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Assembly of Outer Membrane β -Barrel Proteins: the Bam Complex

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INTRODUCTION

The outer membrane (OM) is a distinguishing feature of Gram-negative bacteria. It serves as an essential layer of protection against the entry of toxic small molecules while also providing a sieve-like function that helps to control the influx and efflux of essential metabolic nutrients, solutes, and waste products. The OM encases a thin, aqueous layer termed the periplasm, which separates the OM from an inner membrane (IM), which in turn surrounds the cytoplasm. In most Gram-negative bacteria, a thin, rigid meshwork of peptidoglycan attaches to the inner face of the OM and provides the cell with its shape and a counterbalance to the osmotic pressure from the cytoplasm (Fig. 1).

Composition of the Outer Membrane (OM)

All of the components of the OM, the peptidoglycan, and the periplasmic compartment are synthesized in the cytoplasm or at the cytosolic face of the IM and are subsequently transported to their appropriate destinations by various dedicated pathways. The protein machines that assemble OM components are quite remarkable in that they must direct the transit of amphipathic molecules across both hydrophobic and aqueous compartments as well as bypass the peptidoglycan layer. Amazingly, all of this is accomplished in the extracytoplasmic compartments that lack obvious access to energy sources such as ATP (218).

This Chapter focuses on the transport and assembly of one of the components of the OM, integral OM β -barrel proteins, or OMPs. The reader is referred to other Chapters in this compendium for more detailed analysis of the composition of the OM, periplasmic space, and IM. Here, we provide a cursory overview of the OM to delineate the environment in which OMP assembly takes place.

OM lipids—Most biological membranes, including the IMs of bacteria, are composed of a phospholipid bilayer that contains integral or peripherally associated proteins with a wide range of activities, including the initiation of membrane biogenesis, energy production, respiratory, metabolic, or structural functions, and the transport of molecules into or out of a cell or organelle. Unlike the IM, the OM is an asymmetric bilayer composed of an inner leaflet of phospholipids and an outer leaflet of lipopolysaccharide (LPS), or endotoxin (Fig.

1) (97). Although the composition and decoration of an LPS molecule can be highly variable even among different subspecies, it is this LPS layer that generally provides a remarkably efficient barrier function for the cell (132). LPS consists of a hydrophobic lipid A core that is further conjugated to a core oligosaccharide and an O-antigen glycan chain that impart a hydrophilic character to the molecule as well (132). The amphipathic nature of LPS and the strong lateral interactions between LPS molecules prevent the non-specific entry of many small molecules (134).

The phospholipid composition of both the IM and the OM has been extensively characterized in both *Escherichia* and *Salmonella* (e.g., 82, 138). Membranes of both of these genera share similar phospholipid profiles, with a majority of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), lower levels of cardiolipin (CL), and only trace amounts of other phospholipids in the cell. Both LPS and phospholipids have been implicated as molecular chaperones that contribute to the folding status of certain OMPs (e.g., 17, 18, 22, 35, 38, 114, 154, 170, 171), and deficiencies in either lipid component leads to a disruption of the cells' barrier function and eventual cell death.

In *E. coli* and *Salmonella*, the pathway for phospholipid transport to the OM is not known however much more is understood about how LPS is assembled at the cell surface. Once synthesized, LPS must be flipped from the cytosolic surface of the IM to the periplasmic surface via the integral IM protein, MsbA, in an ATP-dependent fashion (43, 145, 221). The subsequent transport of LPS molecules from the periplasmic surface of the IM to the outer surface of the cell proceeds via a multi-component ABC transport complex termed the Lpt machine (see 162 for review). Depletion of any of the Lpt components leads to the same phenotypes: lack of de novo LPS assembly at the cell surface, the formation of extramembranous compartments within the periplasm, filamentation, and eventually, cell death (21, 161, 182, 183, 217).

OM proteins—The OM of Gram-negative bacteria contain three classes of proteins: OM lipoproteins that are peripherally associated with the OM via a lipidated N-terminal anchor; and two types of integral OM proteins; one minor class enriched in α -helical transmembrane segments, and the far more abundant class of OM β -barrel proteins (or OMPs) enriched in transmembrane β -strands. Surface proteins such as pilins are associated with the outer membrane, but this association is mediated by protein-protein interactions with an OMP and consequently, will not be discussed here. All three types of OM proteins, as well as most periplasmic proteins, commonly utilize the post-translational secretion pathway for translocation from the cytoplasm in enteric bacteria (see 46 for review). The substrates are first synthesized in the cytoplasm, maintained in an unfolded conformation via the tetrameric cytoplasmic chaperone SecB (220), and subsequently delivered to the membrane associated SecA ATPase (Fig. 1). Recognition of precursor OM and periplasmic proteins by the SecA protein typically occurs via a ~20–25 amino acid N-terminal signal sequence with three central context-dependent features; a polar N-terminal region, a stretch of hydrophobic residues, and a more polar C-terminal region that contains a signal cleavage site (46). SecA provides the energy to translocate the unfolded preprotein through the SecYEG translocon, a multi-component protein channel integral to the IM. Once translocated, the substrate's signal sequence is cleaved on the periplasmic side of the IM by a signal peptidase (140), and

sorting of the different protein types to their appropriate destinations is subsequently accomplished via dedicated pathways.

The biosynthesis of lipoproteins is covered extensively elsewhere (Tokuda Chapter X). Briefly, prolipoproteins are characterized by a conserved N-terminal peptide motif termed the lipobox (69). Upon IM translocation, this sequence is recognized by Lgt, a membrane protein that transfers a diacylglycerol moiety to a cysteine residue within the lipobox (56). This modification is required for the recognition of the signal sequence by a dedicated peptidase, LspA (also called signal peptidase II, or SPase II) (140). Cleavage of the signal sequence exposes the modified cysteine residue as the mature N-terminus of the protein (80), and maturation of the lipoprotein occurs upon aminoacylation of the cysteine with a palmitate residue by the Lnt enzyme (66). Once mature, lipoproteins destined for the OM are sorted from those that remain at the IM (172) via the essential Lol pathway, composed of an ABC transport machine at the IM (LolCDE), a periplasmic chaperone (LolA) and an OM assembly site (LolB) (see Fig. 1, and Tokuda Chapter X for additional references).

β -barrel proteins (OMPs) are unique to OMs, and encompass the vast majority of integral proteins in that compartment. These proteins typically contain 8–24 multipass antiparallel β -strands (85) in which adjacent strands are hydrogen bonded to form a β -sheet. This sheet wraps into a cylindrical barrel structure that is stabilized via similar hydrogen bonds that connects the first and last β -strands. The resulting OMP has a hydrophobic protein exterior that can interface with the insoluble lipids of the OM and a more hydrophilic central pore. This pore can serve as a portal for specific solutes or substrates, while other OMPs have partially or fully occluded central cavities that result in little to no pore activity (132). Some OMPs also contain an additional periplasmic or surface exposed domain at the N- or C-terminus that is soluble in nature.

Processing of the N-terminal signal sequence of newly translocated OMPs and periplasmic proteins occurs via LepB, (also called signal peptidase I, or SPaseI) (Fig. 1) (140). While periplasmic proteins complete their folding in the aqueous compartment, OMPs are recognized by periplasmic chaperones that maintain them in an unfolded conformation and deliver them to the OMP assembly machine at the OM (Fig. 1). This assembly site is a highly conserved, multi-component machine called the Bam complex, for β -barrel assembly machine, and is detailed further in the sections below.

The final class of OM proteins is the α -helical integral proteins. Thus far, only a small subset of Gram-negative OM proteins fall into this category, such as the Wza protein of *E. coli* and *Salmonella* (45) and possibly the dodecameric PulD secretin of a Type-II secretion system in *Klebsiella oxytoca* (29). How these proteins are assembled into the OM remains unclear. PulD insertion into the OM has been demonstrated to be independent of the Bam machinery (29), although PulD does require a dedicated chaperone-like lipoprotein, PulS, to direct the protein to the OM (65). It is not yet known if the Wza protein requires the Bam complex for insertion (215). To further complicate matters, the Wza protein is also an OM lipoprotein (47), thus it is possible that this protein is initially processed and sorted as a lipoprotein prior to the insertion of its transmembrane helices.

Introduction to the Bam Complex

The Bam complex is likely responsible for the assembly of β -barrel proteins into the OM of virtually all Gram-negative bacteria (with all fully sequenced representatives carrying at least one homolog of BamA with the rare exceptions thus far of the phylum Chloroflexus (25) and the endosymbiont *Buchnera aphidicola* subsp. *Baizongia pistaciae* (unpublished observations)). Chlorobacteria and *Buchnera aphidicola* both contain only a few putative OMPs, and thus may not require a dedicated assembly machine. In *E. coli*, the machine consists of a central OMP, BamA (formerly YaeT) (216), and four associated OM lipoproteins, BamB, -C, -D, and -E (formerly YfgL, NlpB, YfiO, and SmpA, respectively, with the proteins renamed according to size from largest to smallest) (174, 187, 216). The interactions between subunits of the Bam complex are strong enough to withstand biochemical purification even in the absence of chemical crosslinking reagents (174, 187, 216).

The BamA protein is the most highly conserved member of the Bam complex across Gram-negative genera (Table 1). Indeed, the BamA protein has predicted functional and structural homologs in the OM of chloroplasts (48, 75, 78) and in mitochondria (59, 111, 142). SynToc75 of cyanobacteria was the first Gram-negative BamA homolog demonstrated to be essential (151). Subsequent experiments that depleted the BamA homologs Tob55/Sam50 from the OM of fungal mitochondria (59, 111, 142) and Omp85/BamA from the Gram-negative organisms *Neisseria meningitidis* and *E. coli*, respectively (44, 205, 214, 216), verified that these proteins are essential and that they are required for the assembly of OMPs in their respective organelles.

σ^E - and Cpx- Regulation of OMP Assembly

Bam-mediated OMP assembly is likely to be an imperfect process even under the best of conditions, occasionally leading to the production of misassembled OMPs in the periplasm. In addition, environmental fluctuations can lead to destabilizing conditions that further contribute to OMP misfolding. Enterobacteria have evolved at least two signal transduction systems designed to sense and/or alleviate the periplasmic stress associated with the accumulation of these aberrant molecules, the σ^E and Cpx extracytoplasmic stress response pathways.

Historically, the σ^E and Cpx pathways have been characterized as serving distinct functions; with the σ^E pathway responsible for monitoring misfolded β -barrel peptides (122, 208) and the Cpx pathway responsible for sensing misfolded pilin subunits and periplasmic proteins (77, 93). A host of extracytoplasmic stresses, including changes in extracellular pH (32, 131), the accumulation of some lipoproteins (128, 181) and disturbances in the localization of enterobacterial common antigen (31) or phospholipid content (123) induce the Cpx response, perhaps by causing disturbances in envelope protein folding (42). Both pathways share some overlap in the ability to respond to defective LPS molecules (102, 194) and changes in osmolarity (12, 94, 147). Although most of the environmental disturbances listed above mainly feed into a singular extracytoplasmic stress response, the partial overlap in the σ^E - and Cpx- regulated target genes and functional redundancy in some of their encoded

gene products intertwines the two stress responses and can blur the lines between such distinct cellular roles (61, 146, 163).

The σ^E pathway directly senses aberrant OMPs in the periplasm through the recognition of a conserved C-terminal sequence that is revealed in unfolded OMPs (reviewed in 1). This sequence is recognized by a PDZ domain found in the IM-spanning anti- σ^E complex (208), and binding triggers proteolysis of the complex and the release of σ^E into the cytoplasm (2, 3, 40, 122, 127). σ^E is then available to increase the transcription of a large collection of genes encoding extracytoplasmic proteins involved in maintaining the OM (153). Two σ^E -inducible genes whose products are particularly critical in coping with unfolded OMPs once they accumulate in the periplasm are *degP* (33, 34, 41, 96, 144, 146, 178) and *surA* (33, 34, 41, 96, 144, 146, 153, 178), which function to degrade and/or prevent OMP aggregation, respectively (115, 185, 188). The contribution of these proteins to OMP assembly is discussed in further detail in later sections.

Interestingly, each gene encoding a component of the Bam complex in *E. coli* (*bamA* through *bamE*) is at least partly controlled by the σ^E -stress response (34, 96, 130, 136, 152, 153), and the same is true for most of the *bam* complex genes in *S. typhimurium* (130, 178). In addition to the up-regulation of genes involved in OMP biogenesis, the σ^E pathway increases the production of small RNAs (sRNA's) that inhibit the translation of the major OMPs in both *E. coli* (89, 90, 130, 153, 200) and in *S. typhimurium* (20, 130, 141). Therefore, the σ^E -stress response associated with perturbations in OMP assembly reduces the negative effects of periplasmic OMP accumulation in at least four ways: increased proteolysis of misfolded OMPs, chaperone-dependent prevention of OMP aggregation, transcriptional modulation of *bam* genes and other factors that regulate envelope biogenesis, and sRNA-mediated reduction in OMP synthesis under membrane-stress conditions.

In combination, the various mechanisms utilized by the σ^E pathway are so central to the regulation of envelope perturbations that it is an essential pathway in *E. coli* even under non-stress conditions (23, 39). Although it is not a required pathway in *Salmonella typhimurium*, cells lacking the σ^E pathway in these organisms nevertheless demonstrate aberrant phenotypes such as altered growth rate, decreased virulence, and increased OM permeability and superoxide sensitivity under certain conditions (76, 91). The σ^E pathway is the primary regulator of OMP-induced periplasmic stress, but it also shares a collaborative relationship with the Cpx pathway in this particular regard (30, 61). Both stress response pathways regulate gene clusters that are involved in different aspects of OM homeostasis and they demonstrate a significant level of overlapping regulation for certain target genes (146). In contrast to the σ^E pathway, the Cpx pathway is not essential in *E. coli*, and the principle Cpx-regulated contribution to alleviating OMP-mediated periplasmic stress in that organism is the activation of *degP* (30, 61). The combined expression of *degP* by both the σ^E and Cpx stress response pathways is required to produce the DegP protease in quantities that are sufficient for the effective management of defective OMPs, although it is possible that other Cpx-regulated genes also contribute somewhat to the regulation of OMP stress (61).

Induction of the Cpx pathway occurs via activation of the IM CpxA sensor kinase and the subsequent phosphorylation of its cognate response regulator, CpxR (reviewed in 163). The

mechanisms responsible for transducing the various Cpx-inducing stresses to the CpxA kinase have yet to be determined, but two envelope-derived factors that influence CpxA activity are the sensing of adhesion to hydrophobic surfaces via the OM lipoprotein NlpE (139, 181) and binding of CpxA by the periplasmic CpxP protein (52, 150). It is unclear if the contribution of the Cpx pathway in the recognition of abnormal OMPs involves the direct sensing of unfolded OMPs or if their accumulation indirectly triggers an alteration in one of the conditions already known to induce the Cpx response (61).

STRUCTURE AND FUNCTION OF THE BAM COMPLEX

BamA belongs to the “bacterial surface antigen” superfamily, a cluster of proteins that all contain a somewhat conserved C-terminal β -barrel domain and one or more N-terminal polypeptide transport associated (POTRA) domains (164). The superfamily can be subdivided into two functionally distinct groups (129). The first includes the BamA/Omp85 proteins of Gram-negative bacteria as well as their homologs in the OM of mitochondria (59, 111, 142) and presumably chloroplasts (75, 78, 168) which have been demonstrated and predicted, respectively, to share a functional role in the assembly of β -barrel proteins. The second group consists of proteins that transport peptides across an OM, such as some members of the TpsB-type proteins of Gram-negative two-partner secretion (TPS) systems and Toc75 polypeptide importers of chloroplasts (129). Below, we focus on dissecting the features of the N- and C-termini of bacterial BamA homologs and draw some comparisons among other members of the superfamily where appropriate.

BamA POTRA domains

All Gram-negative BamA homologs are predicted to have five periplasmic POTRA domains (57, 101, 164) followed by a C-terminal β -barrel domain in the OM (59, 186, 205) (depicted in Fig. 2). Each POTRA domain is comprised of approximately 75 amino acid residues that form a $\beta\alpha\alpha\beta$ fold, and although the sequence identity of any two given POTRA domains within the BamA protein can be very low, the overall architecture of all of the domains is conserved (28, 58, 101, 108, 212).

The five POTRA domains are referred to as P1 through P5 starting from BamA's N-terminus (Fig. 2). Two separate crystal structure determinations demonstrate that the β 2 strand of POTRA domain 3 (P3) can template an incoming β -strand in either a parallel (58) or anti-parallel (101) orientation in a process termed β -augmentation (68), while the neighboring α 2- β 2 hydrophobic groove could potentially accommodate the side chains of a bound peptide strand (58). P3 has distinct structural differences from the other BamA POTRA domains, including a β -bulge central to the β 2-strand that is thought to facilitate β -augmentation and play a role in the stabilization of the Bam complex (58, 101). Although these data specifically implicate P3 in β -strand interactions, it is likely that all of the POTRA domains are capable of templating β -strands to some extent (101, 108). Structural similarities between the POTRA domain groove and that of the SecB cytoplasmic chaperone (58, 220) are particularly interesting in light of the fact that the role of SecB is to maintain OMPs in an unfolded conformation (Fig. 1) (46); thus it may be that the POTRA domains serve as periplasmic chaperones critical to the scaffolding of unfolded OMPs prior to assembly.

In *E. coli*, each of the POTRA domains contributes to OMP assembly, either directly or indirectly. Deletion of domains P1 and P2 results in drastically lower OMP levels and increased σ^E activity, but only POTRA domains 3–5 are essential for growth (101). The POTRA domains not only contribute to OMP assembly but also to the interactions among Bam complex members. Deletion of P2, P3, or P4 disrupts the interaction between BamA and the non-essential lipoprotein BamB, while deletion of P5 abolishes binding between BamA and all of the other essential and non-essential components of the Bam complex. The fact that P3 and P4 are essential in *E. coli* despite the fact that they do not bind an essential member of the complex highlights their importance in OMP assembly function (101). Since the BamA homolog in *N. meningitidis* requires only P5 for viability (19), the individual contributions of the POTRA domains to OMP assembly may vary in distantly related organisms.

The relative orientations of the BamA POTRA domains have been determined by two crystal structures (58, 101), one nuclear magnetic resonance spectrometry (NMR)/small-angle X-ray scattering (SAXS) study (108), and one pulsed electron paramagnetic resonance (EPR) study (212). The current consensus is that domains P1-P2 and P3-P4 interactions are relatively rigid with respect to one another, while a putative hinge point between POTRA domains P2 and P3 may induce a conformational change during the OMP assembly process (58, 101, 108, 212). These alternate configurations between a straightened and bent conformation are illustrated in Fig. 2. A complete P5 domain is not represented in the current crystal structures, but based on the structural similarity of the POTRA domains, SAXS data suggests that P5 is sharply folded back with respect to P4 and the mobility of this domain may be flexible (108).

The BamA β -barrel

The periplasmic POTRA domains of BamA are connected to a C-terminal β -barrel domain. While no atomic resolution structure is available for the β -barrel portion of BamA, it most likely contains 16 transmembrane domains based on prediction algorithms (60, 85). Fortunately, a full crystal structure is available for FhaC of *Bordetella pertussis*, a protein that is part of a two-partner secretion (TPS) system and a distant BamA homolog within the same bacterial surface antigen superfamily (28). In these secretion systems, a TpsB β -barrel protein with two periplasmic POTRA domains specifically recognizes a TpsA partner protein. It is thought that the TpsA protein is translocated from the periplasm to the bacterial surface through the TpsB pore in an unfolded conformation and subsequently adopts a β -helical fold post-secretion (72, 85). Although the barrel domain of TPS proteins have some structural characteristics that distinguish them from BamA-like proteins, the two classes of proteins share a number of conserved features as well (60, 85, 129). Therefore, the FhaC crystal structure and evidence obtained from sequence prediction programs have been used to make reasonable predictions concerning the BamA barrel architecture.

Like all members of the bacterial surface antigen superfamily, BamA β -barrel homologs contain a conserved motif 3 and motif 4 sequence (129). Based on the crystal structure of FhaC (28), both of these regions lie in proximity to “Loop 6”, a long extracellular loop that reaches back through the barrel’s pore and likely extends into the periplasm (Fig. 2). It has

been suggested that Loop 6 undergoes a conformational change that allows gating of the FhaC pore by adopting either an extracellular extended state or one that remains buried within the pore (28, 64, 85). Loop 6 in the BamA/Omp85 family is longer than in TpsB proteins and its function is unknown (85), but deletion of this loop in the FhaC protein abolishes secretion of its TpsA partner protein, filamentous hemagglutinin (FHA) (28). It is unclear if OMP substrates utilize the BamA pore in folding per se, Loop 6 may function directly in OMP assembly or by allowing BamA to adopt critical conformations (85). Clear answers as to the significance of Loop 6 and other conserved regions in the BamA barrel await future genetic and biochemical analyses.

Lipoproteins of the Bam complex

The composition of the accessory components of the Bam complex varies among Gram-negative bacteria (for example, see 5, 204), even among members belonging to the same sub-phylum (57) or genus (our observations). Table 1 indicates Gram-negative phyla or sub-phyla that contain at least one genus with a given Bam protein and/or Bam accessory factor as identified by the clusters of orthologous groups database (COG) (<http://www.ncbi.nlm.nih.gov/COG/>). Some organisms lack homologs of one or more *E. coli* Bam complex member(s). Likewise, as more Bam complexes are analyzed in other organisms, there may be novel interacting partners that play a role in OMP assembly but have no homolog in *E. coli*. Although the findings presented in this section cannot necessarily be extended to all Gram-negative organisms, we attempt to generalize what we think are shared features of the Bam complex among most Gram-negatives and to highlight features specific to the enterics and their close relatives where appropriate. For those readers with a specific interest in the Bam complex of *Salmonella*, we note that homologs of all of the Bam complex members identified in *E. coli* can be found in the completed genomes of *S. typhimurium*, *S. enterica*, and *S. proteamaculans* isolates.

As noted above, depletion of BamA results in the accumulation of unfolded OMPs in the periplasmic compartment, a strong induction of the σ^E extracytoplasmic stress response, and eventually cell death (44, 214, 216). BamD is the only other essential component of the Bam complex (119, 136), and depletion of this lipoprotein results in a phenotype identical to that of BamA depletion in *E. coli* (119). Loss-of-function mutations in the genes encoding the remaining Bam complex partners demonstrate that they are not required for OMP assembly nor are they necessary for cell viability. However, loss of even these non-essential Bam complex members results in lower OMP levels and an induction of the σ^E stress response to varying degrees (119, 136, 174, 216). The resulting disruption in OM composition leads to an increase in OM permeability to a number of toxic small molecules such as bile salts and a range of antibiotics, demonstrating the physiological contributions of the accessory OM lipoproteins to a functioning Bam complex.

Though the precise function of the Bam OM lipoproteins is unknown, it is clear that their interactions with BamA can be divided into two categories; those between BamA-BamB, and those that exist between BamA and BamC-D-E (see Figs. 1 and 2).

BamB—The 42 kDa BamB OM lipoprotein directly interacts with the BamA protein independently of the other Bam complex members (101, 119, 174). Residues necessary for BamB's interaction with BamA have been identified in both proteins. For example, residues near the β -bulge of BamA's P3 domain likely form direct interactions with the BamB protein, although there are additional direct or indirect requirements mediated by P2, P3, P4, and P5 (101). Mutagenesis of the BamB protein have identified residues that are necessary for function and that strongly affect the BamA-BamB interaction (57, 206), as well as residues that are required for BamB structural stability (206).

E. coli and *Salmonella enterica* serovar Enteritidis lacking BamB have a moderate increase in the σ^E -stress response and significantly reduced levels of most major OMPs (26, 50, 136, 216), with the notable exception of TolC in *E. coli* which actually increases in abundance in the absence of BamB (26). The reason TolC levels increase in the absence of BamB is unclear, but may be due to the unique oligomeric β -barrel structure of TolC that differentiates it from the three-dimensional structure of most other OMPs of *E. coli*, thus allowing it to compete more effectively for Bam complexes under certain circumstances (26). Another possibility is that unlike the major OMPs, there may not be a σ^E -inducible sRNA that reduces TolC levels under OMP assembly stress.

Other phenotypes that have been associated with a lack of BamB in *E. coli* are a hypersensitivity to antibiotics such as vancomycin, bacitracin, rifampicin, and novobiocin (136, 160), an elevation of LPS levels (26), a loss of swarming motility (79), and surprisingly, impairment in homologous DNA recombination (99). A *bamB* deletion also affects the pathogenesis of the adherent-invasive *E. coli* strain LF82 (157) and *S. Enteritidis* (4, 50). These pathogenic strains exhibit a reduction in Type III secretion (T3S) and flagellar proteins and a consequent decrease in motility and invasion of intestinal epithelial cells (4, 157). At least in *S. Enteritidis*, loss of BamB inhibits the expression of genes encoding the central regulators of TSS and flagellar genes in this organism via an unknown mechanism (50).

BamB is predicted to belong to the pyrrolo-quinoline quinone (PQQ) enzyme repeat family (<http://pfam.sanger.ac.uk>), part of a larger group of proteins that contain multiple repeats of a β -propeller fold implicated in protein-protein interactions (54). PQQ is a prosthetic group utilized by some bacterial dehydrogenases (121). In these quinoproteins, PQQ binds to the center of a disc-like structure created by the β -propeller folds and is a co-factor in oxidation-reduction reactions (54). Although BamB is capable of physically binding to PQQ (99), the physiological relevance of BamB's classification in this family of enzymes is unclear, as *E. coli* cannot normally synthesize the PQQ molecule (120), BamB lacks a PQQ active site residue (57), and PQQ is clearly not necessary for BamB's OMP assembly function. Perhaps more interesting in the context of Bam complex interactions are the eight predicted β -propeller folds of BamB (57), as these domains have been suggested to influence contacts with other proteins through surface-exposed main-chain hydrogen bonds between β -strands (87). As noted above, BamA crystal structures agree that the β -bulge near the P3 domain can template β -strands (58, 101), and site-directed mutagenesis of the β -bulge disrupts the BamA-BamB interaction (101). Therefore, it is possible that the same surface of P3 templates both BamB and incoming peptides in a competitive or synchronous manner.

BamC-BamD-BamE—The remaining OM lipoproteins of the Bam complex, BamC, -D, and-E, have been demonstrated to form a distinct set of contacts from the BamA-BamB interaction; i.e., loss of BamB does not affect BamA-BamCDE contacts and vice-versa. The C-terminal portion of BamD is necessary but not sufficient for strong, direct contacts between BamA and the BamCDE subset of proteins (119, 174), and the site of this interaction most likely occurs between BamD and the P5 domain of BamA (101). BamC and BamE also provide stabilization of the Bam complex, although to a lesser extent than BamD (174). The contribution of each of the subcomplex members to OMP assembly and OM impermeability follows a similar pattern, with BamD being the most important, followed by BamE, and finally BamC (51, 119, 136, 174).

BamD of *E. coli* is a 28 kDa protein that belongs to the ComL family of proteins, named after its homolog in *Neisseria* that was found to influence competence, or the natural uptake of DNA (55). Like BamD in *E. coli*, ComL is an essential member of the Bam complex in *Neisseria* (55, 204). Although ComL is covalently attached to the peptidoglycan in *N. gonorrhoeae*, this is not the case in *E. coli* (55; Malinverni, unpublished observations). Although deletion of *bamD* leads to severe growth defects in *S. Enteritidis*, it has been reported to not be essential for viability in that organism (51).

BamD is predicted to contain 5 or 6 tetratricopeptide repeats (TPR) (57), a ubiquitous fold that is often associated with protein-protein interactions in multi-component complexes (16). Although the structure of BamD is unknown, multiple TPR domains typically form a right-handed super-helical structure creating a shallow groove that could accommodate an α -helix of a substrate or partner protein (16). The C-terminal portion of BamD has been shown to be important for interactions between the BamCDE proteins and BamA (174).

BamC and BamE are the least conserved of the Bam complex accessory proteins (57; Table 1). *E. coli* cells lacking *bamC* have only a modest increase in OM permeability to rifampicin (136) and a mild increase in σ^E activity (119, 136), but this protein clearly plays a role in OMP assembly as deletion of *bamC* exacerbates phenotypes resulting from loss-of-function mutations in other *bam* genes (51, 119, 174, 216). Loss of BamC moderately destabilizes BamE interactions with the remainder of the Bam complex (174), and it may be this altered interaction that accounts for the mild *bamC* phenotypes.

Deletion of *bamE* in *E. coli* or in *S. Enteritidis* results in a more severe defect in OM permeability and lower OMP levels than does loss of *bamC* (51, 174), and homologs of *bamE* have been also been shown to affect OM integrity in *Pseudomonas aeruginosa* (135) and *N. meningitidis* (204).

The structure of the BamE homolog in *P. aeruginosa*, OmlA, has been solved (202) and is comprised of two antiparallel α -helices packed against a β -sheet comprised of three antiparallel strands. The overall fold is reminiscent of the POTRA domain architecture (209), but differs somewhat in that OmlA has an $\alpha\alpha\beta\beta\beta$ topology (202) rather than the $\beta\alpha\alpha\beta\beta$ fold seen in the POTRA structures (28, 58, 101, 108). Nevertheless, BamE greatly stabilizes the BamA-BamCDE subcomplex (174) and it could be that the BamE fold is

required for these BamA-related protein-protein contacts rather than with interactions with incoming OMP substrates.

THE PERIPLASMIC OMP CHAPERONES

The periplasm is filled with soluble chaperone proteins which function to shield the hydrophobic residues of transient proteins such as OMPs and pilin subunits. If left unguarded, these proteins are prone to misfolding in the aqueous compartment due to their exposed hydrophobic residues. Periplasmic stress response pathways like the aforementioned σ^E and Cpx pathways (see 1, 163 for reviews) sense the inappropriate accumulation of transient proteins in the periplasm and regulate the production of cognate periplasmic chaperones that prevent aggregation of the offending substrate. Some chaperone proteins recognize and bind to a narrow range of substrates, while others are less restrictive in nature and can complex with a wide array of proteins.

Although many periplasmic chaperones have been identified, we focus on the main chaperones implicated in the OMP assembly pathway, SurA, Skp, and DegP. All three of the genes encoding these proteins are up-regulated in part by the σ^E pathway (34, 71, 117, 130, 153), and *degP* and *skp* are additionally regulated by the Cpx pathway (33, 34), indicating that they are part of a network required to maintain the envelope under stress conditions. SurA, Skp, and DegP have all been found to associate with various unfolded OMPs in the periplasm (see refs. below). The extent to which these factors play a passive holding function versus an active role in the folding, targeting, and assembly of OMPs is unclear, and we leave it to the next section to speculate how these proteins could influence Bam-mediated OMP biogenesis.

SurA

The periplasmic protein SurA demonstrates chaperone activity in vitro (11) and facilitates the maturation and/or promotes the efficient production of several OMPs in vivo (51, 115, 126, 136, 155, 158, 174, 201, 203). Loss of SurA results in the accumulation of aberrant OMPs in the periplasm, a concomitant increase in the σ^E -stress response and OM permeability defects (11, 115, 126, 136, 158), an increase in the Cpx stress response (61), and a reduced ability to survive during stationary phase (198).

SurA contains four domains; a relatively large N-terminal domain followed by two parvulin-like domains (P1 and P2) (149, 159) and a short C-terminal domain (11, 14). The first crystal structure of SurA revealed that these four domains constitute an asymmetric bipartite structure, with the N, P1, and C segments comprising a core module that connects to the smaller P2 domain by a polypeptide linker region (14). The core module exhibits an extended crevice and could form a binding pocket for target molecules based on crystal packing interactions (14, 219).

The reduction of OMPs associated with cells lacking SurA was originally ascribed to a loss of SurA folding function in the periplasm (115) as the parvulin domains exhibit modest PPIase activity in vitro (11, 126, 158), a reaction that catalyzes the *cis/trans* isomerization of the peptidyl-prolyl bonds (63). However, P1 and P2 seem to play at best a minor role in

most OMP folding pathways given that the N- and C-terminal domains are necessary and sufficient for SurA's binding and chaperone activities and for preventing the OM defects associated with the loss of SurA function (11). In addition, the N-terminal fragment of SurA alone can bind to peptides devoid of prolines (70, 213), further calling into question the importance of SurA's proposed PPIase function.

Whether or not SurA exhibits physiologically relevant foldase activity in addition to its chaperone activity has yet to be demonstrated directly in vivo. Even if SurA does not actively fold OMP substrates, it could be that SurA binding to unfolded or partially folded OMPs confers a "holdase" function that is sufficient to provide periplasmic OMP intermediates with temporary shelter from aggregation until the opportunity arises for the protein to undergo iterative OMP assembly attempts via the Bam complex. In addition, SurA-mediated protection of unfolded OMPs could prevent the recognition of their C-terminal sequences by the anti- σ^E complex at the IM and block unnecessary activation of the σ^E pathway, albeit shielding of these residues would likely be indirect (15, 219).

SurA preferentially binds to porins in vivo (11) and to unfolded OMP peptides over more water-soluble peptides in vitro (13). Moreover, experiments utilizing various peptide libraries demonstrate that SurA substrates display sequential patterns and biased orientation of aromatic residues, similar to those typically found in integral OM β -strands (13, 15, 70). However, the requirements for high-affinity SurA substrate recognition are likely to be more complex than the initial consensus sequences revealed. An additional SurA binding study against a randomized array of slightly longer peptides than those used in initial screens has implicated a role for the P1 domain in substrate selectivity (219), despite the fact that P1 is not required for general chaperone activity of model OMPs in vivo (11). In this study, the P1 domain bound with high-affinity to non-consensus α -helical peptides rich in aromatic residues and conferred peptide-dependent changes in SurA's core domain interactions. Together, these findings suggest that P1 may not be required for all OMP chaperone activity but may be required for high-affinity binding to specific substrates (219).

The rationale that SurA can display selectivity for certain OMP substrates over others has been corroborated in vivo (203), although the role of the P1 domain in this observed specificity remains unclear. Rapid depletion of SurA leads to a shift in the density of the OM (175), reflecting a massive decrease in the bulk levels of properly assembled OMPs (175, 203). Despite this reduction in overall OMP mass, SurA affects the abundance of only a small subset of OMPs, albeit the majority of these proteins represent the major OMPs of *E. coli*, including for example OmpA, OmpF, and LamB (203). In most cases, part of the observed reduction of bulk OMP levels in cells lacking SurA is due to an increase in σ^E -dependent sRNAs that down-regulate OMP transcripts. Two exceptions to this are LptD and FhuA, OMPs that show an increase or no reduction in their associated mRNA levels upon σ^E induction, respectively, but still exhibit a strong reduction in protein levels in the absence of SurA (203). Because LptD is an essential protein involved in the assembly of LPS at the surface of the OM (21, 162), it is likely that many of the OM permeability defects exhibited by strains lacking SurA are an indirect reflection of lower LptD levels (203).

The SurA protein directly interacts with BamA, although this interaction is likely to be relatively transient or weak as cross-linking is required to withstand the biochemical co-purification of a Bam-SurA super-complex (175, 206). Furthermore, disruption of the *surA* gene in combination with loss-of-function mutations with some *bam* complex alleles results in negative synthetic phenotypes (51, 136, 160, 199). Synthetic phenotypes can be used to establish pathway relationships between two encoded proteins, and is used to describe a situation in which two mutations in combination result in a different phenotype than either mutation alone. Thus, *surA*'s synthetic phenotypes with *bam* alleles, the observed OMP defects in a *surA* mutant, and the biochemical SurA-BamA interaction firmly establish SurA as an integral part of the OMP biogenesis pathway.

Skp-DegP

The fact that SurA is not an essential protein despite its major role in the OMP assembly pathway becomes less surprising given that at least two other periplasmic factors, Skp and DegP, have been found to contribute to OMP biogenesis in the absence of SurA (155, 175). In *E. coli*, Skp and DegP must both be present in cells that lack SurA for normal cellular growth, indicating that Skp and DegP can together compensate for the loss of SurA function in a parallel pathway (155, 175, 199). Nevertheless, SurA appears to be the dominant chaperone pathway for the major OMPs under normal conditions (175). Like the loss of SurA, the absence of Skp and/or DegP results in an accumulation of periplasmic OMPs (166), an induction of the σ^E stress response and lowered OMPs (27, 34, 126), and an increase in OM permeability to toxic small molecules (166). However, in contrast to SurA, there is no evidence to date to suggest that DegP or Skp can directly interact with the Bam complex (175).

Skp (for seventeen kilodalton protein (73)) associates with the periplasmic face of the IM and enhances the release of certain OMP substrates from the IM in spheroplasts (67, 166). Skp is a broad-spectrum chaperone, and has been shown to solubilize a number of OMPs during their transit through the periplasm, including the major OMPs such as OmpF, OmpC, OmpA, and LamB (27, 36, 67, 86) as well as more minor OMPs (86, 148, 207) and even some periplasmic proteins (86). In addition to Skp's affinity for OMPs, the chaperone also interacts with LPS and phospholipids in vitro (36, 62, 148). It has been suggested that Skp-lipid interactions can modulate the protein's conformation and the ability of Skp to interact with OMPs (36, 148). Indeed, Skp alone is sufficient for its chaperone function, but LPS has been shown to facilitate Skp-mediated insertion of OmpA into phospholipid bilayers in vitro (22).

Skp forms a stable homo-trimer in solution (148, 167). Crystal structures of *E. coli* Skp demonstrate that each monomer has two domains, an association domain that is responsible for trimerization and a long α -helical domain that resembles a "jellyfish tentacle" and is quite flexible (110, 211). In its trimeric configuration, the association domains come together to form a hydrophobic β -barrel like structure surrounded by short α -helices, with protruding tentacle domains to resemble a three-pronged "grasping forceps" structure (110, 211). Thus, the trimer forms a central cavity that encapsulates unfolded or partially folded OMPs of varying sizes with its flexible forceps (210) in a 1:1 trimer to substrate ratio (148).

Interestingly, a putative LPS binding site has been identified at the external face of the tentacle domains, although further study is needed to address the physiological relevance of these sites in OMP assembly (211).

Mature DegP is a 48 kDa periplasmic chaperone (118, 185, 189) that also exhibits ATP-independent serine endopeptidase activity (118, 193). At lower temperatures, DegP functions mainly as a holdase chaperone, whereas higher temperatures enhance its protease activity (185). DegP protease activity is normally essential following heat shock or other stress conditions that stimulate the accumulation of misfolded periplasmic proteins (109, 116, 188, 189); however a chaperone-competent but proteolytically inactive form of DegP, DegP(S210A) (177), can be sufficient to rescue the cell from the stress of misfolded proteins when produced at abnormally high levels (24, 124, 176, 185).

DegP, like most intracellular proteases, is multimeric. Each monomer of DegP contains an N-terminal domain required for chaperone and protease activity (185), followed by two PDZ domains - PDZ1 binds unfolded substrates via their C-terminal domains and also contributes to DegP's protease activity (83, 184, 185), while PDZ2 is required for oligomerization (83, 88, 92, 165). Several studies concerning the higher order structure of DegP have been reported and the view of its multimeric state has evolved over the years (137). DegP was initially found to form complexes of varying sizes, including trimers, hexamers, and dodecamers (109, 193). The first glimpse of DegP oligomeric structure by electron microscopy revealed a dodecameric multimer consisting of two, layered hexameric rings (100). Later, this view was revised based on a crystallographic study of DegP(S210A), which resolved a hexameric cage (DegP₆) composed of two trimeric rings stacked in a staggered conformation (112). Two additional studies (88, 113) have shown that the presence of unfolded OMP substrates can trigger the formation of massive higher-order DegP₁₂ or DegP₂₄ spherical cages built from DegP trimeric units in solution. These 12-mer and 24-mer structures are capable of substrate proteolysis and may disassociate into the DegP₆ form with reduced peptidase activity once degradation is complete (88, 113, 137). However, the situation in vivo may be more complex, as DegP trimers can also come together to form a range of bowl-like structures in the presence of lipid membranes with higher proteolytic activity and lower chaperone activity than the spherical DegP oligomers in solution (173). Furthermore, the formation of these bowl-structures is independent of substrate (173). Additional studies will be required to determine which of these DegP configurations are dominant in vivo or if the soluble and membrane-bound forms of DegP are exchanged in a regulated fashion.

MODELS OF β -BARREL TRANSPORT AND ASSEMBLY

OMP Transit Across the Periplasm

Despite many years of study, it is still unclear exactly how an unfolded, largely hydrophobic OMP makes its voyage across the aqueous periplasmic compartment and can be targeted for assembly in the OM in the absence of ATP. Two main models exist for this transit between the IM and OM bilayers (133): one that envisions the formation of an OMP-chaperone complex at the IM that is ferried across the periplasm to the Bam complex assembly site at the OM (reminiscent of the well-characterized Lol pathway, Fig. 1); while the other model

evokes a protein bridge that spans the two bilayers, possibly made up of OM chaperones that connect the Bam and Sec machineries. Such a protein bridge could form an insoluble contact point that allows for the passage of most OMPs without the risk of aggregation due to exposure to the periplasmic matrix (analogous to other protein bridge machines such as efflux pumps, the flagellar apparatus, and type three secretion systems). We refer to the former possibility as the soluble intermediate model, and the latter as the trans-envelope complex model. Neither of these models is without flaws nor can they yet be disregarded. Furthermore, these models are not necessarily mutually exclusive, as will be explained after a brief review of existing findings below.

OMP assembly can be divided into several steps based on tracking the formation and conversion of various OMP intermediates. In the case of trimeric porins, there can be at least three major intermediates that represent the precursor, unfolded mature, and folded monomer forms, referred to as p-OMP, u-OMP, f-OMP, respectively (125, 158, 201). Typically, folding intermediates are difficult to detect because the majority of OMP translocation and folding takes place extremely rapidly. However, OMP intermediates can be tracked using pulse-chase and/or immuno-detection methods, and reproducible kinetic data can be obtained under conditions where the maturation pathway is slowed, such as through the use of low temperature conditions or the use of strains that exhibit delayed assembly (201). For example, it has been demonstrated that cells that are defective in proteins such as the cytoplasmic OMP chaperone SecB (Fig. 1), or SecD, an IM protein of unknown function that associates with the SecYEG translocon (46), exhibit a delay in signal sequence processing for both periplasmic proteins and OMPs (201). Therefore, these proteins generally affect IM translocation, but not later assembly steps. On the other hand, most OMPs in cells that lack either SurA or BamB exhibit indistinguishable delays in the interconversion of u-OMP to f-OMP, indicating that these proteins could both function to assist in the delivery of substrates to BamA (201, 206), consistent with the observation that both SurA and BamB can interact with BamA (101, 174, 206, 216). Interestingly, a small fraction of OMP molecules (but not periplasmic proteins) in these mutants also display a defect in the processing of their signal sequences (accumulation of p-OMP). These molecules appear to fall off-pathway, meaning that they are not accessible to further maturation and are essentially “dead-ended” molecules (201). As the simultaneous absence of DegP and Skp lead to no such inhibition in OMP assembly, it has been hypothesized that the role of this redundant chaperone pair could be to rescue the cell from molecules that have fallen off the normal SurA-BamB delivery pathway (175).

How do these observations and others agree with the soluble intermediate and trans-envelope complex models? There is some biochemical evidence to support the existence of protein-spanning bridges that could make up a trans-envelope OMP assembly complex (10, 81, 179), a variation of the Bayer’s patch model, named after the scientist who first visualized putative contact points between the IM and OM using electron microscopy (9). Although the existence of these adhesion sites using some methods has been controversial (10, 98), this model would support a coupled relationship between protein translocation and chaperone-mediated delivery of OMPs to the Bam complex. In this context, most OMPs would be delivered in a directed manner through a channel consisting of Sec/Bam translocons through the assistance of associated chaperones, whereas misdirected,

misassembled, or overproduced OMPs would be complexed with unassociated chaperones in a soluble form in the periplasm. Although this idea is attractive in the sense that it could account for the rapid and efficient folding of most OMPs, no biochemical data exists to support a chaperone-mediated linkage between SecYEG and the OM assembly site.

On the other hand, several lines of evidence support a multi-step periplasmic intermediate model (see 133) that requires the full secretion and release of an OMP from the IM to a periplasmic chaperone prior to its delivery to an unassociated Bam assembly site. First, the artificial overproduction of OMPs results in the accumulation of two intermediates that can be readily isolated from whole cells; p-OMP and u-OMP (e.g., 53). The accumulation of p-OMPs are largely the result of cytoplasmically localized molecules awaiting translocation across the IM through limiting SecYEG translocons, while soluble u-OMPs accumulate in the periplasmic space and could represent a backlog of a transient unfolded or partially folded OMP-chaperone complexes that await subsequent delivery to limiting OM assembly sites. Proof that u-OMP molecules in overexpression systems are at least partially accessible to the periplasmic space comes from the observation that the periplasmic disulfide isomerase, DsbA, can modulate cysteine pairs of susceptible u-OMP substrates (49). Although it could be argued that the aforementioned overexpression data is inherently non-physiological, more convincing still is that radiolabeled u-OMPs expressed at native levels can be released by spheroplasts (cells that have shed a large portion of their OMs) into water-soluble media (170, 196). However, it could be argued that the process of spheroplast formation could disturb hypothetical trans-envelope interactions that may exist between the Sec and Bam machineries.

OMP Recognition and Proposed Models of OM β -barrel Insertion

Regardless of the initial stages of OMP transit across the periplasm, ultimately OMPs must reach the Bam complex in either an unfolded or partially folded form prior to assembly. We previously addressed how the POTRA domains may form complexes with β -strand peptides of periodic hydrophobicity (101), but there is evidence that the C-termini of OMPs contains additional conserved features that are required either for substrate recognition by the Bam complex or for the final maturation folded monomers. In particular, the C-terminal residues of most OMPs feature the Ar-X-Ar tripeptide motif (where Ar is an aromatic residue and X is any residue) (15, 208). Replacement or removal of these residues, particularly the last amino acid, leads to drastic defects in the ability of OMPs to fold properly (37, 84, 124, 156, 190) but they do not affect the further trimerization of folded monomers (124). Additional properties of the C-terminal sequence may contribute to OMP recognition by BamA in a species-specific manner (156).

Once an OMP is recognized, how do the later steps of OMP maturation (the formation of a β -sheet and the wrapping of the sheet into a barrel-structure) proceed? Insertion of the α -helical transmembrane domains of IM proteins likely proceeds via sequential threading and the subsequent lateral escape of the hydrophobic transmembrane regions from the SecYEG translocon into the phospholipid bilayer (46). It seems highly unlikely that OMP assembly occurs in an analogous fashion. Integral β -barrel domains such as the C-terminus of BamA are extremely stable structures. Although it is conceivable that the pore size of individual

BamA monomers could accommodate passage of one or more β -strands (perhaps via the displacement of Loop 6 (Fig. 2)), it is improbable that the hydrogen bonds that stabilize the cylindrical barrel portion of BamA could dynamically open and reclose to allow the sequential exit of substrate β -strands. Moreover, the possibility that β -sheets are synthesized within the membrane itself prior to barrel formation is highly unlikely for at least two reasons. The hydrophobic nature of membranes requires that hydrogen bonds be immediately formed between the amide groups of one transmembrane β -strand and the carbonyl groups of a neighboring strand, thus transmembrane β -strands or hairpins in isolation would not be stable structures (103). In addition, the β -sheets of OMPs have one hydrophobic surface and one hydrophilic surface (195), a geometry that is incompatible with the concept of temporal strand synthesis in a membrane environment unless the hydrophilic face was somehow shielded during the process. It is interesting to note that reconstituted BamA in lipid bilayers demonstrates alterations in pore activity in response to binding C-terminal peptides (156), but the physiological relevance of this observation to in vivo folding events remains unclear in light of the thermodynamic constraints above.

An alternative hypothesis is that OMP assembly could proceed to near completion within homo-oligomeric Bam complexes that act in concert to provide a protected, proteinaceous folding environment followed by lateral exit of the OMP between complex subunits (197). However, this possibility is also not satisfactory, at least not for OMP assembly in *E. coli*. Although purified BamA (156) and other proteins belonging to the bacterial surface antigen superfamily (142, 168, 191) have been reconstituted in vitro as oligomers, there is no evidence that the Bam complex of *E. coli* forms homo-oligomeric structures in vivo (101, 187, 216).

Although the community awaits additional evidence to clarify the mechanisms behind OMP folding in vivo, reconstitution experiments in the presence of lipid bilayers (106, 107, 143) and in lipid vesicles (for example, 192) could reveal valuable insight into this process. These experiments demonstrate that OMP assembly can occur in a spontaneous fashion in the absence of additional proteins, albeit folding in lipid bilayers is vastly slower than the assembly rates observed in biological membranes (195). The folding pathways observed in the artificial membrane system suggests that OMP folding and β -barrel insertion is a concerted process (105). In this model (reviewed in 195), an unfolded, water soluble OMP is converted to a membrane-associated “molten disk” with some secondary β -hairpin structure. The β -hairpins of this disk are then simultaneously driven upwards into the membrane by interactions with the lipid interface to assume a partial tertiary structure termed a “molten globule”, with hydrophilic residues clustered within the structure and hydrophobic residues directed outwards towards the membrane bilayer. Finally, the β -hairpins complete their transmembrane migration to expose outer loops and stable hydrogen bonds can form between the hairpins. It is not unreasonable to propose that the Bam complex and chaperone proteins near the protein-lipid interface may catalyze similar events in vivo (104, 143, 195).

RECEPTOR FUNCTIONS OF BAMA IN *E. COLI*

BamA can be used to potentiate the recruitment of exogenously synthesized pathogenic molecules (6, 180). Although phages and toxic molecules have been known to utilize many

different pore-forming OMPs as portals into the cell, the characterization of factors that employ BamA as a receptor has likely been hampered due to the essential nature of the BamA protein. Despite this obstacle, BamA has recently been demonstrated to serve as a receptor for the majority of Shiga toxin-encoding (Stx) phages (180) and the TpsA protein involved in contact-dependent inhibition (CDI) among different populations of *E. coli* (6, 7, 180).

Infection of *E. coli* by Stx phages results in the transfer of genetic material that converts a commensal strain of bacteria into a pathogenic Shiga-toxin encoding *E. coli* (or STEC) strain (169). A genetic selection for *E. coli* mutants resistant to Stx infection followed by identification of the *bamA* gene as the receptor determinant in an expression library led to the discovery that some Stx phages recognize the BamA protein prior to infection, specifically the short-tailed Stx phages (180). Whole cells exposed to anti-BamA antiserum become resistant to subsequent short-tailed Stx infection, suggesting that the antibody is capable of blocking the BamA receptor residues required for phage adsorption (180).

Contact-dependent inhibition (CDI) describes a process by which strains of *E. coli* cells carrying the CdiA/B two partner secretion (TPS) pathway can reversibly inhibit the growth of other populations of *E. coli* that lack the system through the down-regulation of aerobic respiration, proton-motive force (PMF), and steady-state levels of ATP (7, 8). This mode of cellular competition may be a mechanism by which some strains of *E. coli* persist despite the use of antibiotics intended to sterilize the bacterial population. The CdiA/B+ cells, or inhibitor cells, must make physical contacts with a CdiA/B- cell, or target cell, prior to inhibition of growth (6, 7). The TpsA component of the CDI pathway, CdiA, is a large protein that is secreted from the inhibitor cell in an unfolded conformation through the channel of its cognate TpsB protein, CdiB (7). Presumably, CdiA or some fragment of the protein must bypass the OM of the target cell and ultimately reach its intracellular target to inhibit growth (6, 8).

A genetic selection to identify suppressors of the CDI phenotype in target cells identified mutations that led to the reduction in the levels or accessibility of BamA in target cells, as well as loss-of-function mutations in the IM protein AcrB (6). The ligand of BamA and AcrB is not known, but it is likely that they bind to CdiA or some fragment of CdiA, with BamA and AcrB serving as the OM receptor and IM target, respectively (6, 8). As with Stx infections (180), external blockage of target BamA receptors via anti-BamA antibodies prevents CDI infection (6). It is formally possible that BamA acts to recruit the toxic CDI molecule but another target cell component is expatriated for the importation of the molecule across the OM (as is the case reported for one class of colicins that uses one OMP as a primary receptor and a different OMP as a secondary translocon (74), however this seems unlikely given that the suppressor screens have never implicated the existence of a secondary translocation factor in CDI (6). Interestingly, CdiA is predicted to adopt a large β -helical structure typical of TpsA proteins upon secretion at the surface of the cell, and it could be that the BamA protein of target cells is co-opted to import and catalyze CdiA folding upon importation. However, BamA mutants lacking POTRA domains critical for OMP assembly are still capable of mediating the CDI phenotype, indicating that the OMP folding function of BamA and the import of CdiA are likely to be uncoupled (6).

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Figure 1. Biogenesis of OM proteins

In *E. coli*, most OM lipoproteins and OMPs are synthesized and transported to the IM secretion machinery post-translationally. SecB chaperone molecules bind to hydrophobic segments of OM proteins to prevent their aggregation in the cytoplasm. OM proteins are delivered to the SecA ATPase complexed with the IM translocation machinery, SecYEG, as well as other associated proteins of unknown function that not depicted here (see 46). OM lipoproteins and OMPs are distinguished by the composition of their signal sequences and sorted for processing by separate pathways (95, 140). *OM lipoprotein biogenesis pathway* (depicted to the left of the IM secretion machinery): The asterisk indicates a lipoprotein without an Asp amino acid at the +2 position, the main signal for sorting by the LolABCDE pathway to the OM in *E. coli* (172). *OMP biogenesis pathway*: (depicted to the right of the IM secretion machinery): After signal sequence processing, the SurA chaperone is thought to complex with the bulk of OMPs in the periplasmic space and deliver them to the OM assembly site, the Bam complex. In the absence of SurA, the Skp/DegP pathway become an indispensable substitute. Details concerning the mechanisms behind Bam-mediated OMP assembly into the OM are unknown. See text for references and further details.

Figure 2. Model of the Bam Complex

The Bam complex is composed of the integral OMP, BamA, and four OM lipoproteins, BamBCDE (174, 216). BamA consists of an essential N-terminal periplasmic region with five POTRA domains and a C-terminal β -barrel domain (101, 164, 186). The arrangement of the periplasmic domain may be dynamic, with a putative hinge region between P2 and P3 that ranges from a comparatively straight (130°) (58) vs. bent (100°) (101) conformation. The relative orientation of P5 (shaded in white) with respect to P1-P4 is not represented in the existing crystal structures, but is portrayed in a sharply kinked conformation in agreement with SAX data (see text for details) (108). The C-terminal portion of BamA is predicted to be a β -barrel with several extra-cellular loops. Extra-cellular loop 6 (L6) is particularly interesting in that portions of L6 are highly conserved and are predicted to interact with other conserved features of the barrel (85, 129). Biochemical and structural data from a BamA homolog, FhaC, implies that L6 may be a dynamic loop capable of extending into the barrel and reaching the periplasmic space (28, 64, 85).

Table 1

gram-negative phyla containing Bam complex and periplasmic chaperone proteins

	BamA	BamB*	BamC	BamD	BamE	SurA [‡]	Skp	DegP
COG	4775	1520	3317	4105	2913	0760	2825	0265
<i>Proteobacteria</i>								
α-proteobacteria	+	+	-	+	+	+	+	+
β-proteobacteria	+	+	+	+	+	+	+	+
γ-proteobacteria	+	+	+	+	+	+	+	+
δ-proteobacteria	+	+	-	+	-	+	+	+
ε-proteobacteria	+	-	-	+	-	+	-	+
<i>Bacteroidetes</i>	+	+	-	+	-	+	+	+
<i>Chlorobi</i>	+	-	-	+	-	+	+	+
<i>Aquificae</i>	+	-	-	+	-	+	+	+
<i>Deinococcus</i>	+	+	-	-	-	+	+	+
<i>Spirochaetes</i>	+	-	-	-	-	+	+	+
<i>Thermotogae</i>	+	+	-	-	-	+	-	+
<i>Cyanobacteria</i>	+	-	-	-	-	+	-	+
<i>Chlamydiae</i>	+	-	-	-	-	-	+	+
<i>Chloroflexi</i>	-	+	-	-	-	+	-	+

* Sequences recognized as COG1520 may include 8-bladed repeats that do not have BamB function

[‡] Sequences recognized as COG0760 include all PpiC-type peptidyl-prolyl cis-trans isomerases