superimposable 12- stranded β barrels (10–15). The two domains are connected by a short α-helical “linker” that is embedded inside the β barrel domain (10, 12, 13, 16). Many passenger domains are released from the cell surface by a proteolytic cleavage following their secretion (17).

Several other pathways have been described that appear to be variations on the same theme (Fig. 1). Trimeric autotransporters (type Vc pathway) are comprised of three identical subunits that each contain an N-terminal passenger domain that can exceed 4000 residues in length and a ~80 residue C-terminal segment that contributes four β strands to a single 12-stranded β barrel. Although the structure of the β barrel domains is very similar to those of classical autotransporters (18, 19), the three passenger domains assemble into a long coiled-coil “stalk” that emerges from the β barrel domain. The stalk is interspersed with and/or terminated by globular β-roll or β-prism “head” domains that function as adhesins (20–27). In the intimin/invasin (type Ve) pathway, the order of the domains is reversed. These “inverted autotransporters” contain a 12-stranded β barrel domain at (or near) the N terminus and a passenger domain comprised of multiple immunoglobulin (Ig)-like repeats at the C terminus (28–30). Although the structure of the β barrel domain resembles that of classical and trimeric autotransporters, the linker does not form an α helix (29). In the type Vb or two-partner secretion (TPS) pathway, a single “exoprotein” is secreted by a coordinately expressed OM transporter. While exoproteins have the same β-helical architecture as the passenger domains of classical autotransporters, the transporters are members of the “Omp85” superfamily, a group of proteins that have 16-stranded β barrel domains and 1–7 periplasmic POTRA (polypeptide transport- associated) domains that are believed to mediate protein-protein interactions (31, 32). The TPS pathway is the only type V pathway in which a β barrel protein secretes a non-covalently linked polypeptide (for details see chapter X). The type Vd pathway is related to the type Vb pathway in that the C-terminal domains are similar to TpsB proteins, but the covalently linked passenger domains are patatin-like lipases that are released into the environment (33–35). Finally, a family of *Helicobacter pylori* proteins (at least some of which are adhesins) has been described in which an extracellular α-helical domain of up to ~1000 amino acids is situated between β strands 1 and 2 of a putative 8-stranded β barrel (36–40). These proteins have been proposed to represent a “type V-like” pathway based on their modular organization (40), but they do not have a clear phylogenetic relationship to other autotransporters and their structure is unique.

AUTOTRANSPORTER ASSEMBLY AND THE MECHANISM OF PASSENGER DOMAIN SECRETION

Although the first classical autotransporter was discovered over 30 years ago (2), the mechanism(s) by which passenger domains are translocated across the OM through the type V pathways is (are) still not well understood. It was originally proposed that passenger domains are secreted through a channel formed solely by the covalently linked β barrel domain (whence the name “autotransporter”) (2). Indeed it is easy to imagine how translocation in the type Va pathway, which proceeds in a C to N-terminal direction (41, 42), might involve the insertion of a C-terminal hairpin into the β barrel pore followed by the

progressive secretion of more distal segments. The resolution of the hairpin following the completion of translocation would explain why the two domains are connected by an intra-barrel linker. It should be noted, however, that the self-transport model was proposed before significant insights into the biogenesis of bacterial OMPs had emerged. Our view of autotransporter secretion has evolved considerably in recent years and has been strongly influenced both by new experimental data (that focuses primarily on the type Va pathway) and by the identification and characterization of the machinery that catalyzes OMP assembly.

Based on all of the available evidence, it now appears that the β barrel domain does play a role in translocation, but that the process by which passenger domains are transported across the OM is more complex than originally envisioned. On a fundamental level, the finding that translocation is abolished by the replacement of the C terminus of an autotransporter with the β barrel of another OMP suggests that the native β barrel domain does not simply target the passenger domain to an unlinked transporter (43). Furthermore, the finding that mutations that slow the folding and/or membrane integration of the β barrel domain concomitantly delay the initiation of passenger domain translocation also suggests that the β barrel domain promotes the transport reaction (44, 45). The idea that autotransporters are completely autonomous secretion systems, however, was first challenged by two contradictory lines of evidence. Crystal structures revealed that the β barrel pore of classical autotransporters is only ~10 Å in diameter and therefore only wide enough to accommodate a single α helix or a hairpin in an extended conformation (10–15). Molecular dynamic simulations also indicated that β barrel domains are relatively rigid and are unlikely to expand significantly without an input of energy (46, 47). Paradoxically, considerable evidence has emerged that polypeptides that have local tertiary structure can be secreted by the type Va pathway. A subset of native type Va and type Ve passenger domains undergo disulfide bonding in the periplasm, and at least some ~10–20 kD heterologous polypeptides that fold in the periplasm are secreted effectively when they are fused to passenger domains (29, 49–52). An analysis of the secretion of peptides that vary in length and structural complexity also suggests that the translocation channel is ~17–20 Å wide (53). Furthermore, evidence that the linker is already embedded inside the β barrel in an α- helical conformation during translocation strongly suggests that the active transport channel contains at least an α helix and an extended polypeptide (54, 55). Finally, several studies have indicated that the β barrel domain reaches its native state only after the passenger domain is completely secreted (56–58). Taken together, the results imply that during translocation the β barrel domain is in an open or distorted conformation that would be incompatible with stable integration into a lipid bilayer.

A plausible alternative to the self-transport hypothesis that accounts for the secretion of folded polypeptides arose from an analysis of stalled translocation intermediates. One study exploited the fortuitous discovery that the insertion of a peptide linker near the middle of the passenger domain of a classical autotransporter (the *E. coli* O157:H7 EspP protein) did not affect the initiation of translocation, but transiently stalled translocation when the inserted peptide was in the vicinity of the transport channel (41). Site-specific photocrosslinking experiments showed that passenger domain residues located near the site of stalling are in close proximity to BamA, a member of the Omp85 superfamily. BamA is an essential

component of the barrel assembly machinery (Bam) complex, a heterooligomer that catalyzes the membrane insertion of essentially all β barrel proteins, including autotransporters (59–62). In a second study, chemical crosslinking experiments showed that a related autotransporter (Hbp) was close to the Bam complex when the secretion of the passenger domain was stalled by a different method (56). Interestingly, the crystal structure of BamA together with molecular dynamics simulations strongly suggests that the BamA β barrel can open laterally (63). Although the function of the BamA lateral gate is still unclear, the results raise the intriguing possibility that passenger domains are secreted through a hybrid channel composed of open forms of both the linked β barrel domain and the BamA β barrel. Such a channel would presumably be wide enough to accommodate the transport of polypeptides that have local tertiary structure.

The analysis of these and other assembly intermediates has led to a detailed model for the biogenesis of classical autotransporters. The finding that the EspP linker becomes protected from proteolysis and chemical modification (54) prior to the initiation of passenger domain translocation suggests that the β barrel domain begins to fold in the periplasm (Fig. 2, step I). Consistent with this idea, a recent study indicated that the trimeric β barrel of a type Vc autotransporter begins to assemble in the periplasm (64). The observation that the linker is required for the membrane integration of the EspP β barrel domain in vivo (54) and accelerates assembly in an in vitro assay (65) suggests that it nucleates early folding events. Photocrosslinking experiments (44, 57) have shown that at this stage the EspP β barrel domain interacts with the periplasmic chaperone Skp, a jellyfish-like homotrimer that binds to both small and large β barrels in a 1:1 or 2:1 ratio (66, 67). Subsequently the β barrel domain is targeted to the Bam complex (Fig. 2, step II). Crosslinks between specific residues of the EspP β barrel domain and two lipoprotein subunits of the Bam complex, BamB and BamD, can be detected at this step (44). Interestingly, a map generated by projecting the molecular interactions implied by the photocrosslinking experiments onto the crystal structure of the Bam holocomplex supports the idea that the β barrel domain is already folded into a cylinder-like structure (68). The initiation of passenger domain translocation requires an additional assembly step that appears to correspond to the movement (but not full integration) of the β barrel domain into the OM (44, 69) (Fig. 2, step III). As suggested above, the passenger domain might be transported through a hybrid channel that contains the BamA β barrel in an open conformation. Available evidence indicates that translocation involves a stepwise transfer of passenger domain segments from the chaperone SurA, which binds to the first POTRA domain (70), to membrane proximal POTRA domains and then to the transport channel (44) (Fig. 2, step IV). In the TPS pathway, exoproteins use a similar path to traverse the cognate transporter (71). Following the completion of translocation (Fig. 2, step V), a surface exposed basic or large polar residue stimulates a final step in the folding of at least some classical autotransporters that may correspond to the closing of the β barrel domain (58) (Fig. 2, step VI). Ultimately the β barrel domain is released from the Bam complex and the passenger domain is cleaved (Fig. 2, step VII).

ENERGETICS OF PASSENGER DOMAIN SECRETION

Because the periplasm is devoid of ATP and there is no electrochemical gradient across the OM, the source of energy for passenger domain translocation has remained unclear. It is

possible that in some cases an interaction between the passenger domain or specialized components of the OM transport machinery and an energized IM protein drives the translocation reaction. The observation that the Bam complex and SurA are sufficient to promote passenger domain translocation into proteoliposomes, however, suggests that autotransporter assembly does not strictly require an exogenous energy source (72).

To explain the energetics of autotransporter secretion, it was proposed years ago that small segments of the passenger domain passively diffuse through the transport channel and then fold in the extracellular space (73). Folding would trap the passenger domain on the cell surface and thereby provide the driving force for translocation. This hypothesis is especially attractive given that most passenger domains are composed of modular β helices that might fold in a stepwise fashion. A subset of passenger domains contain so-called passenger-associated transport repeats (PATRs) that might also contribute to progressive folding (74). Indeed even the passenger domain of a classical autotransporter that has a globular structure has been predicted to fold sequentially based on the arrangement of its secondary structure elements (12). During the last decade the “vectorial folding” hypothesis has been supported by several observations. Studies that have analyzed the refolding or unfolding of passenger domains in vitro or the effect of mutations on passenger domain secretion in vivo have demonstrated that the folding of a conserved ~20–25 kD “stable core” segment located at the C terminus of many passenger domains plays a key role in driving translocation (6, 55, 69, 75–77). The results of kinetic simulations also suggest that passenger domain secretion is driven by the free energy of folding in the extracellular milieu (78). Furthermore, an analysis of insertions and deletions in the intimin passenger domain suggested that secretion by the type Ve pathway is driven by sequential folding of the Ig-like domains (79). A recent study provided intriguing evidence that the folding of classical autotransporter passenger domains on the cell surface is not spontaneous, but is nucleated by the fifth extracellular loop of the β barrel domain (80).

Despite the evidence that supports the vectorial folding model, several observations have strongly suggested that autotransporter secretion is not driven solely by passenger domain folding. It has been shown, for example, that multiple point mutations introduced into the middle of the EspP passenger domain destabilize the protein but only moderately impair translocation (81). In addition, an intrinsically disordered polypeptide fused to the C terminus of EspP was secreted as rapidly and efficiently as the native passenger domain (52). The disordered polypeptide is unusually acidic, and the neutralization of multiple acidic amino acids was shown to stall translocation. Taken together with the finding that many native passenger domains are acidic, this observation suggests that charge interactions and/or the Donnan potential across the OM (82) might help to drive translocation. Furthermore, it seems likely that the secretion of ~10–20 kD folded polypeptides that has been reported would also require the input of energy from an alternative source. In this regard it is noteworthy that structural studies on the OM transporters associated with the chaperone/usher and type VIII secretion pathways suggest that they drive translocation by defining a low-energy pathway or using an entropy-based diffusion mechanism (83, 84).

ACCESSORY FACTORS IN AUTOTRANSPORTER ASSEMBLY

Like all OMPs, autotransporters must remain in an assembly-competent conformation in the periplasm. Consistent with this expectation, several periplasmic chaperones that play a broad role in OMP biogenesis including DegP, FkpA, Skp and SurA have been shown to interact with autotransporters in vivo and/or in vitro (41, 57, 85, 86). Presumably because periplasmic chaperones have redundant or at least partially overlapping functions (87), however, the requirement for specific chaperones in autotransporter assembly appears to be protein, organism and condition-dependent (41, 64, 85, 88, 89).

It is still unclear whether factors other than periplasmic chaperones and the Bam complex play a general role in autotransporter assembly. The finding that both EspP and the *E. coli* autotransporter Ag43 can be assembled into proteoliposomes that contain only the Bam complex in purified protein or spheroplast-based assays (72, 90) certainly suggests that no other factors are absolutely essential. There is evidence, however, that a member of the Omp85 superfamily (TamA) and an IM-anchored protein (TamB) that interacts with TamA facilitate the assembly of a subset of autotransporters including the *Citrobacter* p1121 protein and Ag43 (91, 92). Interestingly, TamA/B has also been implicated in the assembly of inverse autotransporters (93). Although different models for the function of TamA/B have been proposed (68, 94), the observation that the Tam system facilitates the biogenesis of the fimbrial usher protein FimD (95) suggests that it is not an autotransporter-specific assembly factor. In addition, the efficient assembly of a subset of trimeric autotransporters requires the activity of a trimeric IM lipoprotein that is encoded in the same operon (96).

CONCLUDING REMARKS

Studies on type V secretion suggest that autotransporter β barrel domains are not autonomous transporters, but that the assembly of the β barrel domain and the secretion of the passenger domain are catalyzed by the Bam complex in a concerted reaction. Although it is possible that BamA promotes passenger domain translocation indirectly by keeping the autotransporter β barrel domain in an open conformation, the fact that some members of the Omp85 superfamily catalyze secretion reactions suggests that BamA might play a direct role in translocation. In any case, to obtain a better understanding of autotransporter biogenesis it will likely be necessary to elucidate the function of the Bam complex, which so far has remained elusive (97). Of course the degree to which the assembly of type Vc-Ve and “autotransporter- like” proteins resembles the assembly of the better characterized classical autotransporters also remains to be investigated. Indeed it should be interesting to determine why features of the Omp85 superfamily are found only in the type Vd pathway and if the folding of predominantly α- helical passenger domains drives their secretion. Given that the loops of most OMPs are relatively short (<75 residues) and that at least in some cases only small insertions are tolerated (98), the presence of the large loop structures in “autotransporter-like” proteins is intriguing. Based on the discovery of these proteins, the structural similarity of the β barrel domains found in multiple type V pathways, and the unique structures of passenger domains, it is tempting to speculate that the Bam complex evolved to facilitate the efficient export of a range of specialized polypeptides that are paired with specific types of β barrels.

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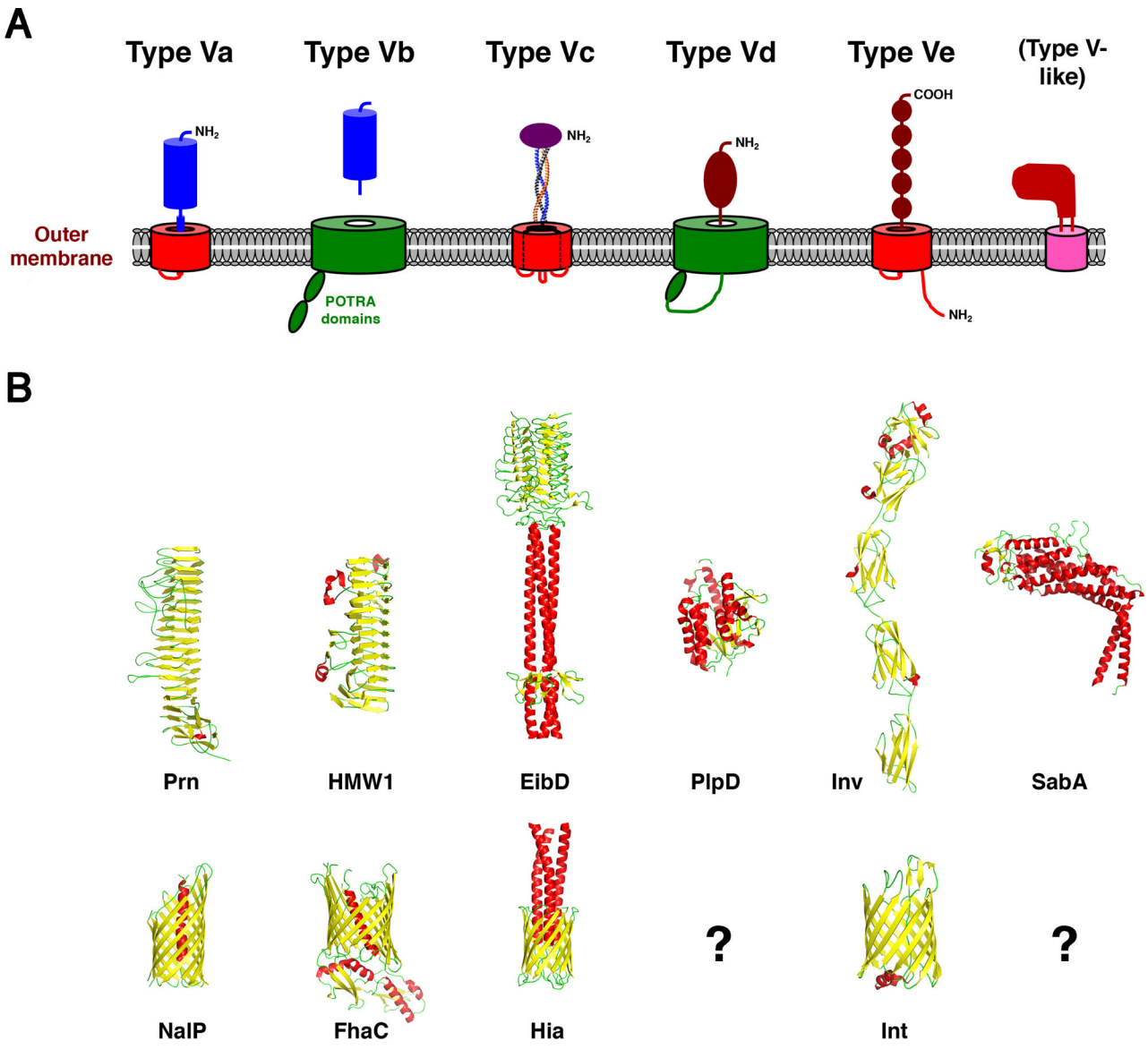

FIG 1.

Illustration of type V secretion pathways. (A) Proteins in type V (and type V-like) secretion pathways consist of a 12-stranded (red), 16-stranded (green) or predicted 8-stranded (pink) β barrel domain and an extracellular (“passenger”) domain that typically folds into a β-helical (blue), mixed coiled-coil/β roll/β prism (purple) or globular (brown) structure. The 16-stranded β barrel domains are members of the Omp85 superfamily and contain periplasmic POTRA domains. In most cases the β barrel and passenger domains are covalently linked, but in the type Vb pathway the β barrel domain and the extracellular component (“exoprotein”) are separate polypeptides. In the type Vc pathway both domains are formed through the assembly of three identical subunits. The passenger domain is located at the N terminus of the protein in the type Va, Vb, Vc and Vd pathways, but is found at the C terminus in the type Ve pathway. In the type V-like pathway the extracellular domain is located in a loop that connects the first two β strands of the β barrel domain. (B) Crystal

structures of representative polypeptides from each pathway are shown. α-helical segments are colored red and β strands are colored yellow. The structures include the pertactin (Prn) passenger domain (4; PDB ID: 1DAB), a fragment of the HMW1 exoprotein (99; PDB ID: 2ODL), a fragment of the EibD passenger domain (24; PDB ID: 2XQH), the phospholipase D (PlpD) passenger domain (34; PDB ID: 5FYA), the invasin (Inv) passenger domain (28; 1CWV), the SabA extracellular domain (36; PDB ID: 4O5J), and the NalP, FhaC, Hia and intimin (Int) β barrel domains (10, 100, 18, 29; PDB IDs: 1UYO, 4QKY, 2GR7, 4E1S). The helix inside the FhaC β barrel was generated from a neighboring asymmetric unit in the crystal lattice. No structures of β barrel domains of type Vd or type V-like proteins have been reported. Modified from *Molecular Microbiology* (101) with the permission of the publisher.

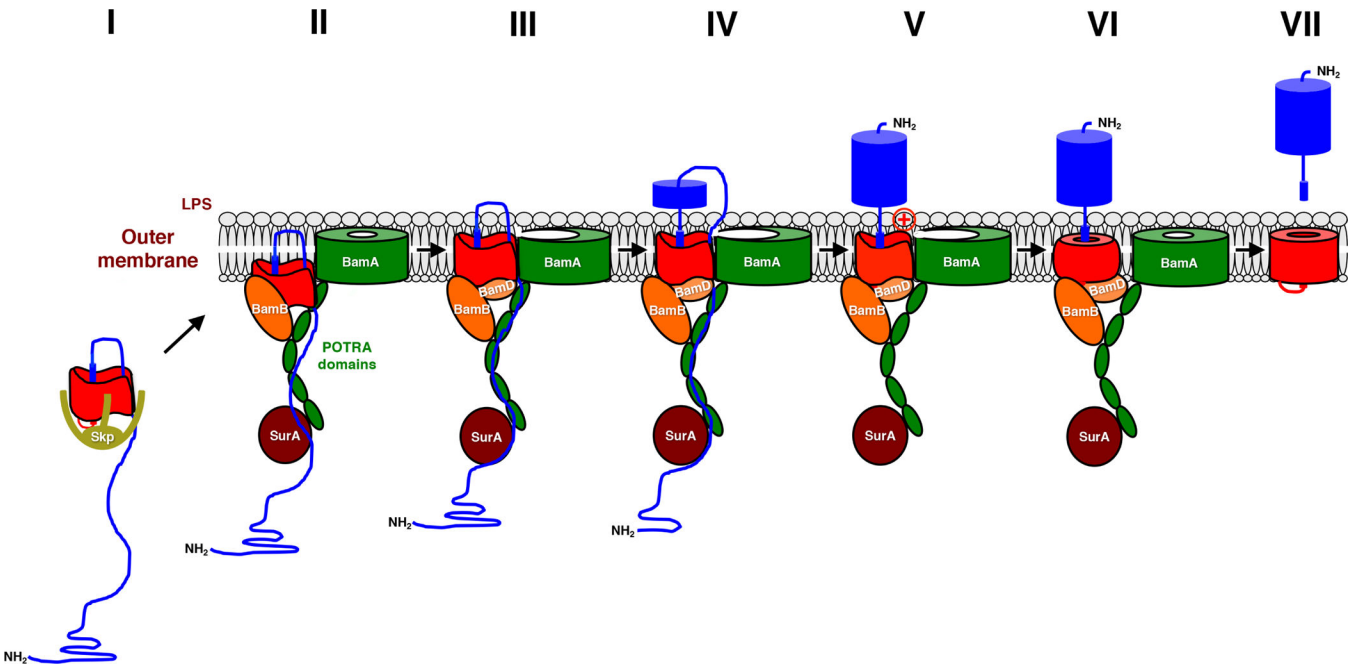

FIG 2.

Model for the assembly of a classical autotransporter. Available evidence suggests that the β barrel domain (red) begins to fold in the periplasm (step I) and incorporates the C terminus of the passenger domain (blue) in a hairpin conformation. At this stage the β barrel domain interacts with the molecular chaperone Skp. The partially folded β barrel domain is then targeted to the OM where it binds to BamA, BamB and BamD in a stereospecific fashion (step II). The surface exposure of the passenger domain and the initiation of translocation requires an additional assembly step in which the β barrel domain moves into the membrane (step III). Both autotransporter and BamA β barrels are in an open conformation at this stage. Translocation involves the progressive movement of passenger domain segments from the chaperone SurA to the POTRA domains of BamA to the transport channel and is driven at least in part by vectorial folding (step IV). Following the completion of translocation the hairpin is resolved (step V), and an unusual lipid-facing basic or large polar residue found in at least a subset of autotransporters facilitates the completion of β barrel domain assembly (step VI). The β barrel domain is then released from the Bam complex and, in some cases, the two domains are separated by an intrabarrel cleavage or an extrabarrel cleavage mediated by a trans-acting protease (step VII). In *E. coli* the Bam complex contains five subunits, but BamC and BamE have been omitted for clarity. Modified from *Molecular Microbiology* (58, 101) with the permission of the publisher.