

## The bacterial outer membrane $\beta$ -barrel assembly machinery

Kelly H. Kim, Suraaj Aulakh, and Mark Paetzel\*

Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Received 26 January 2012; Accepted 20 March 2012

DOI: 10.1002/pro.2069

Published online 30 March 2012 proteinscience.org

**Abstract:**  $\beta$ -Barrel proteins found in the outer membrane of Gram-negative bacteria serve a variety of cellular functions. Proper folding and assembly of these proteins are essential for the viability of bacteria and can also play an important role in virulence. The  $\beta$ -barrel assembly machinery (BAM) complex, which is responsible for the proper assembly of  $\beta$ -barrels into the outer membrane of Gram-negative bacteria, has been the focus of many recent studies. This review summarizes the significant progress that has been made toward understanding the structure and function of the bacterial BAM complex.

**Keywords:** outer membrane proteins;  $\beta$ -barrel assembly machinery; outer membrane; protein folding;  $\beta$ -barrels

### Introduction

In Gram-negative bacteria, proteins can be divided into different categories according to their subcellular location. These are cytosolic proteins, secreted proteins, integral membrane proteins (found either in the inner or the outer membrane), periplasmic proteins (soluble proteins in the periplasm, a peptidoglycan-rich space between the inner and the outer membranes), peripheral membrane proteins that are temporarily associated with lipid bilayer, and finally lipoproteins, which are soluble lipid-anchored proteins associated with the inner or outer membrane.<sup>1,2</sup>

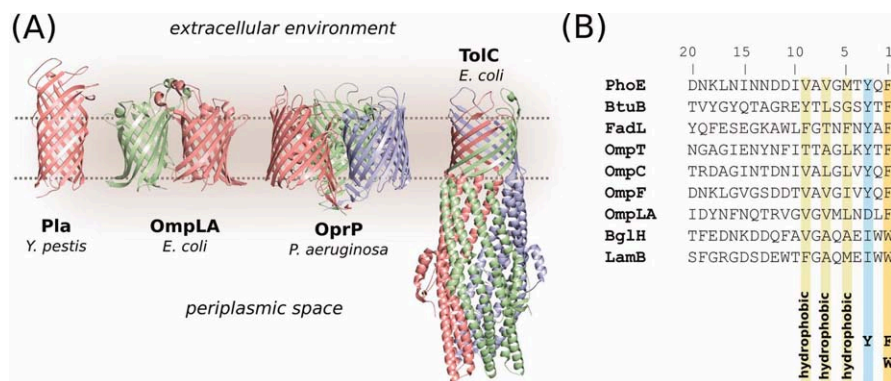
*Abbreviations:* BAM, beta-barrel assembly machinery; IM, inner membrane; OM, outer membrane; OMP, outer membrane protein; POTRA, polypeptide transport associated; SAM, sorting and assembly machinery; TOC, translocon at the outer envelope of chloroplasts; TPR, tetratricopeptide repeat.

\*Correspondence to: Mark Paetzel, Department of Molecular Biology and Biochemistry, Simon Fraser University, South Science Building 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6. E-mail: mpaetzel@sfu.ca.

Grant sponsors: Canadian Institutes of Health Research (M. P.), National Science and Engineering Research Council of Canada (M. P.), Michael Smith Foundation for Health Research (M. P.).

Biogenesis of all prokaryotic proteins begins in the cytosol where they are first synthesized, but all noncytosolic proteins use at least one or more translocation systems to reach their final destinations. Depending on the protein, the folding of secreted proteins and assembly of membrane proteins can take place before, during, or after the translocation process. In Gram-negative bacteria, one of the least understood processes of membrane protein assembly is that of outer membrane proteins (OMPs), which are predominantly  $\beta$ -barrels.<sup>3</sup> A significant breakthrough came, however, with the identification of a protein complex that catalyzes the coordinated events of OMP membrane insertion and folding. This protein complex is known as the  $\beta$ -barrel assembly machinery (BAM) complex.<sup>3–6</sup>

Since its discovery, the BAM complex has attracted much attention from the research community mainly for its potential to serve as a novel antibiotic target, as well as the expectation that it will advance our understanding of  $\beta$ -barrel membrane protein biogenesis. The fact that systems homologous to the BAM complex exist in mitochondrial and chloroplastic outer membranes of eukaryotes has



**Figure 1.** Outer membrane proteins (OMPs) are found predominantly as  $\beta$ -barrels. A: Some examples of OMPs from different Gram-negative bacteria are shown. OMPs can be found either as a monomer (e.g., Pla from *Yersinia pestis*; PDB: 2X55), an oligomer where each subunit creates its own  $\beta$ -barrel (e.g., OmpLA from *Escherichia coli* and OprP from *Pseudomonas aeruginosa*; PDB: 1QD6 and 2O4V), or an oligomer where the multiple subunits come together to form one  $\beta$ -barrel (e.g., TolC from *E. coli*; PDB: 1EK9). B: OMPs share a conserved sequence of hydrophobic and aromatic residues at their C-terminus. A sequence alignment of the last 20 residues of *E. coli* OMPs PhoE (UniProt ID: P02932), BtuB (P06129), FadL (P10384), OmpT (P09169), OmpC (P06996), OmpF (P02931), OmpLA (P0A921), BglH (P26218), and LamB (B7UPJ7) is shown. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

further fueled the research effort.<sup>7,8</sup> Here, we summarize the great advances that have been made in both the structural and functional understanding of the bacterial BAM complex.

### Bacterial Outer Membrane Proteins

The outer membranes of Gram-negative bacteria, mitochondria, and chloroplasts all contain transmembrane  $\beta$ -barrel proteins known as OMPs as previously described (Fig. 1).<sup>3</sup> Typically, OMPs consist of an even number of  $\beta$ -strands, ranging from 8 to 24, that are arranged in an antiparallel fashion.<sup>3,9</sup> Depending on the protein, OMPs may or may not have soluble domains extending away from the transmembrane  $\beta$ -barrel domain. The  $\beta$ -barrel domains are formed by either a single chain of amino acids or multiple chains assembled into one large  $\beta$ -barrel [Fig. 1(A)]. For example, the transmembrane domain of the *Escherichia coli* multidrug efflux pump TolC is formed by three monomers that contribute four  $\beta$ -strands each to make a 12-stranded  $\beta$ -barrel.<sup>10</sup> In other OMPs, oligomerization of  $\beta$ -barrels may be required for function, such as in the case of the *E. coli* phospholipase OmpLA that is only functional upon dimerization.<sup>11</sup>

While most OMPs share common  $\beta$ -barrel architecture, they are functionally very diverse. Based on their function, OMPs can fall into one of these six categories: nonspecific porins, substrate specific channels, translocons for substrate export, autotransporters, enzymes, and structural OMPs. Nonspecific porins allow passive diffusion of a variety of small hydrophilic molecules.<sup>12,13</sup> On the other hand, substrate-specific channels allow the transport of specific molecules, either through passive diffusion or active transport (the latter which involves cooperation with the TonB complex at the inner mem-

brane).<sup>13–15</sup> Translocons include OMPs that are involved in the export of proteins, drugs, and other molecules, with examples including TolC as well as members of the two-partner secretion system.<sup>16,17</sup> Autotransporters cover the category of OMPs that have a transporter domain embedded in the outer membrane that secretes a passenger domain found on the same polypeptide; this secreted protein is usually a virulence factor, and thus, autotransporters are commonly found in pathogenic strains of Gram-negative bacteria.<sup>18</sup> Enzymatic OMPs discovered to date have functions that include proteases and phospholipases.<sup>19</sup> The final category includes OMPs that contribute to the formation of the cell wall, and thus play a structural role. These structural OMPs include proteins involved in peptidoglycan formation, OMP assembly, as well as usher proteins that transport and polymerize pili subunits required at the bacterium's exterior.<sup>15</sup> Essentially, OMPs assist the outer membrane to fulfill its role as a protective physical barrier that regulates the traffic of molecules across the lipid bilayer. Furthermore, some OMPs play direct roles in virulence, extending the importance of studying bacterial OMPs and their biogenesis to medical interest.

### Bacterial OMP Biogenesis

Bacterial OMP biogenesis begins in the cytosol where OMPs are synthesized with a cleavable N-terminal signal sequence that targets them to the inner membrane.<sup>20–22</sup> Once there, the OMPs are translocated across the membrane via the Sec translocation system into the periplasm [Fig. 2(A)].<sup>1,20</sup> Removal of the N-terminal signal sequence by signal peptidase I (SPase I) releases the OMPs from the inner membrane.<sup>1,23,24</sup> The released OMPs are then transported across the periplasmic space to the outer membrane via either the SurA pathway or the Skp/DegP

pathway.<sup>25–27</sup> SurA, Skp, and DegP are periplasmic chaperones that keep the proteins in a protected unfolded state to prevent misfolding and aggregation.<sup>28,29</sup> Studies have shown that the two pathways function in parallel and that cells are viable when either one of the pathways are missing.<sup>25,26</sup> It has been suggested that the SurA pathway plays a more important role under normal conditions and that the role of the Skp/DegP pathway becomes more important when the cells are under stress.<sup>26</sup> Regardless of the pathway taken, the journey of all OMPs ends at the outer membrane, their final destination.

Once at the outer membrane, protein folding and membrane insertion take place in concert to complete the OMP maturation. *In vitro* studies show that

OMPs are able to fold and insert themselves spontaneously into synthetic phospholipid bilayer membranes without help from any proteinaceous machinery.<sup>30–32</sup> This suggests that OMP folding does not require an external energy source and that the information for folding is encoded in their amino acid sequence. However, the folding occurs too slowly *in vitro* to be biologically relevant, and hence, *in vivo* OMP folding and membrane insertion require proteinaceous machinery known as the BAM complex to increase the kinetics of the whole process.<sup>32</sup> Absence of the BAM complex results in misfolded OMPs aggregating in the periplasm, eventually leading to cell death.<sup>33,34</sup> The OMP substrates are recognized by the BAM complex via their C-terminal targeting signal that has a consensus sequence of X-Z-X-Z-X-Z-Tyr-Z-Phe/Trp, where X is hydrophobic and Z is any amino acid [Fig. 1(B)].<sup>35–38</sup> The exact consensus sequence varies slightly from one bacterial species to another, and the BAM complex of one species is only capable of recognizing the OMPs from the same species.<sup>36</sup> OMPs, along with lipoproteins, account for ~50% of the outer membrane mass.<sup>39</sup> Table I lists the *E. coli* OMP substrates that have been shown to require the BAM complex for

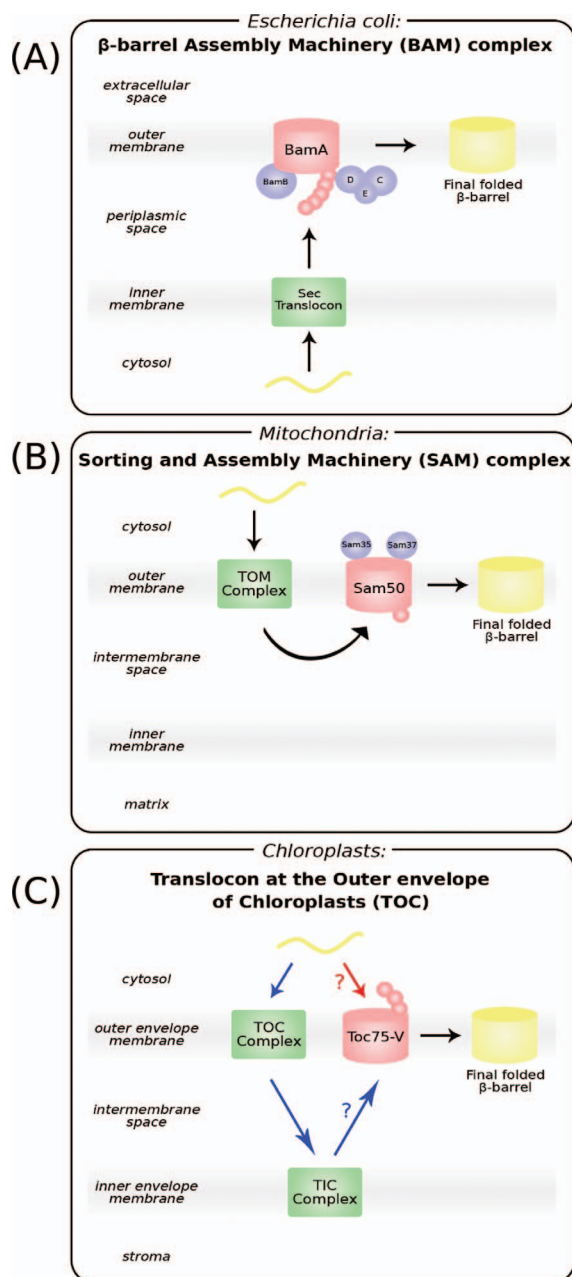


Figure 2.

**Figure 2.** The *E. coli* BAM complex and homologous systems. In both Gram-negative bacteria and eukaryotes, outer membrane  $\beta$ -barrel proteins are first synthesized in the cytosol of the cell and then targeted to either the inner membrane (bacteria) or the proper organelle (mitochondria or chloroplasts). This figure compares the three pathways as the unfolded substrate protein (yellow curve) is directed by associated translocons (green) to the assembly complex consisting of the core BamA homologue (pink) and accessory proteins (purple), to form the final folded  $\beta$ -barrel (yellow cylinder). For simplicity, other proteins and chaperones involved in the pathways are not shown. A: The *E. coli*  $\beta$ -barrel assembly machinery (BAM) complex consists of membrane embedded BamA, and four accessory lipoproteins: BamB, C, D, and E. Substrate proteins cross the inner membrane via the Sec translocase and travel through the periplasmic space before being assembled by the BAM complex at the outer membrane. B: In the mitochondrial system, the substrate proteins enter via the translocase of outer mitochondrial membrane (TOM) and are assembled by the sorting and assembly machinery (SAM) complex. The BamA homologue is Sam50, which works together with cytosolic proteins Sam35 and Sam37 for insertion of OMPs into the outer mitochondrial membrane. C: In chloroplasts, the translocons at the outer and inner envelopes of chloroplasts (TOC/TIC complexes) are believed to be involved in assembly of OMPs. The BamA homologue is Toc75-V, with accessory proteins yet to be identified. It is unclear if the substrate proteins travel to the stroma before being assembled (blue arrows) or if they are directly assembled into the outer envelope membrane from the cytosol (red arrow). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

**Table I.** *E. coli* OMP Substrates Used in the BAM Complex Studies<sup>a</sup>

| Substrate | UniProt ID | No. of $\beta$ -strands | MW (kDa) | Oligomeric state               | Function           | PDB ID   | Reference |
|-----------|------------|-------------------------|----------|--------------------------------|--------------------|--|-----------|
| OmpA      | P0A910     | 8                       | 37.2     | Dimer                          | Porin and receptor | 1BXW, 1G90, 1QJP, 2GE4, 2JMM   | 40        |
| OmpT      | P09169     | 10                      | 35.6     | Possible pentamer <sup>b</sup> | Protease           | 1I78   | 41        |
| Ag43      | P39180     | 12 <sup>c</sup>         | 106.8    | Monomer <sup>d</sup>           | Autotransporter    | (Unsolved)   | 42        |
| AIDA-I    | Q03155     | 12 <sup>c</sup>         | 132.3    | Monomer <sup>d</sup>           | Autotransporter    | (Unsolved)   | 43        |
| Hbp       | O88093     | 12                      | 148.3    | Monomer <sup>d</sup>           | Autotransporter    | 1WXR, 3AEH, 3AK5   | 44        |
| Intimin   | P43261     | 12 <sup>c</sup>         | 101.8    | Dimer                          | Autotransporter    | ( $\beta$ -Barrel unsolved)  | 45        |
| Pet       | O68900     | 12 <sup>c</sup>         | 139.8    | Monomer <sup>d</sup>           | Autotransporter    | (Unsolved)   | 42        |
| TolC      | P02930     | 12                      | 53.7     | Trimer                         | Transporter        | 1EK9, 1TQQ, 2VDD, 2VDE, 2WMZ, 2XMN   | 40        |
| OmpC      | P06996     | 16                      | 40.4     | Trimer                         | Porin              | 2J1N, 2J4U, 2XE1, 2XE2, 2XE3, 2XE5, 2XG6   | 40        |
| OmpF      | P02931     | 16                      | 39.3     | Trimer                         | Porin              | 1BT9, 1GFM, 1GFN, 1GFO, 1GFP, 1Gfq, 1HXT, 1HXU, 1HXX, 1MPF, 1OPF, 2OMF, 2ZFG, 2ZLD, 3FYX, 3HW9, 3HWB, 3K19, 3K1B, 3O0E | 40        |
| PhoE      | P02932     | 16                      | 38.9     | Trimer                         | Porin              | 1PHO   | 36        |
| LamB      | P02943     | 18                      | 49.9     | Trimer                         | Porin              | 1AF6, 1MAL, 1MPM, 1MPN, 1MPO, 1MPQ   | 40        |
| FimD      | P30130     | 24                      | 96.5     | Dimer                          | Transporter        | 1ZDV, 1ZDX, 1ZE3, 3BWU, 3OHN, 3RFZ   | 46        |

<sup>a</sup> This table only lists *E. coli* OMPs that have been shown to require the BAM complex for correct assembly. Although not listed here, it should be noted that OMPs from other species of Gram-negative bacteria such as PilQ and PorA from *N. meningitidis* have been used in the functional studies of the BAM complex.<sup>4</sup>

<sup>b</sup> Gel filtration analysis of OmpT suggests possible pentamer formation.<sup>47</sup>

<sup>c</sup> Based on currently available structures, most autotransporters are predicted to have 12-stranded  $\beta$ -barrels.

<sup>d</sup> These proteins have been classified as monomeric proteins.<sup>48</sup>

proper assembly into the outer membrane. Other *E. coli*  $\beta$ -barrel OMPs not listed in Table I are also likely to require the BAM complex. It should be noted that some OMPs have been shown to assemble independently of the BAM complex. These proteins include Wza that is unique in that it spans the outer membrane by forming  $\alpha$ -helices<sup>49</sup> and PulD, which lacks the conserved C-terminal aromatic residue.<sup>50</sup>

### BAM Complex: The $\beta$ -Barrel Assembly Machinery

The first discovered component of the BAM complex is the only integral membrane protein in the complex, known as BamA.<sup>4</sup> It was first identified in *Neisseria meningitidis*, but its homologues are found in all Gram-negative bacteria, as well as in the mitochondria and chloroplasts of eukaryotes.<sup>51,52</sup> Later on, it was discovered that BamA associates with various numbers of accessory proteins to form a larger complex known as the BAM complex in Gram-negative bacteria,<sup>53,54</sup> sorting and assembly machinery (SAM) complex in mitochondria,<sup>33,51</sup> and translocon at the outer envelope of chloroplasts (TOC) complex in chloroplasts<sup>55</sup> (Fig. 2).

In the *E. coli* BAM complex, BamA is associated with four lipoproteins: BamB, BamC, BamD, and BamE [Fig. 2(A) and Table II].<sup>53,54</sup> Unlike BamA that spans the outer membrane with its  $\beta$ -barrel domain,

the lipoproteins (BamB/C/D/E) are anchored to the inner leaflet of the outer membrane via lipidation of the invariant cysteine residue at their mature N-termini (once processed by SPase II).<sup>75</sup> Mutagenesis and co-immunoprecipitation studies suggest that BamA associates with BamB independently from BamC/D/E,<sup>40,56</sup> but the exact oligomeric state of the BAM complex is as yet unclear. Although size exclusion chromatography and native gel analysis show that BamA may form a tetramer,<sup>36</sup> a functional unit of the BAM complex reconstituted in proteoliposomes *in vitro* was shown to have a BamA:B:C:D:E ratio of 1:1:1:1:(or 2).<sup>41,76</sup> In any case, the BAM complex is most efficient when all five components are present.<sup>41,56</sup> Although cells cannot tolerate genetic deletion of BamA or BamD, lack of BamB, BamC, or BamE leads to nonlethal yet noticeable defects in OMP assembly.<sup>54,77-79</sup>

In the mitochondrial system, the OMPs destined for the outer mitochondrial membrane are imported via the translocase of outer mitochondrial membrane and enter the intermembrane space, where they are transported to the outer membrane for assembly by the SAM complex [Fig. 2(B)]. The primary component of this complex is Sam50 (the BamA homologue) which contains only one polypeptide transport associated (POTRA) domain facing the intermembrane space. Interestingly, Sam50 works together

**Table II.** Components of the *E. coli* BAM Complex

|                  | Protein name | Length (aa) | MW (kDa) | UniProt ID | Structure   |  |  |             |            |
|------------------|--------------|-------------|----------|------------|---|--|--|-------------|------------|
|                  |              |             |          |            | PDB ID  | Description  | Resolution   | Space group | References |
| Membrane protein | BamA         | 810         | 90.6     | P0A940     | 2QDF  | POTRA 1-4 ( <i>E. coli</i> )   | 2.20   | P 21 21 2   | 48         |
|                  |              |             |          |            | 2QCZ  | POTRA 1-4 ( <i>E. coli</i> )   | 2.70   | P 21 21 2   | 48         |
|                  |              |             |          |            | 3EFC  | POTRA 1-4 ( <i>E. coli</i> )   | 3.30   | P 31 2 1    | 56         |
|                  |              |             |          |            | 3OG5  | POTRA 4-5 ( <i>E. coli</i> )   | 2.69   | P 32 2 1    | 57         |
|                  |              |             |          |            | 3Q6B  | POTRA 4-5 ( <i>E. coli</i> )   | 1.50   | C 2         | 58         |
|                  |              |             |          |            | 2V9H  | POTRA 1-2 ( <i>E. coli</i> )   | NMR structure                                      |             | 59         |
|                  |              |             |          |            | 3MC8  | POTRA 1-3 ( <i>Anabaena sp.</i> )                                      | 2.59   | P 43 21 2   | 60         |
|                  |              |             |          |            | 3MC9  | POTRA 1-3 ( <i>Anabaena sp.</i> )                                      | 2.20   | P 63        | 60         |
|                  |              |             |          |            | 2X8X  | POTRA 1-3 ( <i>Thermosynechococcus elongates</i> )                     | 1.97   | I 4 3 2     | 61         |
|                  |              |             |          |            |   | 2QDZ   | FhaC (homologue from <i>Bordetella pertussis</i> ) | 3.15        | C 2 2 21   |
| Lipoproteins     | BamB         | 391         | 41.7     | P77774     | 2YH3  | <i>E. coli</i>   | 2.60   | P 43 21 2   | 63         |
|                  |              |             |          |            | 3PRW  | <i>E. coli</i>   | 1.80   | I 2 2 2     | 64         |
|                  |              |             |          |            | 3PIL  | <i>E. coli</i>   | 2.60   | P43 21 2    | 65         |
|                  |              |             |          |            | 3Q7M  | <i>E. coli</i>   | 1.65   | I 2 2 2     | 66         |
|                  |              |             |          |            | 3Q7N  | <i>E. coli</i>   | 1.77   | P 21 21 21  | 66         |
|                  |              |             |          |            | 3Q7Q  | <i>E. coli</i>   | 2.09   | P 21 3      | 66         |
|                  |              |             |          |            |   |  |  |             |            |
|                  | BamC         | 344         | 36.8     | P0A903     | 2YH5  | C-terminal domain  | 1.25   | P 21        | 63         |
|                  |              |             |          |            | 2YH6  | N-terminal domain  | 1.55   | P 1         | 63         |
|                  |              |             |          |            | 3SNS  | C-terminal domain  | 1.50   | H 3         | 67         |
|                  |              |             |          |            | 3TGO  | Unstructured N-terminus and the N-terminal domain in complex with BamD | 2.90   | I 1 2 1     | 68         |
|                  |              |             |          |            |   |  |  |             |            |
|                  |              |             |          |            |   |  |  |             |            |
|                  |              |             |          |            |   |  |  |             |            |
|                  |              |             |          |            |   |  |  |             |            |
|                  | BamD         | 245         | 27.8     | P0AC02     | 2YHC  | <i>E. coli</i>   | 1.80   | P 21        | 63         |
|                  |              |             |          |            | 3QKY  | Homologue from <i>Rhodothermus marinus</i>                             | 2.15   | H 3         | 70         |
| 3TGO             |              |             |          |            | <i>E. coli</i> BamD in complex with BamC                              | 2.90   | I 1 2 1  | 71          |            |
|                  |              |             |          |            |   |  |  |             |            |
|                  |              |             |          |            |   |  |  |             |            |
| BamE             | 113          | 12.3        | P0A937   | 2YH9       | <i>E. coli</i>  | 1.80   | C 2  | 63          |            |
|                  |              |             |          | 2KXX       | <i>E. coli</i>  | NMR structure  |  | 72          |            |
|                  |              |             |          | 2KM7       | <i>E. coli</i>  | NMR structure  |  | 73          |            |
|                  |              |             |          | 2PXG       | OmlA (homologue from <i>Xanthomonas axonopodis</i> pv. <i>citri</i> ) | NMR structure  |  | 74          |            |

with cytosolic proteins Sam35 and Sam37 that are essential for the function of the SAM complex, but no homologues have yet been found in bacteria. Sam35 appears to be important for substrate recognition, whereas Sam37 is involved in the release of the substrate from the complex.<sup>80,81</sup>

The homologous pathway in chloroplasts is less understood. It was proposed that the substrate proteins may first be imported into the stroma via the translocons at the outer and inner envelopes of chloroplasts (the TOC/TIC complexes), and then sent back to the outer envelope membrane for assembly by the BamA homologue, Toc75-V [Fig. 2(C), blue arrows].<sup>82</sup> However, recent research suggests that the three POTRA domains of Toc75-V may be facing the cytosol, and hence, it is possible that Toc75-V may assemble the  $\beta$ -barrels directly from the cytosol, without using the TOC/TIC pathway [Fig. 2(C), red

arrow].<sup>83</sup> The accessory proteins involved with Toc75-V have yet to be identified.

The exact molecular mechanism by which the BAM complex facilitates OMP folding and insertion remains poorly understood. However, a flux of new structural and biochemical information that has emerged in recent years suggests possible roles for each component of the BAM complex, and also provides a firm platform upon which further experiments can be designed. Below are the summaries of what is known about the structure and function of each BAM complex protein.

### BamA

BamA belongs to a protein superfamily known as Omp85.<sup>4,52</sup> The Omp85 superfamily includes other proteins whose functions are closely associated with protein translocation, as well as BamA homologues

in mitochondria (Sam50) and chloroplasts (Toc75-V) (Fig. 2).<sup>8,84</sup> All Omp85 proteins consist of a transmembrane  $\beta$ -barrel domain at the C-terminus and a varying number of POTRA domains at the N-terminus.<sup>8</sup> Most bacterial BamA homologues contain five POTRA domains, whereas Toc75-V of chloroplasts has three and Sam50 of mitochondria has one [Fig. 4(E)].<sup>51,61,85</sup>

In *E. coli*, BamA is predicted to form a 16-stranded  $\beta$ -barrel that spans the outer membrane. BamA exhibits a channel activity when reconstituted in planar lipid membranes, and the channel conductivity has been shown to increase upon binding denatured OMPs.<sup>86</sup> Although the  $\beta$ -barrel structure of BamA is not known, it is expected to look similar to that of FhaC (13.5% sequence identity), an Omp85 protein that mediates secretion of filamentous hemagglutinin (FHA) in *Bordetella pertussis*. The crystal structure of FhaC shows that its  $\beta$ -barrel is composed of 16 anti-parallel  $\beta$ -strands, with a long conserved loop folding over into the pore [Fig. 4(D)].<sup>63</sup> The tip of the loop contains a conserved sequence (VRGY) that is exposed to the periplasm in its folded form.<sup>63,87</sup> Mutating the conserved VRGY tetrad sequence of FhaC significantly reduces its ability to secrete FHA,<sup>88</sup> suggesting it is functionally important. It is currently not understood exactly how the conserved loop facilitates the protein translocation mechanism. Because the same conserved loop is found in BamA [Figs. 3 and 4(E)], it may also play an important role in the function of BamA.

All Gram-negative bacterial BamA proteins have up to five POTRA domains at their N-termini, numbered 1–5 in the N- to C-terminus direction [Fig. 4(A,E)]. Although proteobacteria have five POTRA domains, cyanobacteria have three.<sup>62</sup> The POTRA domains extend away from the  $\beta$ -barrel domain into the periplasm.<sup>89</sup> In *E. coli*, the five POTRA domains, despite low sequence similarity, have the same overall structure with each domain consisting of a three-stranded  $\beta$ -sheet and two  $\alpha$ -helices.<sup>34,90</sup> The protein–protein interaction interfaces between adjacent POTRA domains keep POTRA1-2 and POTRA3-4-5 rigid; however, a flexible linker between POTRA2 and POTRA3 results in the POTRA domains existing either in a bent or an extended form [Fig. 4(B)].<sup>56,58</sup> The functional implication of this conformational flexibility is not known, and there are conflicting data on which POTRA domains are essential. Although POTRA3, 4, and 5 are required for cell viability in *E. coli*,<sup>56</sup> only POTRA5 is essential in *N. meningitidis*.<sup>91</sup>

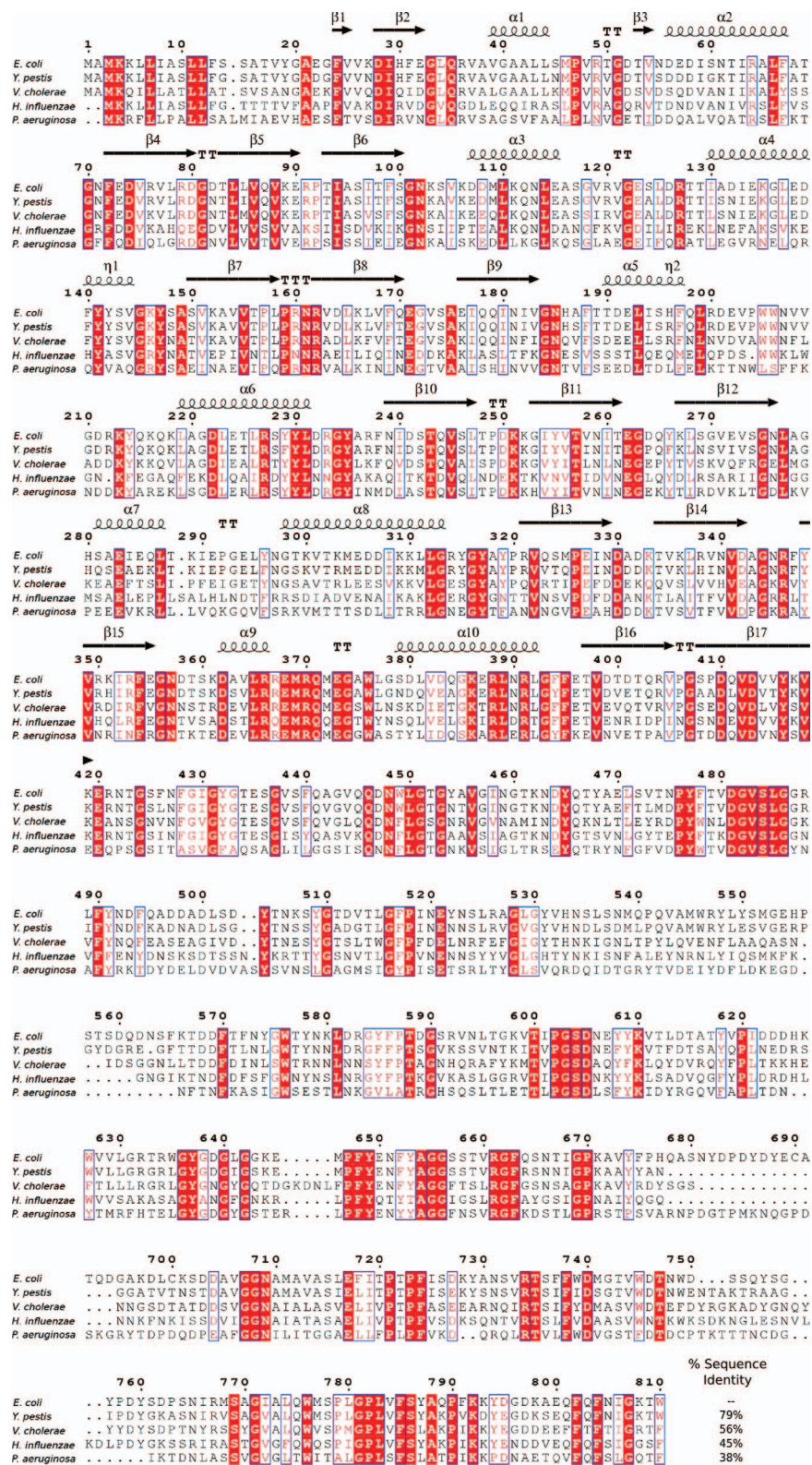
The exact roles of the POTRA domains are not clear, but experimental evidence suggests that they serve as docking sites for the BamB/C/D/E lipoproteins and have chaperone-like function for the substrates.<sup>56</sup> In *E. coli*, a deletion analysis of the POTRA domains has shown that POTRA2-5 are

required for BamB association and POTRA5 is required for BamC/D/E association.<sup>56</sup> POTRA1, on the other hand, has been shown to interact with SurA, a major periplasmic chaperone.<sup>92</sup> Ability of the POTRA domains to bind unfolded OMPs has been demonstrated by an NMR titration analysis that showed  $\beta$ -strands with varying sequences derived from PhoE, a BAM complex substrate, bind to the POTRA domains.<sup>60</sup>

How do POTRA domains of BamA interact with the other components of the BAM complex and the substrates? Based on crystal packing analysis, it has been proposed that exposed  $\beta$ -strands of POTRA domains can interact with strands from other proteins, via a process known as  $\beta$ -augmentation.<sup>56,61,89</sup>  $\beta$ -Augmentation is a mode of protein–protein interaction where a  $\beta$ -strand of one protein is added to an existing  $\beta$ -sheet of another protein.<sup>93</sup> This hypothesis of POTRA domains interacting with substrates via  $\beta$ -augmentation is supported by the observation that an exposed  $\beta$ -strand of POTRA3 is involved in forming one of the crystal packing contacts via  $\beta$ -augmentation [Fig. 4(C)].<sup>56,57</sup> Inducing a change in the backbone conformation of the same  $\beta$ -strand of POTRA3 abolishes its ability to interact with BamB,<sup>56</sup> implying that BamA and BamB may interact via  $\beta$ -augmentation. Also, an NMR titration study showed that  $\beta$ -strands of PhoE bind along the exposed edges of the  $\beta$ -sheets of POTRA1 and POTRA2.<sup>60</sup> It has been suggested that conformational change of the POTRA domains from the extended to the bent state (when it is bound to an unfolded OMP) could facilitate formation of  $\beta$ -hairpins in the substrate.<sup>57</sup> However, more concrete experimental evidence is needed to validate if this is how the POTRA domains interact with other proteins *in vivo*.

### **BamB**

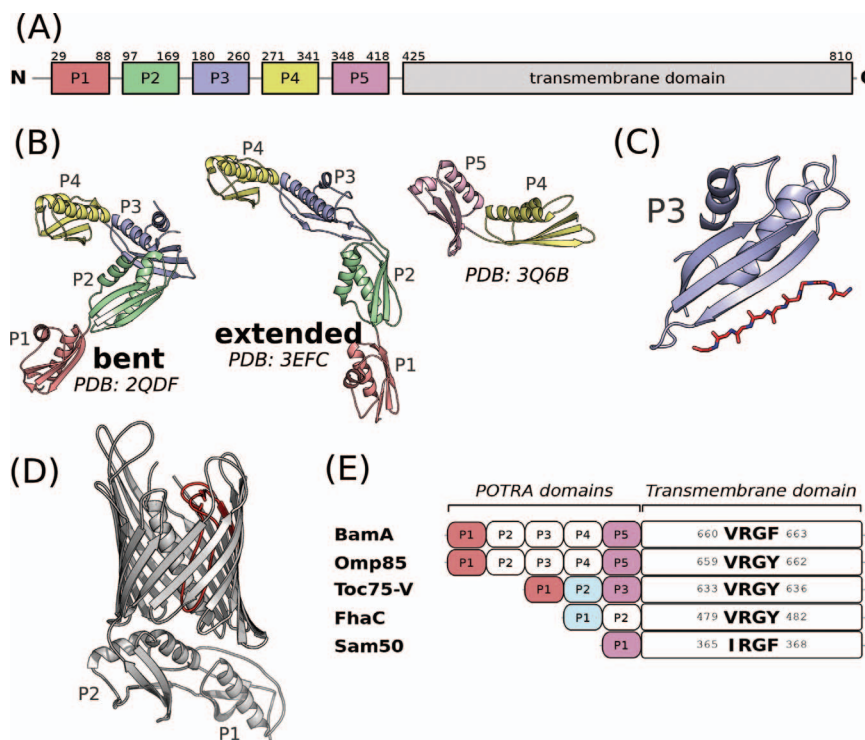
Among all the lipoprotein members of the BAM complex, BamB is functionally and structurally the most well characterized. Although BamB is not essential for cell viability, OMP assembly is significantly reduced in its absence.<sup>78,94</sup> BamB and SurA (a periplasmic chaperone) deletion mutants are almost indistinguishable from each other in phenotype,<sup>95</sup> suggesting that BamB may work in the SurA pathway to facilitate the delivery of  $\beta$ -barrel precursors to BamA. Accordingly, simultaneous absence of BamB and SurA results in a synthetic lethal phenotype.<sup>78</sup> BamB shows synthetic lethality also with deletion of DegP, a chaperone/protease that works in the Skp/DegP pathway that is thought to “rescue” proteins falling off the SurA pathway.<sup>94</sup> Taken together, these results imply that BamB is involved in the earlier steps of OMP assembly. As the protein is not essential, it is unlikely for BamB to be involved in substrate recognition and more likely for it to be involved in the delivery of  $\beta$ -barrel precursors to BamA.



**Figure 3.** Sequence alignment of BamA. A sequence alignment is shown of *E. coli* BamA (UniProt ID: P0A940) with homologues from *Yersinia pestis* (Q8ZH58), *Vibrio cholerae* (Q9KPW0), *Haemophilus influenzae* (O32625), and *Pseudomonas aeruginosa* (Q9HX4). Red boxes show absolutely conserved residues, red text shows similar residues, and blue boxes show stretches of similar residues. The secondary structure of the POTRA domains is shown above the sequence. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

As the OMPs that are most affected by BamB deletion are relatively large (16–24 stranded  $\beta$ -barrels), it has been suggested that BamB could aid

BamA function by increasing the substrate binding capacity.<sup>65</sup> Structural analysis of BamB provides clues for where potential substrate and BamA



**Figure 4.** Structural features of BamA. BamA forms the core of the BAM complex and is the only component that spans the outer membrane. A: The domain structure of BamA shows an N-terminal periplasmic region that contains five POTRA domains along with a C-terminal transmembrane domain. The exact boundaries of the transmembrane domain are not known as the structure has yet to be determined, but the  $\beta$ -barrel is predicted to begin around Ser425. B: Ribbon diagrams of the POTRA1-4 and POTRA4-5 domains are shown. The structures for POTRA1-4 show the possibility for bent and extended conformations. C: A close up view of POTRA3 is shown, where an exposed  $\beta$ -strand is involved in making a crystal contact. This suggests the possibility of POTRA3 interacting with substrates or other BAM components via a process known as  $\beta$ -augmentation. D: The structure of FhaC from *Bordetella pertussis* shows the presence of only two POTRA domains, as well as a helix that can insert itself inside the  $\beta$ -barrel. The conserved long loop is highlighted in red. E: A domain structure comparison is shown for the BamA homologues from bacteria and eukaryotes. BamA (*Escherichia coli*, UniProt ID: P0A940) and Omp85 (*Neisseria meningitidis*, Q9K1H0) have five POTRA domains, whereas Toc75-V (in chloroplasts of *Arabidopsis thaliana*, Q9C5J8) has three, FhaC (*Bordetella pertussis*, P35077) has two, and Sam50 (in mitochondria of *Saccharomyces cerevisiae*, P53969) only has one. Cluster analysis of the different POTRA sequences suggests that POTRA1 of BamA, Omp85, and Toc75-V are closely related (red), whereas POTRA1 of FhaC is similar to POTRA2 of Toc75-V (cyan). The POTRA domains nearest to the membrane, for all homologues except FhaC, are closely related to each other (purple).<sup>62</sup> The important VRGY motif seen in the transmembrane domain of FhaC is also conserved among the other homologues. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

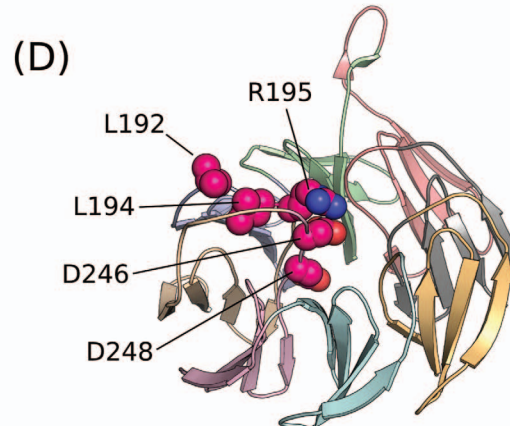
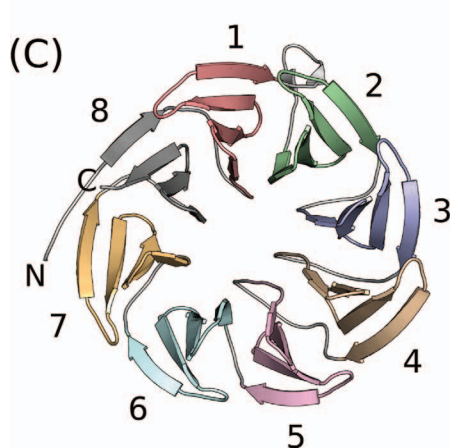
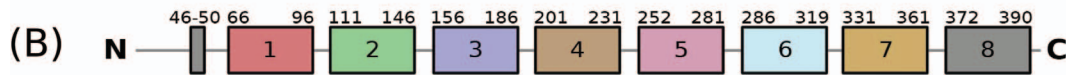
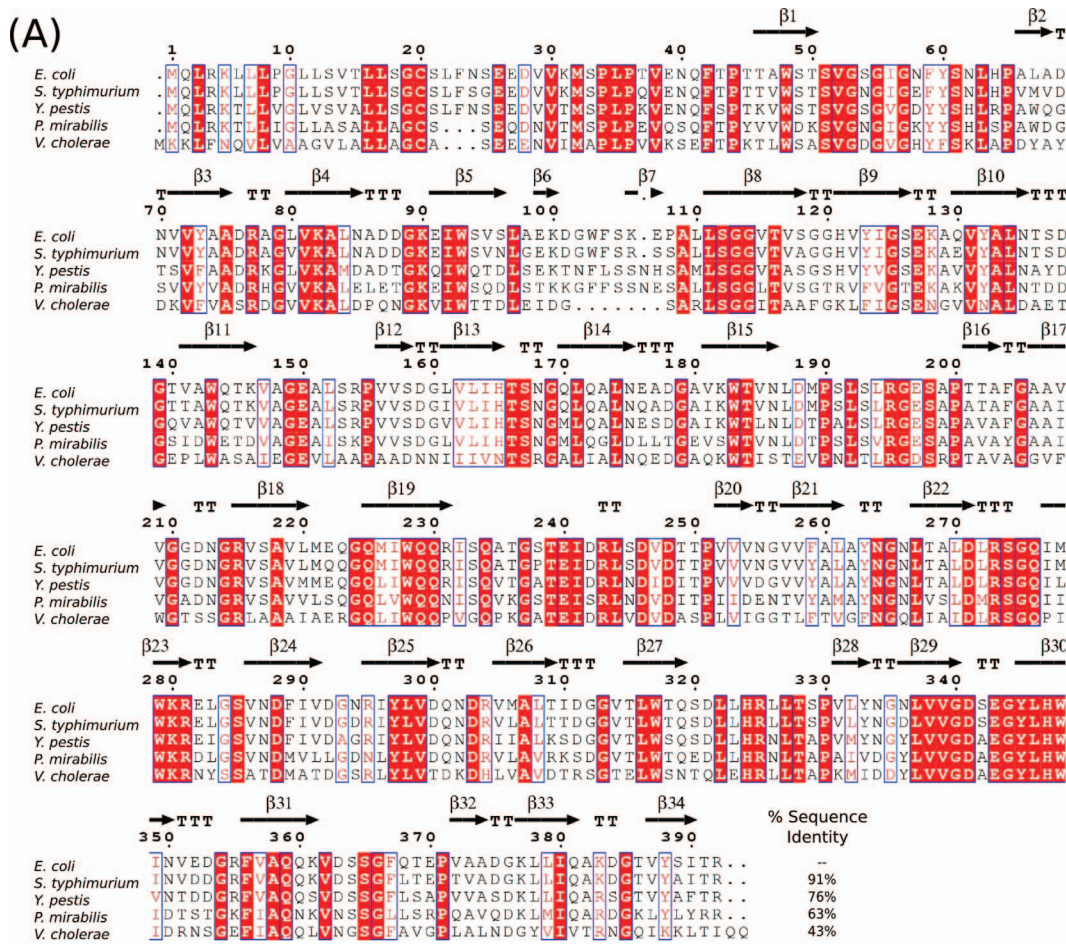
binding sites are located. BamB forms an eight-bladed  $\beta$ -propeller structure, with a four-stranded anti-parallel  $\beta$ -sheet making up each blade (Fig. 5).<sup>65-67</sup> The blades are arranged in a ring-like structure, and the neighboring blades are connected by long loops. Conserved residues are found concentrated on one face of the  $\beta$ -propeller structure, and this area contains residues that have been shown by a mutagenesis study to be important for BamA interaction [Fig. 5(D)].<sup>96</sup> Crystal contacts are observed on the exposed outermost  $\beta$ -strands of the blades.<sup>65</sup> Because BamB has eight blades, there are eight exposed outermost  $\beta$ -strands that could potentially make protein interactions via  $\beta$ -augmentation. There has not been a study yet examining the ability of BamB to bind  $\beta$ -barrel precursors. However, if the function of BamB does indeed involve substrate binding, it could provide multiple binding surfaces

for substrates with a larger number of  $\beta$ -strands. Also, as BamB physically interacts with POTRA2-4, these potential binding sites could be the additional contact points between BamB and BamA.

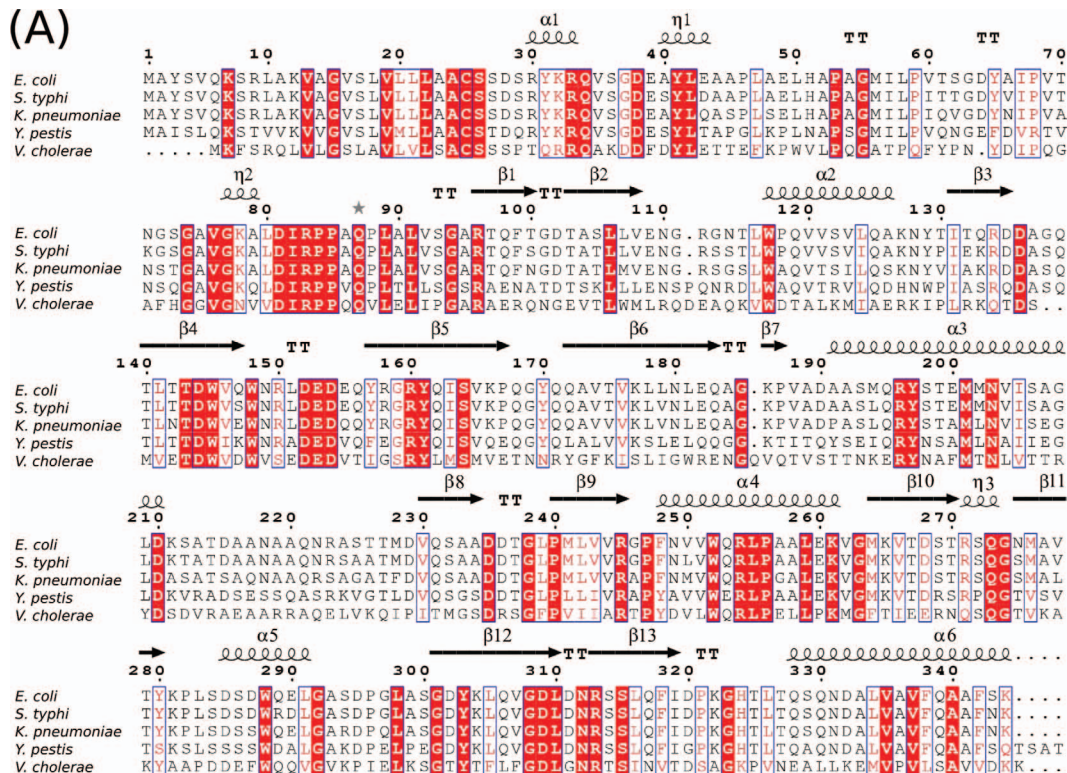
### BamC

Similar to BamB, absence of BamC results in impairment of OMP assembly, although not as severe. Mutants lacking BamC display outer membrane permeability defects and reduced levels of OMPs in the outer membrane.<sup>77</sup> Limited proteolysis experiments suggest that BamC has two independently folded domains (N- and C-terminal domains), preceded by a long (~70 residues) unstructured N-terminus (Fig. 6).<sup>68,97,98</sup> The two folded domains have the same helix-grip folds despite low sequence identity (12%), and they are connected by a highly flexible linker.<sup>64,70</sup> The N-terminus of BamC lacks



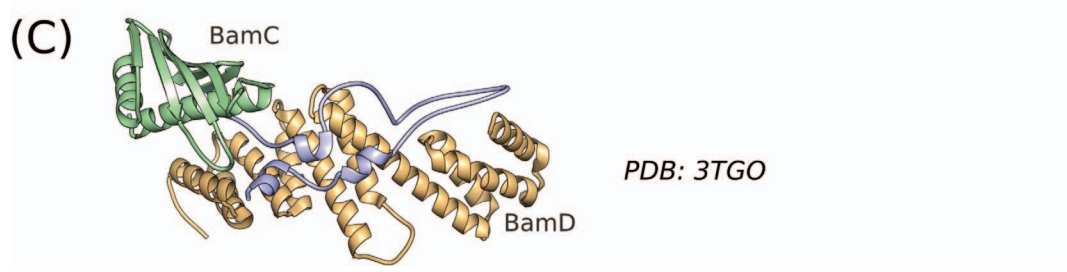
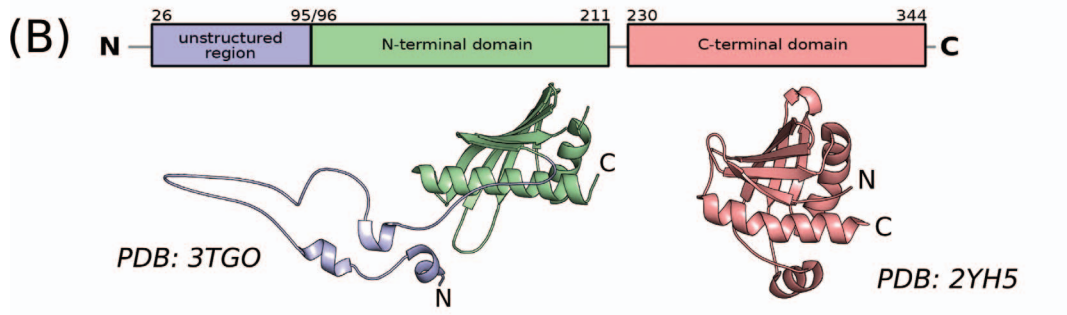


**Figure 5.** Structural features of BamB. A: A sequence alignment is shown of *E. coli* BamB (UniProt ID: P77774) with homologues from *Salmonella typhimurium* (F5ZUU9), *Yersinia pestis* (G0JC13), *Proteus mirabilis* (C2LH28), and *Vibrio cholerae* (F9ADD9). Red boxes show absolutely conserved residues, red text shows similar residues, and blue boxes show stretches of similar residues. The secondary structure is shown above the sequence. B: The domain structure of BamB shows the presence of eight domains that together form the  $\beta$ -propeller structure. Note that residues 46–50 form a strand that is a part of blade 8, both shown in gray. C: Ribbon diagram of BamB shows the eight-bladed  $\beta$ -propeller structure, with each blade numbered as in (B). The N- and C-terminus come together to form blade 8 (gray). D: Conserved residues are shown as spheres and are found clustered on one face of the  $\beta$ -propeller structure. These residues are believed to be important for interaction with BamA. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

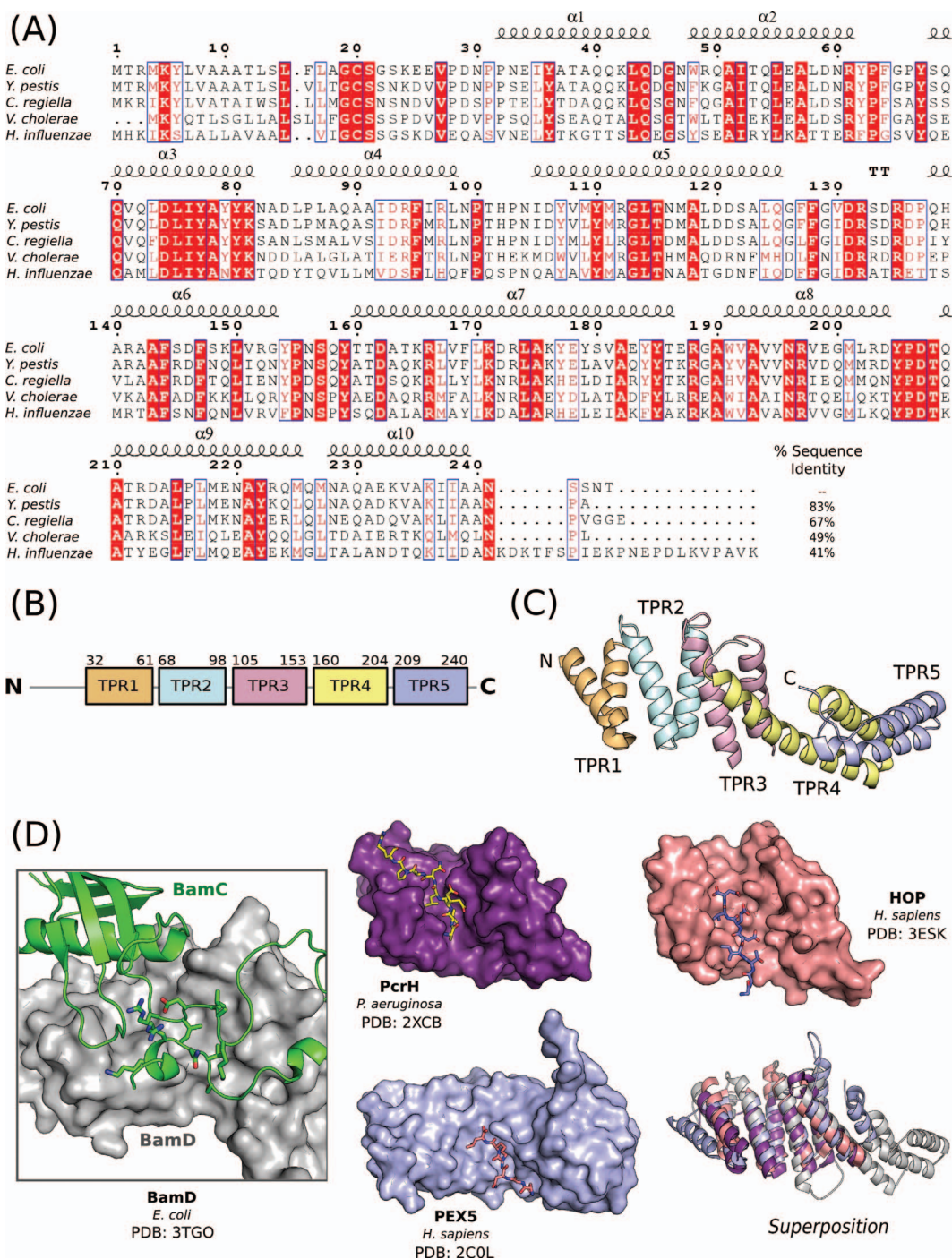


**% Sequence Identity**

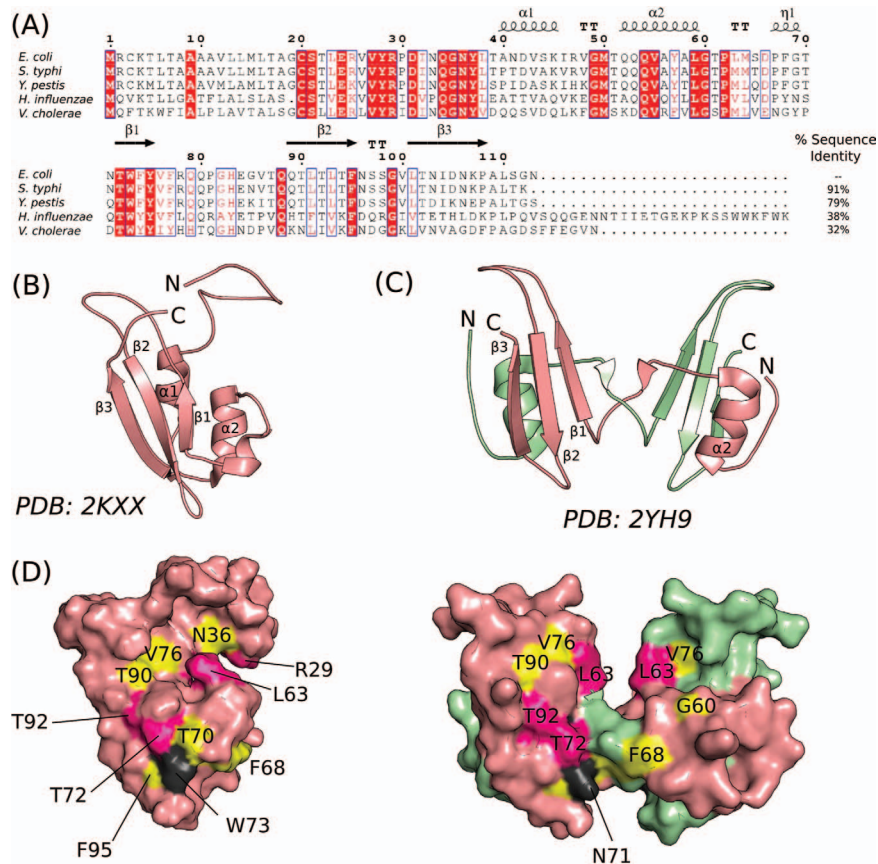
|                      |       |     |
|----------------------|-------|-----|
| <i>E. coli</i>       | . . . | --  |
| <i>S. typhi</i>      | . . . | 92% |
| <i>K. pneumoniae</i> | . . . | 85% |
| <i>Y. pestis</i>     | AIK   | 62% |
| <i>V. cholerae</i>   | . . . | 27% |



**Figure 6.** Structural features of BamC. A: A sequence alignment is shown of *E. coli* BamC (UniProt ID: P0A903) with homologues from *Salmonella typhi* (Q83T79), *Klebsiella pneumoniae* (B5XVM8), *Yersinia pestis* (D1TUX3), and *Vibrio cholerae* (Q9KQ48). Red boxes show absolutely conserved residues, red text shows similar residues, and blue boxes show stretches of similar residues. The secondary structure is shown above the sequence. B: The domain structure of BamC shows the presence of three domains: an unstructured region at the N-terminus followed by two domains known as the N-terminal domain and the C-terminal domain. The C-terminal domain (pink) was solved separately and shows to have a similar helix-grip fold as the N-terminal domain (light blue) and N-terminal domain (green). C: The BamC unstructured region (light blue) and N-terminal domain (green) was co-crystallized with BamD. The resulting structure shows the unstructured region to form a long loop that interacts with BamD (shown in beige). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**Figure 7.** Structural features of BamD. A: A sequence alignment is shown of *E. coli* BamD (UniProt ID: P0AC02) with homologues from *Yersinia pestis* (G0JE21), *Candidatus Regiella insecticola* (E0WTN2), *Vibrio cholerae* (D0I076), and *Haemophilus influenzae* (E4QXC3). Red boxes show absolutely conserved residues, red text shows similar residues, and blue boxes show stretches of similar residues. The secondary structure is shown above the sequence. B: The domain structure of BamD shows the presence of five TPR motifs. C: The ribbon diagram of the BamD structure is shown. Ten helices form the five TPR motifs which are numbered as in (B). D: (Left) A close up of the BamCD binding site is shown, where BamC (green) binds to a region of BamD (gray) that superimposes with the binding sites of the structural homologues. (Right) Superposition of BamD (gray), PcrH<sup>99</sup> from *Pseudomonas aeruginosa* (purple), HOP<sup>100</sup> from *Homo sapiens* (pink), and PEX5<sup>101</sup> from *Homo sapiens* (blue) show high structural similarity. These structural homologues are involved in binding to protein targeting sequences. The binding sites of these proteins are individually shown as surface models with substrate peptides as stick models. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**Figure 8.** Structural features of BamE. A: A sequence alignment is shown of *E. coli* BamE (UniProt ID: P0A937) with homologues from *Salmonella typhi* (Q8XF17), *Yersinia pestis* (G0JGU1), *Haemophilus influenzae* (P44057), and *Vibrio cholerae* (P0C6Q9). Red boxes show absolutely conserved residues, red text shows similar residues, and blue boxes show stretches of similar residues. The secondary structure is shown above the sequence. B: The ribbon diagram of a BamE monomer shows the presence of an unstructured N-terminus as well as a long flexible loop between strands  $\beta 1$  and  $\beta 2$ . C: The ribbon diagram of a BamE dimer shows how the two monomers (shown in pink and green) exchange  $\alpha$ -helices to form a domain-swapped dimer. D: The residues previously identified to be important for lipid binding are shown in yellow, and those important for BamD interaction are shown in dark pink on the surface diagrams of the BamE monomer (left) and the dimer (right). The residues shown in dark gray are important for both lipid and BamD interactions. The protein structures are shown in the same orientations as in (B) and (C). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

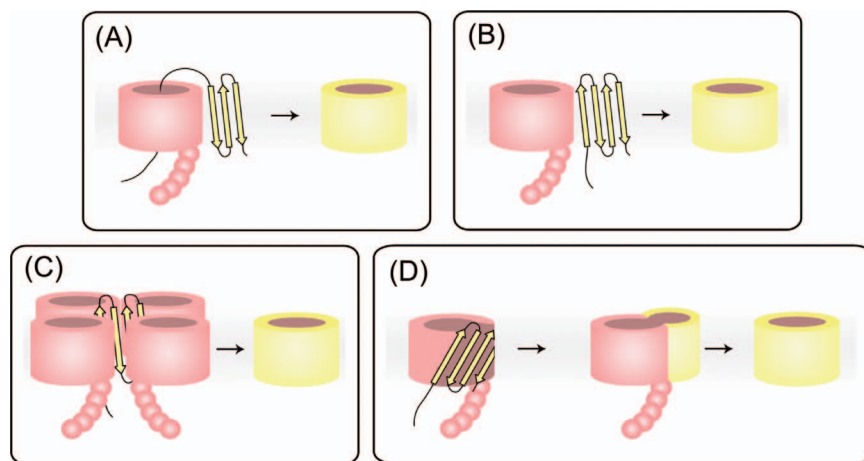
secondary structural elements, as shown both via NMR chemical shift analysis and crystallographic analysis.<sup>69,98</sup> Thus, BamC forms a modular structure exhibiting conformational flexibility.

It is currently unclear what role BamC plays in the BAM complex. Interestingly, a multiple sequence alignment shows that the unstructured N-terminus is the most conserved region, followed by the C-terminal domain [Fig. 6(A)]. A co-crystal structure of a BamC construct (the unstructured region and the N-terminal domain) with full length BamD reveals that the conserved N-terminus of BamC is important for the formation of the BamCD subcomplex [Fig. 6(C)].<sup>69</sup> Furthermore, the unstructured N-terminus is observed to bind BamD in a region that is proposed to interact with the outer membrane targeting sequence of  $\beta$ -barrel precursor substrates [Fig. 7(D)], suggesting the possibility of BamC playing a regulatory role.<sup>69,71,97</sup> In the C-terminal domain of BamC,

the conserved residues are found clustered in a groove that could potentially be a site of protein interaction.<sup>68</sup> This may be an additional binding site for BamD or perhaps other BAM protein components. Further biochemical studies are needed to examine if the function of BamC is associated with regulating the substrate binding ability of BamD.

### BamD

Like BamA, BamD is absolutely essential for proper OMP assembly and its absence results in cell death.<sup>40,78</sup> It is also the most highly conserved among the BAM lipoproteins, implying that it serves a vital function for the BAM complex. Mutagenesis data combined with pull-down assays show that BamD interacts with BamA via the POTRA5 domain (the POTRA domain closest to the membrane),<sup>56</sup> and with other BAM lipoproteins, namely, BamC and BamE.<sup>40,54</sup>



**Figure 9.** Models of  $\beta$ -barrel assembly. Four different models of how the BAM complex may facilitate the folding and insertion of OMPs are shown. BamA is shown in pink, and the substrate protein is shown in yellow. The lipoproteins BamB/C/D/E are not shown in these models for clarity. The outer membrane is represented by the gray rectangle, with the extracellular space above and the periplasmic space below. A: In the first model, the substrate protein is first translocated across the outer membrane through the channel formed by the  $\beta$ -barrel domain of BamA. The substrate then inserts and folds into the outer membrane lipid bilayer from outside the cell. B: In the second model, the substrate inserts into the lipid bilayer from the periplasmic face of the outer membrane. Instead of using the channel of BamA, the insertion and folding of OMPs occur at the BamA-lipid interface. In this model, the outer wall of the  $\beta$ -barrel of BamA provides a scaffold for the substrate folding. C: This model is similar to the second model, but assumes that BamA forms an oligomeric structure. The coordinated events of substrate folding and membrane insertion are contained within the space formed by the BamA tetramer. The mature, folded OMP substrate is then released laterally into the lipid bilayer of the outer membrane. D: In the last model, the OMP substrate uses the N- and the C-terminal  $\beta$ -strands of BamA as folding templates. The hydrogen bonds between the two terminal  $\beta$ -strands of BamA are displaced by the incoming substrate that becomes part of the BamA structure as it folds into a  $\beta$ -sheet. The  $\beta$ -sheet of the substrate is then closed to form a  $\beta$ -barrel, and the substrate is released into the lipid bilayer. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

Presently, existing data suggests BamD may act as an initial substrate recognition protein that binds the C-terminal targeting sequence on the  $\beta$ -barrel precursors.<sup>64,71</sup> BamD, which is predominantly  $\alpha$ -helical and consists of five tetratricopeptide repeat (TPR) motifs [Fig. 7(B,C)], shares structural similarity with other proteins whose functions involve binding to targeting sequences [Fig. 7(D)].<sup>64,69,71</sup> A pocket formed by the N-terminal half (TPR1-3) of BamD superimposes very closely with the binding pockets of the other proteins where targeting signal sequences in extended conformations bind [Fig. 7(D)]. Furthermore, a truncated form of BamD consisting only of TPR1-3 has been shown to crosslink with synthetic peptides harboring the C-terminal targeting sequence.<sup>64</sup> While these data favor the hypothesis that the N-terminal binding pocket of BamD functions in targeting signal recognition, the recently solved BamCD subcomplex crystal structure revealed that the unstructured N-terminus of BamC binds to the exact same pocket of BamD [Figs. 6(C) and 7(D)].<sup>69</sup> It is clear from the BamCD structure that BamD will not be able to bind targeting signal sequence, at least in the proposed binding pocket, as the pocket is completely occluded by BamC. Nevertheless, the structural similarity between BamD and those of other targeting signal recognition proteins is conspicuous, which gives rise to a question of

whether the substrate binding activity of BamD is regulated by BamC interaction.

### BamE

BamE is the smallest and the most recently discovered component of the BAM complex. It is not an essential member of the complex, but the loss of BamE causes mild OMP assembly defects.<sup>54,102</sup> Furthermore, the stability of the BAM complex is compromised in the absence of BamE, suggesting that it plays an important structural role.<sup>54</sup> On the other hand, NMR spectroscopic analysis has shown that BamE binds specifically to phosphatidylglycerol,<sup>73</sup> which has previously been shown to enhance the insertion of OMPs into liposomes, and that the lipid binding site partially overlaps with that of the BamD binding surface [Fig. 8(D)].<sup>73</sup> On the basis of these experimental results, it has been hypothesized that the function of BamE may be to recruit phosphatidylglycerol to enhance OMP membrane insertion.<sup>103</sup>

Interestingly, BamE is observed to exist in both monomeric and dimeric forms in solution.<sup>64,72,73</sup> NMR and crystal structures of BamE show that monomeric BamE consists of a small three-stranded anti-parallel  $\beta$ -sheet against which two  $\alpha$ -helices are packed (Fig. 8).<sup>64,72,73,104</sup> Crystal structure of BamE in its dimeric form revealed that it forms a domain-swapped

dimer, in which the  $\alpha$ -helices of the two monomers are exchanged [Fig. 8(C)].<sup>64</sup> The dimer formation of BamE is irreversible under various pH and salt concentration conditions,<sup>72</sup> and there are conflicting data on which oligomeric form of BamE is biologically relevant. Although one study reported that the formation of BamE dimer is a result of protein misfolding under temperature stress,<sup>73</sup> another study reported that BamE purified from a native outer membrane exhibits a dimeric state.<sup>64</sup> Further, experiments are required to identify which oligomeric form of BamE is found *in vivo*, or if both forms are functional in the BAM complex.

## Conclusion

The field of OMP biogenesis research has experienced exciting advances in recent years. Although genetics and protein-protein interaction studies identified the BAM complex and its components, emerging structural and biochemical studies are revealing how the BAM proteins may interact with each other and with  $\beta$ -barrel precursor substrates. In addition, the structures of all five members of the BAM complex (with exception of the  $\beta$ -barrel domain of BamA) are now available, giving us a clearer picture of what the individual components of the BAM complex look like.

So how does the BAM complex facilitate the folding and membrane insertion of OMPs? The current general consensus is that BamA and BamD perform the essential function of substrate recognition and assembly, whereas BamB, BamC, and BamE increase the efficiency of the process. Based on the most recently published structural and functional data, BamD likely recognizes the  $\beta$ -barrel precursors via their C-terminal targeting sequence. The substrates are then bound by the POTRA domains, and subsequently, guided toward the BamA  $\beta$ -barrel domain which is thought to provide a scaffold to facilitate the formation of  $\beta$ -strands. Perhaps the flexibility of the POTRA domains could play an important role in this step, but the mechanism of  $\beta$ -barrel folding in general remains poorly understood. During the whole OMP assembly process, BamB could provide extra substrate binding surfaces to aid the POTRA domains, and BamE may recruit phosphatidylglycerols to enhance the folding efficiency. Finally, BamC could use its unstructured N-terminus to regulate the targeting signal binding activity of BamD.

The least understood aspect of the BAM complex function is the molecular mechanism by which the substrates are folded into  $\beta$ -barrels. Based on *in vitro*  $\beta$ -barrel folding studies, folding and membrane insertion of OMPs probably take place in a concerted manner.<sup>30,31</sup> Several different OMP folding mechanisms have been suggested to date. In the first model, the pore of the  $\beta$ -barrel domain of BamA is

thought to serve as a conduit for the substrate that will be translocated across the lipid bilayer into the extracellular space in an unfolded form, before being assembled into the outer membrane [Fig. 9(A)]. This model is largely based on the structural studies of FhaC, a BamA homolog. The channel formed by the  $\beta$ -barrel of FhaC is  $\sim 3$  Å in diameter, which is too small for accommodating even an unfolded polypeptide.<sup>63</sup> However, studies suggest binding of the substrate (FHA) causes a conformational change in FhaC, which could subsequently increase the diameter of the pore from 3 Å to 16 Å.<sup>63,88</sup> This increase in the pore size could make enough room for the substrate to enter the channel within the barrel in an extended form.<sup>63</sup> As it has been shown that substrate binding increases the channel conductivity of BamA,<sup>86</sup> it is possible that BamA also undergoes a similar conformational change as in FhaC to allow an OMP substrate to move through. According to Robert *et al.*,<sup>36</sup> the diameter of the BamA channel is estimated to be 25 Å, which is significantly larger than that estimated for FhaC. However, this model implies that the substrate folding takes place on the outside of the cell, and it is hard to imagine OMPs assembling efficiently without extra folding factors on the extracellular surface of the outer membrane.

Another model of OMP assembly by the BAM complex predicts that the substrate uses the outer wall of the BamA  $\beta$ -barrel as a scaffold for folding and membrane insertion. In this model, the unfolded substrate will start to insert between the BamA-lipid interface as it starts acquiring secondary structure [Fig. 9(B)]. Alternatively, if BamA forms a tetramer *in vivo*, the substrate folding could be contained in the space formed by the four BamA subunits [Fig. 9(C)]. The limited folding space may facilitate the closing of the  $\beta$ -sheet into a  $\beta$ -barrel, and the  $\beta$ -barrel would be released laterally into the lipid bilayer. This model requires that BamA subunits within the proposed tetramer be able to associate and dissociate with each other to allow substrate release.

Finally, we propose another model of  $\beta$ -barrel assembly that is modified from an earlier model. When the BAM complex was first discovered, it was first speculated that a substrate folds into a  $\beta$ -barrel inside BamA and that the  $\beta$ -barrel of BamA would open up to release the folded product laterally into the outer membrane.<sup>105</sup> However, the structural information now tells us that the channel formed by BamA is nowhere close to being large enough to hold a folded OMP. In addition, breaking hydrogen bonds of a  $\beta$ -barrel to release the substrate seems energetically very costly. To attune to the new structural data and to minimize the energy cost, we suggest the model be modified as follows. Instead of the unfolded substrate folding within the  $\beta$ -barrel of BamA, the N- and the C-terminal  $\beta$ -strands of BamA (the two strands that hydrogen bond with

each other to close the  $\beta$ -sheet into a  $\beta$ -barrel) could serve as folding templates for the substrate. In this model [Fig. 9(D)], the hydrogen bonds between the N- and the C-terminal  $\beta$ -strands of BamA would be interrupted and replaced by an incoming substrate, which would form new hydrogen bonds with the terminal strands of BamA. As the rest of the substrate folds, it starts forming a  $\beta$ -sheet held between the two terminal strands of BamA until it closes into a  $\beta$ -barrel and then released into the lipid bilayer. Although this model requires the  $\beta$ -barrel of BamA to open up, the energy cost of breaking hydrogen bonds is compensated by having the substrate forming new hydrogen bonds with BamA via  $\beta$ -augmentation.

There are still many other aspects of the BAM complex that remain to be elucidated, which would help in deciphering the molecular mechanism of  $\beta$ -barrel assembly. A crystal structure of BamD bound to the C-terminal targeting sequence of a  $\beta$ -barrel precursor substrate would provide a great deal of insight into the BAM-OMP specificity. Once the functional role of BamD is established, its substrate binding properties could be further examined, for example, by binding kinetics. Substrate binding to BamB via  $\beta$ -augmentation also requires experimental evidence to validate the hypothesis that it acts in concert with SurA to deliver the substrates to BamA. The structural investigation of the BAM complex should now be focused on determining the structure of the BamA  $\beta$ -barrel domain, and how the BAM components are arranged within the complex. Together, these studies will help us better understand  $\beta$ -barrel protein assembly into the Gram-negative bacterial outer membrane and the homologous systems in eukaryotes.

Could better understanding of the BAM complex be used for medical applications? New drug development inevitably starts with the challenging task of identifying a suitable drug target. In this respect, the BAM complex could serve as potential candidate, as it is essential for the survival of Gram-negative bacteria and it is functionally nonredundant (i.e., there is no other back-up system in the cell that can perform the same function). Furthermore, the BAM complex is found in the outer membrane, implying uncomplicated drug delivery strategy and its potential to serve as an antigen for novel vaccine design. Because the SAM complex exists in human cells, the differences between the SAM and the BAM complex pathways should be exploited for the development of potential antimicrobial agents. Potential antimicrobial compounds that target the BAM complex would need to be carefully screened against the SAM complex to avoid compounds with cross reactivity. To realize its potential as a drug target, the detailed molecular mechanism of the BAM complex still needs to be elucidated. We have almost all of the pieces of

the puzzle—it is now time to discover how they fit together.

## References

1. Silhavy TJ, Kahne D, Walker S (2010) The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2: a000414:1–16.
2. Ricci DP, Silhavy TJ (2012) The Bam machine: a molecular cooper. *Biochim Biophys Acta* 1818:1067–1084.
3. Fairman JW, Noinaj N, Buchanan SK (2011) The structural biology of beta-barrel membrane proteins: a summary of recent reports. *Curr Opin Struct Biol* 21: 523–531.
4. Voulhoux R, Bos MP, Geurtsen J, Mols M, Tommassen J (2003) Role of a highly conserved bacterial protein in outer membrane protein assembly. *Science* 299:262–265.
5. Werner J, Misra R (2005) YaeT (Omp85) affects the assembly of lipid-dependent and lipid-independent outer membrane proteins of *Escherichia coli*. *Mol Microbiol* 57:1450–1459.
6. Doerrler WT, Raetz CR (2005) Loss of outer membrane proteins without inhibition of lipid export in an *Escherichia coli* YaeT mutant. *J Biol Chem* 280: 27679–27687.
7. Schleiff E, Maier UG, Becker T (2011) Omp85 in eukaryotic systems: one protein family with distinct functions. *Biol Chem* 392:21–27.
8. Gentle I E, Burri L, Lithgow T (2005) Molecular architecture and function of the Omp85 family of proteins. *Mol Microbiol* 58:1216–1225.
9. Walther DM, Rapaport D, Tommassen J (2009) Biogenesis of beta-barrel membrane proteins in bacteria and eukaryotes: evolutionary conservation and divergence. *Cell Mol Life Sci* 66:2789–2804.
10. Bavro VN, Pietras Z, Furnham N, Perez-Cano L, Fernandez-Recio J, Pei XY, Misra R, Luisi B (2008) Assembly and channel opening in a bacterial drug efflux machine. *Mol Cell* 30:114–121.
11. Snijder HJ, Ubarretxena-Belandia I, Blaauw M, Kalk KH, Verheij HM, Egmond MR, Dekker N, Dijkstra BW (1999) Structural evidence for dimerization-regulated activation of an integral membrane phospholipase. *Nature* 401:717–721.
12. Nakae T (1976) Identification of the outer membrane protein of *E. coli* that produces transmembrane channels in reconstituted vesicle membranes. *Biochem Biophys Res Commun* 71:877–884.
13. Nikaido H (1994) Porins and specific diffusion channels in bacterial outer membranes. *J Biol Chem* 269: 3905–3908.
14. Noinaj N, Guillier M, Barnard TJ, Buchanan SK (2010) TonB-dependent transporters: regulation, structure, and function. *Annu Rev Microbiol* 64:43–60.
15. Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* 67:593–656.
16. Zgurskaya HI, Krishnamoorthy G, Ntrel A, Lu S (2011) Mechanism and function of the outer membrane channel TolC in multidrug resistance and physiology of enterobacteria. *Front Microbiol* 2:189:1–23.
17. Jacob-Dubuisson F, Loch C, Antoine R (2001) Two-partner secretion in Gram-negative bacteria: a thrifty, specific pathway for large virulence proteins. *Mol Microbiol* 40:306–313.
18. Desvaux M, Parham NJ, Henderson IR (2004) The autotransporter secretion system. *Res Microbiol* 155: 53–60.

19. Bishop RE (2008) Structural biology of membrane-intrinsic beta-barrel enzymes: sentinels of the bacterial outer membrane. *Biochim Biophys Acta* 1778: 1881–1896.
20. Danese PN, Silhavy TJ (1998) Targeting and assembly of periplasmic and outer-membrane proteins in *Escherichia coli*. *Annu Rev Genet* 32:59–94.
21. Osborn MJ, Rick PD, Lehmann V, Rupprecht E, Singh M (1974) Structure and biogenesis of the cell envelope of gram-negative bacteria. *Ann NY Acad Sci* 235:52–65.
22. Tokuda H (2009) Biogenesis of outer membranes in Gram-negative bacteria. *Biosci Biotechnol Biochem* 73:465–473.
23. Bos MP, Robert V, Tommassen J (2007) Biogenesis of the gram-negative bacterial outer membrane. *Annu Rev Microbiol* 61:191–214.
24. Paetzel M, Karla A, Strynadka NC, Dalbey RE (2002) Signal peptidases. *Chem Rev* 102:4549–4580.
25. Volokhina EB, Grijpstra J, Stork M, Schilders I, Tommassen J, Bos MP (2011) Role of the periplasmic chaperones Skp, SurA, and DegQ in outer membrane protein biogenesis in *Neisseria meningitidis*. *J Bacteriol* 193:1612–1621.
26. Sklar JG, Wu T, Kahne D, Silhavy TJ (2007) Defining the roles of the periplasmic chaperones SurA, Skp, and DegP in *Escherichia coli*. *Genes Dev* 21: 2473–2484.
27. Eppens EF, Nouwen N, Tommassen J (1997) Folding of a bacterial outer membrane protein during passage through the periplasm. *EMBO J* 16:4295–4301.
28. Allen WJ, Phan G, Waksman G (2009) Structural biology of periplasmic chaperones. *Adv Protein Chem Struct Biol* 78:51–97.
29. Qu J, Mayer C, Behrens S, Holst O, Kleinschmidt JH (2007) The trimeric periplasmic chaperone Skp of *Escherichia coli* forms 1:1 complexes with outer membrane proteins via hydrophobic and electrostatic interactions. *J Mol Biol* 374:91–105.
30. Burgess NK, Dao TP, Stanley AM, Fleming KG (2008) Beta-barrel proteins that reside in the *Escherichia coli* outer membrane in vivo demonstrate varied folding behavior in vitro. *J Biol Chem* 283:26748–26758.
31. Kleinschmidt JH (2003) Membrane protein folding on the example of outer membrane protein A of *Escherichia coli*. *Cell Mol Life Sci* 60:1547–1558.
32. Tamm LK, Arora A, Kleinschmidt JH (2001) Structure and assembly of beta-barrel membrane proteins. *J Biol Chem* 276:32399–32402.
33. Tommassen J (2010) Assembly of outer-membrane proteins in bacteria and mitochondria. *Microbiology* 156:2587–2596.
34. Knowles TJ, Scott-Tucker A, Overduin M, Henderson IR (2009) Membrane protein architects: the role of the BAM complex in outer membrane protein assembly. *Nat Rev Microbiol* 7:206–214.
35. Struyve M, Moons M, Tommassen J (1991) Carboxy-terminal phenylalanine is essential for the correct assembly of a bacterial outer membrane protein. *J Mol Biol* 218:141–148.
36. Robert V, Volokhina EB, Senf F, Bos MP, Van Gelder P, Tommassen J (2006) Assembly factor Omp85 recognizes its outer membrane protein substrates by a species-specific C-terminal motif. *PLoS Biol* 4:e377: 1984–1995.
37. de Cock H, Struyve M, Kleerebezem M, van der Krift T, Tommassen J (1997) Role of the carboxy-terminal phenylalanine in the biogenesis of outer membrane protein PhoE of *Escherichia coli* K-12. *J Mol Biol* 269: 473–478.
38. Lehr U, Schutz M, Oberhettinger P, Ruiz-Perez F, Donald JW, Palmer T, Linke D, Henderson IR, Autenrieth IB (2010) C-terminal amino acid residues of the trimeric autotransporter adhesin YadA of *Yersinia enterocolitica* are decisive for its recognition and assembly by BamA. *Mol Microbiol* 78:932–946.
39. Koebnik R, Locher KP, Van Gelder P (2000) Structure and function of bacterial outer membrane proteins: barrels in a nutshell. *Mol Microbiol* 37:239–253.
40. Malinverni JC, Werner J, Kim S, Sklar JG, Kahne D, Misra R, Silhavy TJ (2006) YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in *Escherichia coli*. *Mol Microbiol* 61:151–164.
41. Hagan CL, Kim S, Kahne D (2010) Reconstitution of outer membrane protein assembly from purified components. *Science* 328:890–892.
42. Rossiter AE, Leyton DL, Tveen-Jensen K, Browning DF, Sevastyanovich Y, Knowles TJ, Nichols KB, Cunningham AF, Overduin M, Schembri MA, Henderson IR (2011) The essential  $\beta$ -barrel assembly machinery complex components BamD and BamA are required for autotransporter biogenesis. *J Bacteriol* 193:4250–4253.
43. Jain S, Goldberg MB (2007) Requirement for YaeT in the outer membrane assembly of autotransporter proteins. *J Bacteriol* 189:5393–5398.
44. Sauri A, Soprova Z, Wickström D, de Gier JW, Van der Schors RC, Smit AB, Jong WS, Luirink J (2009) The Bam (Omp85) complex is involved in secretion of the autotransporter haemoglobin protease. *Microbiology* 155:3982–3991.
45. Bodelón G, Marín E, Fernández LA (2009) Role of periplasmic chaperones and BamA (YaeT/Omp85) in folding and secretion of intimin from enteropathogenic *Escherichia coli* strains. *J Bacteriol* 191:5169–5179.
46. Palomino C, Marín E, Fernández LA (2011) The fimbrial usher FimD follows the SurA-BamB pathway for its assembly in the outer membrane of *Escherichia coli*. *J Bacteriol* 193:4250–4253.
47. Sugimura K, Nishihara T (1988) Purification, characterization, and primary structure of *Escherichia coli* protease VII with specificity for paired basic residues: identity of protease VII and OmpT. *J Bacteriol* 170: 5625–5632.
48. van Ulsen P (2011) Protein folding in bacterial adhesion: secretion and folding of classical monomeric autotransporters. *Adv Exp Med Biol* 715:125–142.
49. Collins RF, Derrick JP (2007) Wza: a new structural paradigm for outer membrane secretory proteins? *Trends Microbiol* 15:96–100.
50. Collins S, Guilvout I, Chami M, Pugsley AP (2007) YaeT-independent multimerization and outer membrane association of secretin PulD. *Mol Microbiol* 64:1350–1357.
51. Zeth K (2010) Structure and evolution of mitochondrial outer membrane proteins of beta-barrel topology. *Biochim Biophys Acta* 1797:1292–1299.
52. Gentle I, Gabriel K, Beech P, Waller R, Lithgow T (2004) The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria. *J Cell Biol* 164:19–24.
53. Wu T, Malinverni J, Ruiz N, Kim S, Silhavy TJ, Kahne D (2005) Identification of a multicomponent complex required for outer membrane biogenesis in *Escherichia coli*. *Cell* 121:235–245.
54. Sklar JG, Wu T, Gronenberg LS, Malinverni JC, Kahne D, Silhavy TJ (2007) Lipoprotein SmpA is a component of the YaeT complex that assembles outer membrane proteins in *Escherichia coli*. *Proc Natl Acad Sci USA* 104:6400–6405.



55. Andres C, Agne B, Kessler F (2010) The TOC complex: preprotein gateway to the chloroplast. *Biochim Biophys Acta* 1803:715–723.
56. Kim S, Malinverni JC, Sliz P, Silhavy TJ, Harrison SC, Kahne D (2007) Structure and function of an essential component of the outer membrane protein assembly machine. *Science* 317:961–964.
57. Gatzeva-Topalova PZ, Walton TA, Sousa MC (2008) Crystal structure of YaeT: conformational flexibility and substrate recognition. *Structure* 16:1873–1881.
58. Gatzeva-Topalova PZ, Warner LR, Pardi A, Sousa MC (2010) Structure and flexibility of the complete periplasmic domain of BamA: the protein insertion machine of the outer membrane. *Structure* 18:1492–1501.
59. Zhang H, Gao ZQ, Hou HF, Xu JH, Li LF, Su XD, Dong YH (2011) High-resolution structure of a new crystal form of BamA POTRA4–5 from *Escherichia coli*. *Acta Crystallogr F* 67:734–738.
60. Knowles TJ, Jeeves M, Bobat S, Dancea F, McClelland D, Palmer T, Overduin M, Henderson IR (2008) Fold and function of polypeptide transport-associated domains responsible for delivering unfolded proteins to membranes. *Mol Microbiol* 68:1216–1227.
61. Koenig P, Mirus O, Haarmann R, Sommer MS, Sinning I, Schleiff E, Tews I (2010) Conserved properties of polypeptide transport-associated (POTRA) domains derived from cyanobacterial Omp85. *J Biol Chem* 285:18016–18024.
62. Arnold T, Zeth K, Linke D (2010) Omp85 from the thermophilic cyanobacterium *Thermosynechococcus elongatus* differs from proteobacterial Omp85 in structure and domain composition. *J Biol Chem* 285:18003–18015.
63. Clantin B, Delattre AS, Rucktooa P, Saint N, Meli AC, Loch C, Jacob-Dubuisson F, Villeret V (2007) Structure of the membrane protein FhaC: a member of the Omp85-TpsB transporter superfamily. *Science* 317:957–961.
64. Albrecht R, Zeth K (2011) Structural basis of outer membrane protein biogenesis in bacteria. *J Biol Chem* 286:27792–27803.
65. Heuck A, Schleiff A, Clausen T (2011) Augmenting beta-augmentation: structural basis of how BamB binds BamA and may support folding of outer membrane proteins. *J Mol Biol* 406:659–666.
66. Kim KH, Paetzel M (2011) Crystal structure of *Escherichia coli* BamB, a lipoprotein component of the beta-barrel assembly machinery complex. *J Mol Biol* 406:667–678.
67. Noinaj N, Fairman JW, Buchanan SK (2011) The crystal structure of BamB suggests interactions with BamA and its role within the BAM complex. *J Mol Biol* 407:248–260.
68. Kim KH, Aulakh S, Tan W, Paetzel M (2011) Crystallographic analysis of the C-terminal domain of the *Escherichia coli* lipoprotein BamC. *Acta Crystallogr F* 67:1350–1358.
69. Kim KH, Aulakh S, Paetzel M (2011) Crystal structure of the  $\beta$ -barrel assembly machinery BamCD complex. *J Biol Chem* 286:39116–39121.
70. Warner LR, Varga K, Lange OF, Baker SL, Baker D, Sousa MC, Pardi A (2011) Structure of the BamC two-domain protein obtained by Rosetta with a limited NMR data set. *J Mol Biol* 411:83–95.
71. Sandoval CM, Baker SL, Jansen K, Metzner SI, Sousa MC (2011) Crystal structure of BamD: an essential component of the beta-Barrel assembly machinery of gram-negative bacteria. *J Mol Biol* 409:348–357.
72. Kim KH, Kang HS, Okon M, Escobar-Cabrera E, McIntosh LP, Paetzel M (2011) Structural characterization of *Escherichia coli* BamE, a lipoprotein component of the beta-barrel assembly machinery complex. *Biochemistry* 50:1081–1090.
73. Knowles TJ, Browning DF, Jeeves M, Maderbocus R, Rajesh S, Sridhar P, Manoli E, Emery D, Sommer U, Spencer A, Leyton DL, Squire D, Chaudhuri RR, Viant MR, Cunningham AF, Henderson IR, Overduin M (2011) Structure and function of BamE within the outer membrane and the beta-barrel assembly machine. *EMBO Rep* 12:123–128.
74. Vanini MM, Spisni A, Sforca ML, Pertinhez TA, Benedetti CE (2008) The solution structure of the outer membrane lipoprotein OmlA from *Xanthomonas axonopodis* pv. *citri* reveals a protein fold implicated in protein-protein interaction. *Proteins* 71:2051–2064.
75. Hayashi S, Wu HC (1990) Lipoproteins in bacteria. *J Bioenerg Biomembr* 22:451–471.
76. Hagan CL, Kahne D (2011) The reconstituted *Escherichia coli* Bam complex catalyzes multiple rounds of beta-barrel assembly. *Biochemistry* 50:7444–7446.
77. Hagan CL, Silhavy TJ, Kahne D (2011) beta-Barrel membrane protein assembly by the Bam complex. *Annu Rev Biochem* 80:189–210.
78. Onufryk C, Crouch ML, Fang FC, Gross CA (2005) Characterization of six lipoproteins in the sigmaE regulon. *J Bacteriol* 187:4552–4561.
79. Gatsos X, Perry AJ, Anwari K, Dolezal P, Wolyne PP, Likic VA, Purcell AW, Buchanan SK, Lithgow T (2008) Protein secretion and outer membrane assembly in Alphaproteobacteria. *FEMS Microbiol Rev* 32:995–1009.
80. Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N (2009) Importing mitochondrial proteins: machineries and mechanisms. *Cell* 138:628–644.
81. Paschen SA, Neupert W, Rapaport D (2005) Biogenesis of beta-barrel membrane proteins of mitochondria. *Trends Biochem Sci* 30:575–582.
82. Oreb M, Tews I, Schleiff E (2008) Policing Tic 'n' Toc, the doorway to chloroplasts. *Trends Cell Biol* 18:19–27.
83. Sommer MS, Daum B, Gross LE, Weis BL, Mirus O, Abram L, Maier UG, Kuhlbrandt W, Schleiff E (2011) Chloroplast Omp85 proteins change orientation during evolution. *Proc Natl Acad Sci USA* 108:13841–13846.
84. Schleiff E, Soll J (2005) Membrane protein insertion: mixing eukaryotic and prokaryotic concepts. *EMBO Rep* 6:1023–1027.
85. Sanchez-Pulido L, Devos D, Genevrois S, Vicente M, Valencia A (2003) POTRA: a conserved domain in the FtsQ family and a class of beta-barrel outer membrane proteins. *Trends Biochem Sci* 28:523–526.
86. Stegmeier JF, Andersen C (2006) Characterization of pores formed by YaeT (Omp85) from *Escherichia coli*. *J Biochem* 140:275–283.
87. Jacob-Dubuisson F, Villeret V, Clantin B, Delattre AS, Saint N (2009) First structural insights into the TpsB/Omp85 superfamily. *Biol Chem* 390:675–684.
88. Delattre AS, Clantin B, Saint N, Loch C, Villeret V, Jacob-Dubuisson F (2010) Functional importance of a conserved sequence motif in FhaC, a prototypic member of the TpsB/Omp85 superfamily. *FEBS J* 277:4755–4765.
89. Misra R (2007) First glimpse of the crystal structure of YaeT's POTRA domains. *ACS Chem Biol* 2:649–651.
90. Knowles TJ, Bobat S, Jeeves M, Henderson IR, Overduin M (2007) Secondary structure and <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N resonance assignments of the *Escherichia coli* YaeT POTRA domain. *Biomol NMR Assign* 1:113–115.

91. Bos MP, Robert V, Tommassen J (2007) Functioning of outer membrane protein assembly factor Omp85 requires a single POTRA domain. *EMBO Rep* 8:1149–1154.
92. Bennion D, Charlson ES, Coon E, Misra R (2010) Dissection of beta-barrel outer membrane protein assembly pathways through characterizing BamA POTRA 1 mutants of *Escherichia coli*. *Mol Microbiol* 77:1153–1171.
93. Remaut H, Waksman G (2006) Protein-protein interaction through beta-strand addition. *Trends Biochem Sci* 31:436–444.
94. Charlson ES, Werner JN, Misra R (2006) Differential effects of yfgL mutation on *Escherichia coli* outer membrane proteins and lipopolysaccharide. *J Bacteriol* 188:7186–7194.
95. Ureta AR, Endres RG, Wingreen NS, Silhavy TJ (2007) Kinetic analysis of the assembly of the outer membrane protein LamB in *Escherichia coli* mutants each lacking a secretion or targeting factor in a different cellular compartment. *J Bacteriol* 189:446–454.
96. Vuong P, Bennion D, Mantei J, Frost D, Misra R (2008) Analysis of YfgL and YaeT interactions through bioinformatics, mutagenesis, and biochemistry. *J Bacteriol* 190:1507–1517.
97. Albrecht R, Zeth K (2010) Crystallization and preliminary X-ray data collection of the *Escherichia coli* lipoproteins BamC, BamD and BamE. *Acta Crystallogr F* 66:1586–1590.
98. Knowles TJ, McClelland DM, Rajesh S, Henderson IR, Overduin M (2009) Secondary structure and (1)H, (13)C and (15)N backbone resonance assignments of BamC, a component of the outer membrane protein assembly machinery in *Escherichia coli*. *Biomol NMR Assign* 3:203–206.
99. Job V, Mattei PJ, Lemaire D, Attree I, Dessen A (2010) Structural basis of chaperone recognition of type III secretion system minor translocator proteins. *J Biol Chem* 285:23224–23232.
100. Kajander T, Sachs JN, Goldman A, Regan L (2009) Electrostatic interactions of Hsp-organizing protein tetratricopeptide domains with Hsp70 and Hsp90: computational analysis and protein engineering. *J Biol Chem* 284:25364–25374.
101. Stanley WA, Filipp FV, Kursula P, Schuller N, Erdmann R, Schliebs W, Sattler M, Wilmanns M (2006) Recognition of a functional peroxisome type 1 target by the dynamic import receptor pex5p. *Mol Cell* 24:653–663.
102. Lewis C, Skovierova H, Rowley G, Rezuchova B, Homerova D, Stevenson A, Sherry A, Kormanec J, Roberts M (2008) Small outer-membrane lipoprotein, SmpA, is regulated by sigmaE and has a role in cell envelope integrity and virulence of *Salmonella enterica* serovar Typhimurium. *Microbiology* 154:979–988.
103. Endo T, Kawano S, Yamano K (2011) BamE structure: the assembly of beta-barrel proteins in the outer membranes of bacteria and mitochondria. *EMBO Rep* 12:94–95.
104. Knowles TJ, Sridhar P, Rajesh S, Manoli E, Overduin M, Henderson IR (2010) Secondary structure and 1H, 13C and 15N resonance assignments of BamE, a component of the outer membrane protein assembly machinery in *Escherichia coli*. *Biomol NMR Assign* 4:179–181.
105. Bos MP, Tommassen J (2004) Biogenesis of the Gram-negative bacterial outer membrane. *Curr Opin Microbiol* 7:610–616.