Review

Evolution of the β-barrel assembly machinery

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Proteins from the Omp85 family have roles in membrane biogenesis, and the archetypal protein of this family is the bacterial outer membrane protein BamA. Through evolution, BamA has acquired membrane protein partner subunits, but distinct partner subunits are evident in the various bacterial lineages. As a result, experimental work on several species of bacteria has revealed varietal forms of the β-barrel assembly machinery (BAM complex). This scenario extends even into mitochondria and plastids, organelles of eukaryotic cells that evolved from intracellular bacterial ancestors. In addition to the BAM complex, other molecular machines, namely the two-partner secretion system (TPS) and the translocation and assembly module (the TAM), probably evolved from gene duplication events involving BamA. We discuss what is known about the diverse composition of the BAM complex in various bacterial lineages, and how this diversity impacts on our understanding of the mechanism underlying the assembly of bacterial outer membranes.

An ancient protein family and a function essential to the first bacteria

Gram-negative bacteria are encased by two membranes, and proteins located in the outer membrane are crucial for transfer of substances (e.g., porins) and as the physical point of contact with the environment (e.g., adhesins and other cellular projections). The majority of integral outer membrane proteins are composed from β-strands, which, when stitched together, form a β-barrel structure, a membrane-embedded, cylinder-shaped protein that, in the case of porins, forms a channel for the passage of small molecules. Correct folding and assembly of β-barrel proteins into the lipid phase of the outer membrane is not a trivial task, and depends on a cellular machinery to ensure this process is performed correctly and efficiently. The BAM complex executes this indispensable role, the core component of which is a protein called BamA. Conserved throughout the bacterial kingdom, and probably present in the earliest bacteria, BamA belongs to the Omp85 family of proteins [1–3].

Consistent with the hypothesis that they were present in the earliest forms of bacteria, members of the Omp85 protein family appear to be present in all bacteria with outer membranes. All Omp85 family members consist of a conserved type of β-barrel referred to as the D15 domain (Box 1) or the bacterial surface antigen domain that serves to anchor the protein into the outer membrane. Attached to this C-terminal D15 domain are from one to seven polypeptide-transport associated (POTRA) domains in the N-terminal region of the Omp85 protein [4]. As detailed later, the POTRA domains interact with proteins, the partner subunits of the Omp85 and/or accessory subunits and/or substrate proteins en route into the outer membrane.

All Omp85 proteins described so far function in protein transport, either in protein assembly into or protein translocation through the outer membrane. The prototypical member of the Omp85 family, BamA, was first identified in the outer membranes of cyanobacteria [5]. It was shown that BamA drives the assembly of β-barrel proteins into the outer membrane of *Neisseria meningitidis* and *Escherichia coli* [6–9]. Specific lipoproteins dock to BamA to constitute the BAM complex (Figure 1) and the quaternary structure varies according to the bacterial lineage. Lineage-specific differences in the BAM complex as a molecular machine are the main theme of this review. We discuss what is known of the diversification of the machinery through bacterial groups other than the well-studied experimental models and into organelles that derived from bacterial ancestors. In particular, we address the emerging concept that evolution has resulted in distinct partner proteins that interact with BamA in diverse bacterial taxonomic groups. This diversity in composition offers a better understanding of the key elements required for BAM complex function in general.

The three known classes of the Omp85 protein family in bacteria

The most architecturally simple protein transport system known, which has an Omp85 family protein as its core component, is the two-partner secretion system (TPSS), also referred to as the type Vb secretion system [10,11]. TPSSs have been characterized in just a few groups of proteobacteria, suggesting they are a relatively new evolutionary invention. Each TPSS is dedicated to secreting a unique virulence factor, some of which are characterized toxins and adhesins. The TPSS consists of an Omp85 family protein with only two POTRA domains and this protein is necessary and sufficient for substrate translocation through the outer membrane (Figure 1) [12]. The protein FhaC from *Bordetella pertussis* is the exemplar for this class of protein translocases and was crystallized to

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Box 1. Early identification of BamA through vaccinology

The Omp85 protein family came into the scientific spotlight in 1990, when a protein termed D15 surface antigen, the first member of the Omp85 family described, was recognized as the target of antibodies present in patient sera infected with *Haemophilus influenza* [78]. It was subsequently demonstrated that antibodies against this protein protected rats from infection [79] and that the protein contains immunodominant B cell epitopes [80]. These early findings were not limited to *H. influenzae*; in 1996, Ruffolo and Adler showed a protective effect of murine immunization with the *Pasteurella multicoda* homolog called Oma87 [81]. Recent studies have brought this area of research back in vogue for pathogens such as *Burkholderia* [82], *Edwardsiella* [83], *Actinobacillus* [84], and *Chlamydia* [85]. In addition, the essential nature of the BAM complex has sparked recent investigations into its lipoprotein subunits as antibiotic targets, which might lead to new paths being followed in the fight against bacterial pathogens [86].

provide the only known three-dimensional structure for an Omp85 family protein [13].

A distinct subgroup of the Omp85 family (TamA) was recently discovered in bacteria as the core to the translocation and assembly module (TAM); this TamA component of the TAM is found in bacterial outer membranes (Figure 1). First characterized as YtfM, an outer membrane protein of unknown function [14], it has been shown that TamA is crucial for the assembly of autotransporters onto the surface of the cell (Box 2) [15]. Its partner protein, TamB, is an integral inner membrane protein. Although still speculative, there is a strong indication that the TPSSs and TAM share an evolutionary history. Both systems are encoded in similarly structured operons in which an open reading frame encoding the member of the Omp85 protein family precedes and is transcriptionally linked to an open reading frame encoding a large protein of β-helical domain structure [15,16]. In the case of the TPSS, the β-helical protein has a cleavable signal sequence and is secreted into the periplasm, and its interaction with the Omp85 family member subunit (e.g., FhaC) results in translocation across the outer membrane [17]. In the case of the TAM, secondary structure prediction suggests that TamB also has high β-helical content and has a non-cleavable signal anchor [16], allowing secretion into the periplasm. However, TamB remains anchored in the inner membrane, and its interaction with TamA results in a docking that generates the TAM complex (Figure 1) [18].

Pivotal studies on BamA in *N. meningitidis* (where it was first called simply Omp85) and *E. coli* (first called YaeT) have led to our current understanding of protein assembly into the bacterial outer membrane [6,8,9]. Using both genetic and biochemical approaches, a set of four partner proteins docked to BamA have been identified. BamB, BamC, BamD, and BamE are all lipoproteins targeted to the outer membrane via the Lol translocation pathway and are membrane-anchored through lipidation of an N-terminal cysteine [9,19,20]. The complex from *E. coli* has a molecular size estimated to be ~200 kDa when allowances are made for the likely contribution of detergent, consistent with the size of a complex in which a molecule of BamA carries a single copy of each accessory lipoprotein, although the possibility of more than one BamE subunit in the complex remains possible (BamE is a small protein, so each molecule contributes relatively little to the overall mass of the complex) [21–23]. In the absence of lipoprotein partners, BamA self-associates to form tetramers [24]. The implications of all of this remain speculative, but a dynamic association of BAM complexes mediated through BamA–BamA interactions cannot be ruled out and might be important as models to explain BAM complex handling of β-barrel substrate proteins.

Crystal structures and evolutionary insights

The complete architecture and subunit arrangement within the BAM complex are still to be deciphered, but recent characterization of the structures of BamB, BamC, BamD, and BamE from *E. coli* have contributed exciting new insights into their functional influences and their evolution (Figure 2). BamB exhibits a β-propeller fold that forms a ring-like structure [25–27]. Genetic studies have demonstrated that BamB binds to the POTRA domains of BamA, an interaction supported by modeling studies using individual crystal structures [22,27,28]. Evidence of a direct interaction between BamB and β-barrel substrates comes from chemical crosslinking of nascent β-barrel molecules to BamB [21,29]. BamB is found in essentially all Alpha-, Beta- and Gammaproteobacteria but not in other bacterial groups. This raises the question of where BamB came from. Bioinformatics has been used to track the ancestry of BamB and sequence similarity suggests that the lipoprotein is derived from cytoplasmic dehydrogenases [30]. The β-propeller fold (Figure 2a) shared by these proteins is a common framework requiring a signal sequence and only a little evolutionary tinkering to convert the enzymatic functions of dehydrogenases into protein–protein docking functions [31], such as proposed for BamB [27].

BamC contains two helix-grip domains (Figure 2b), the structures of which have been elucidated using X-ray crystallography and NMR [25,32–34]. This compact region of BamC is preceded by a long, disordered N-terminal segment. BamD forms an elongated bundle of helices termed tetratricopeptide repeats (TPRs) [25,35,36]. In *E. coli*, BamD directly binds BamA via POTRA 5 (the POTRA domain closest to the D15 domain of BamA) and forms a platform on which both BamC and BamE dock the complex [19,22]. This interaction between BamC and BamD has been captured structurally to show that the disordered N-terminal segment of BamC binds to BamD [32]. How BamE interacts with BamD is not yet clear but, as shown in Figure 2c, BamE has an extended, disordered N-terminal segment followed by a small compact domain of ~8 kDa in size [25,37,38]. For an extensive analysis and discussion of subunits of the BAM complex in *E. coli*, we refer readers to current reviews [7,39,40].

The POTRA domains of BamA are essential for binding the lipoprotein partners BamB, C, D and E [22], interacting with the periplasmic chaperone SurA [41], and potentially also binding β-barrel substrate proteins [42,43]. In *N. meningitidis* only POTRA 5 is essential [44], whereas POTRA 3, POTRA 4, and POTRA 5 are all essential in *E. coli* [22]. However, apparently contradictory data from distinct bacterial species need to be viewed in light of (i) which partner lipoproteins are present in each species, (ii) which periplasmic chaperone system the species depends

Box 2. Emergence of a novel bacterial outer membrane protein biogenesis complex

TamA is a member of the Omp85 protein family distinguished by three POTRA domains and serves as the core component of a protein module that drives the translocation of autotransporters across the outer membrane [14,15,51]. TamA depends on the assistance of a partner protein, TamB, which is anchored into the inner membrane, docking to TamA through complementary surfaces projecting into the periplasm.

Autotransporters were once believed to be self-contained transport systems for the delivery of an effector domain to the surface of bacterial cells. The discovery and growing understanding of the BAM complex shattered this dogma, and now numerous examples demonstrate that the autotransporter β-barrel domain needs the BAM complex for assembly into the outer membrane [87]. Functional characterization of the TAM opens the way to a fuller understanding of autotransporter biogenesis; in cells lacking the TAM, autotransporter adhesins are unable to induce cell–cell aggregation as seen in wild-type cells, even though their β-barrel domains are assembled in the outer membrane [15]. A working model to explain these early observations would be that once an autotransporter arrives at the BAM complex and its β-barrel is being assembled into the outer membrane, the TAM assists the passenger domain to breach the outer membrane.

on most, and (iii) what range of β-barrel substrates are required to generate a viable phenotype. These three factors are well understood for *E. coli* [7,39,40] and may generally be similar in other species of the Gammaproteobacteria, but not necessarily in other groups of bacteria.

Comprehensive analysis of Proteobacteria revealed that the subunit composition of the BAM complex is not conserved even within this group of relatively closely related organisms [30,45]. The Gamma- and Betaproteobacteria seem to be the only subgroups with a predicted complete set of the classical BamB–E lipoproteins (BamB is even absent from *N. meningitidis* [46], but is present in other species of Betaproteobacteria [30,45]). Furthermore, there is an apparent absence of BamB, BamC, and BamE from the Epsilon- and Deltaproteobacteria. Although this could indicate a more streamlined molecular machine in the outer membranes of the Epsilon- and Deltaproteobacteria, it could as easily be an indication that distinct partner proteins are found in the BAM complex of these organisms. Evidence supporting the hypothesis that distinct lipoproteins have gained functional significance in the BAM complex during the evolution of distinct lineages comes from studies of the Alphaproteobacteria. Of the 149 Alphaproteobacteria genomes that have been sequenced, none encodes a protein related to BamC [30,45]. Purification of the BAM complex from the Alphaproteobacterium *Caulobacter crescentus* revealed the presence of a novel lipoprotein referred to as BamF as a component of the BAM complex [30]. BamF and BamC are apparently unrelated except for a short sequence segment in the unstructured region that docks BamC onto BamD in *E. coli* [32]; it has also been proposed that this sequence docks BamF onto BamD in Alphaproteobacteria [30].

When considering other bacterial phyla, a global picture begins to emerge for the evolution of the diversity of the BAM complex (Figure 3). A protein showing sequence similarity with BamA was even identified in the highly divergent group of Deinococcus-Thermus bacteria; it is essential for viability and shows similar sequence (including five POTRA domains) and channel conductance characteristics to the proteobacterial BamA [47]. However, whereas BamA is conserved throughout, there are a few groups of bacteria in which the number of POTRA domains deviates from the canonical five, perhaps reflecting differences in the lipoprotein partners in the BAM complex of these organisms and/or the chaperones used to deliver substrate proteins to the BAM complex (Table 1). Here we consider three informative cases in detail.

Case 1: the Spirochaetes

Recent studies have shed light on outer membrane assembly in Spirochaetes, a group of bacteria that includes important pathogens of humans with a radically different outer membrane protein repertoire compared to other

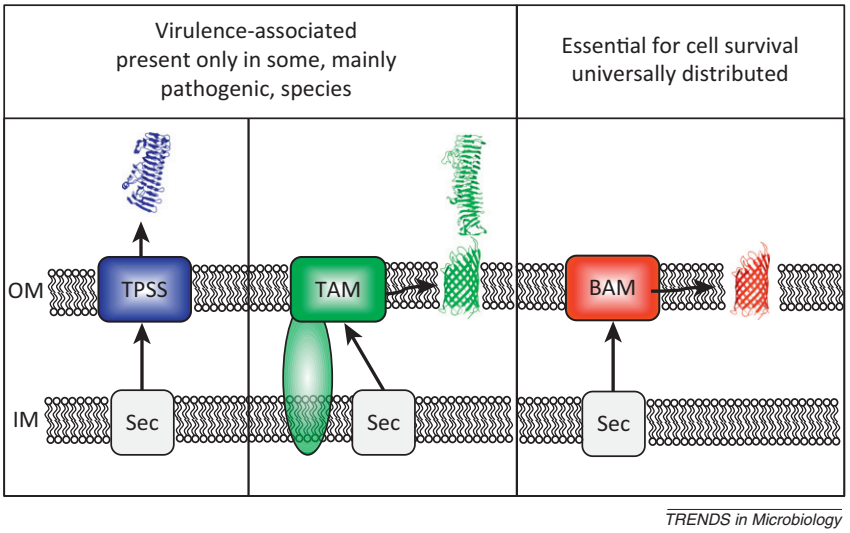

Figure 1. The Omp85 family subgroups. Schematic comparison of the β-barrel assembly machinery (BAM) complex and related molecular machines, the two-partner secretion system (TPSS) and the translocation and assembly module (TAM). In all three, a member of the Omp85 family of proteins serves as the key outer membrane (OM) component. Whereas the TPSS and the TAM are similar with respect to their important roles in the translocation of bacterial virulence factors, the BAM complex is fundamentally important and essential for cell viability. Abbreviations: IM, inner membrane; Sec, Sec protein translocation pathway.

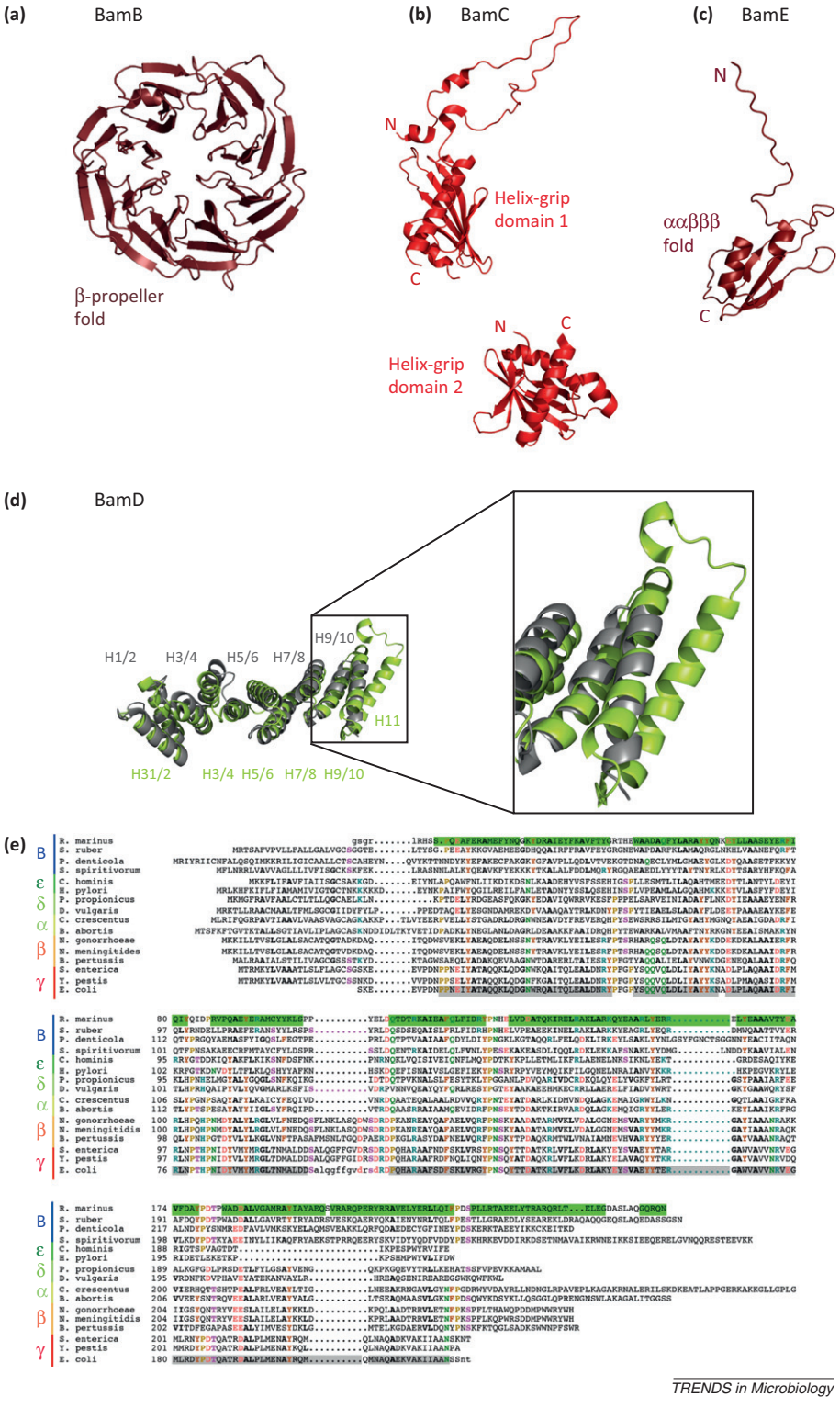

Figure 2. Structural characteristics of the lipoprotein subunits of the β-barrel assembly machinery (BAM) complex. Ribbon diagrams of lipoproteins from *Escherichia coli*. **(a)** BamB (Protein Data Bank [PDB] 3Q7O). **(b)** BamC (residues 28–213, PDB 3TGO, chain C and residues 226–344, PDB 2YH5). **(c)** BamE (PDB 2KM7). **(d)** *E. coli* BamD (PDB 3Q5M) shown in gray is overlaid with BamD from *R. marinus* (PDB 3QKY) shown in green. Distinctions in the helical packing are highlighted in the magnified view. **(e)** Selected BamD homologs from all five groups of Proteobacteria as well as the Bacteroidetes were aligned using STRAP [67], with helices highlighted. The sequences shown are from *E. coli* (PDB 3Q5M, residues 23–245), *Rhodothermus marinus* (PDB 3QKY, residues 25–280), *Yersinia pestis* (NP_668242.1), *Salinibacter ruber* (YP_446179.1), *Prevotella denticola* (ZP_08171093.1), *Campylobacter hominis* (YP_001406138.1), *Helicobacter pylori* (YP_005765874.1), *Pelobacter propionicus* (YP_902305.1), *Desulfovibrio vulgaris* (YP_966770.1), *Bordetella pertussis* (NP_879922.1), *Neisseria meningitidis* (NP_273745.1), *Caulobacter crescentus* (NP_420791.1), *Brucella abortus* (YP_222108.1), *Neisseria gonorrhoeae* (YP_207439.1), *Salmonella enterica* (YP_002244664.1), and *Sphingobacterium spiritivorum* (ZP_03966075.1). Indicated on the left side are the different bacterial (sub)phyla: Bacteroidetes (B), Alphaproteobacteria (α), Betaproteobacteria (β), Gammaproteobacteria (γ), Deltaproteobacteria (δ) and Epsilonproteobacteria (ε).

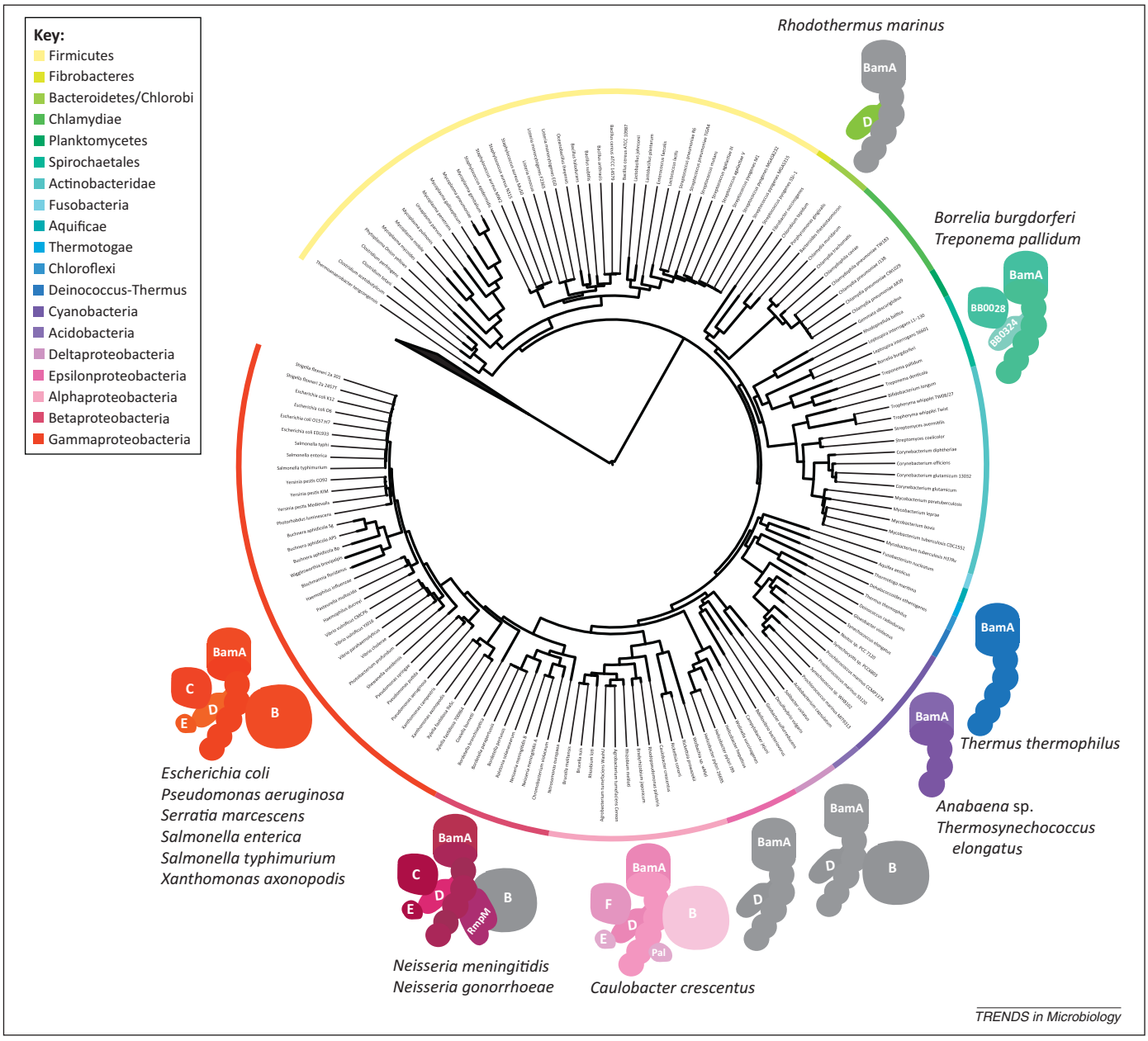

Figure 3. The β-barrel assembly machinery (BAM) complex across bacterial phyla. Our current knowledge of the BAM complex according to data for the known main bacterial taxonomic group; the tree representation was generated using the iTOL website [68]. Shapes labeled B–E represent homologs of BamB, BamC, BamD, and BamE, and unlabeled shapes associated with BamA indicate other partner proteins identified. Colored components indicate where functional studies support the proteins designation as a component of the BAM complex, whereas gray components have been predicted on the basis of sequence similarity but have not been shown to be part of the respective BAM complexes. Details for the components identified and the respective references are given in Table 1.

bacteria [48–50]. BamA from *Borrelia burgdorferi* shares a high level of conservation with the *E. coli* protein, even across all five POTRA domains [49]. The BAM complex purified from *B. burgdorferi* is oligomeric and two partner proteins have been identified [50]. One of them shares sequence similarity to BamD, but the other protein has no obvious homolog in Proteobacteria and bioinformatic analyses did not reveal any signature sequences as clues to the domain structure of the protein. This highlights that the BAM complex has taken a very different shape through evolution. We suggest that appreciation of taxon-specific subunit composition for the BAM complex will be invaluable in deciphering the key activities that the overall machinery must provide to assemble substrate proteins into the outer membrane. Of further interest, it has been noted that in the genome of *B. burgdorferi* there is no gene encoding TamA but that the gene for the inner membrane component of the TAM, TamB, is situated immediately upstream of – and perhaps transcriptionally coupled to – the gene encoding BamA [15]. Hypothetically, the Spirochaetes may have evolved a composite molecular machine combining the activities of both the TAM and BAM complex.

Case 2: the Cyanobacteria

Structural analyses of BamA from two cyanobacterial species, *Thermosynechococcus elongatus* and *Anabaena* sp., revealed only three POTRA domains [51,52]. These domains are highly conserved with respect to their structure, although a unique extended loop in the POTRA

Table 1. Summary of the BAM complex components across diverse bacteria

Taxonomy	Organism	Characterized BAM components	Evidence	Refs
Gammaproteobacteria	*Escherichia coli*	BamA, BamB, BamC, BamD, BamE	Location, function, structure, partner interactions	[7,39,40]
	Pseudomonas aeruginosa	BamA (Opr86), BamE (OmlA)	Location, function	[69,70]
	Serratia marcescens	BamC	Location, function	[71]
	Salmonella enterica	BamA, BamB, BamC, BamD, BamE	Function	[72,73]
	Salmonella enterica serovar Typhimurium	BamE (SmpA)	Function	[74]
	Xanthomonas axonopodis	BamE (OmlA)	Structure	[75]
Betaproteobacteria	*Neisseria meningitidis*	BamA, BamC, BamD, BamE RmpM	Location, function, partner interactions Structure	[6,8,46] [55]
	Neisseria gonorrhoeae	BamD (ComL)	Location, function	[76]
Alphaproteobacteria	*Caulobacter crescentus*	BamA, BamB, BamD, BamE Pal, BamF	Location, partner interactions, biochemical data	[30,56]
Bacteroidetes/Chlorobi	*Rhodothermus marinus*	BamD	Structure	[36]
Spirochaetes	*Borrelia burgdorferi*	BamA BB0324 (BamD), BB0028	Location, function, partner interactions	[49,50]
	Treponema pallidum	BamA	Location, biochemical data	[48]
Cyanobacteria	*Anabaena* sp.	BamA	Location, function, biochemical data	[53,77]
	Thermosynechococcus elongatus	BamA	Location, function, structure	[51]
Deinococcus-Thermus	*Thermus thermophilus*	BamA ($Omp85_{Tt}$)	Location, biochemical data	[47]

directly adjacent to the β-barrel (equivalent to POTRA 5 in *E. coli* and *N. meningitidis*) was observed and it has been suggested that this plays a role in substrate binding or recognition [52]. This large loop is conserved across species in a defined group of Cyanobacteria [52]. Another difference in the cyanobacterial BamA is that its ion conductance suggests a channel in its β-barrel domain greater than that in BamA from *E. coli* [51,53]. It remains unclear what physiological relevance, if any, the ion-conducting channel might have, but it is clear that the BamAs from these two species have some significant structural differences. Finally, chemical crosslinking studies identified a novel protein in *Anabaena* sp. that interacts with BamA [54]. The protein, Tic22, might be a periplasmic chaperone akin to SurA from *E. coli*, or a partner protein that directly functions in the BAM complex [54], or even an accessory factor more transiently bound to the BAM complex such as RmpM from *N. meningitidis* [46,55], Pal/YiaD from *C. crescentus* [56], and YiaD from *E. coli* [57].

Case 3: the Bacteroidetes

The Bacteroidetes are part of the commensal flora of the human gut [58] and in evolutionary terms are very distinct from the Proteobacteria. From this group of bacteria emerged the first study on the structure of BamD [36]. A comparison of BamD from *Rhodothermus marinus* and *E. coli* highlights considerable conservation at both the sequence and structural levels, particularly within the N-terminal TPRs (Figure 2d). The N-terminal region appears reminiscent of other proteins involved in binding substrates, further suggesting that conservation throughout this region is specifically related to function [25,36]. One point of difference is a C-terminal truncation in BamD from *E. coli* in comparison to BamD from *R. marinus* [30,36]. In Epsilonproteobacteria, BamD is even shorter than the protein from *E. coli* (Figure 2e). Given that the other major role of BamD in *E. coli* is to dock BamC and BamE, two partners absent from species other than the Gamma- and Betaproteobacteria, the BamD structures may offer insight into the number and/or type of partner lipoproteins docked into the varietal BAM complexes.

Thus, whereas the BAM complex in these various bacterial lineages is required for the same purpose – to assemble β-barrel proteins into the outer membrane – it appears that quite different, non-homologous partner proteins have evolved independently to assist BamA in this role. This raises important questions about what, in precise biochemical terms, the function of each partner subunit is and how, in structural terms, BAM mediates assembly of β-barrel substrates into the outer membrane (Box 3).

Evolution of protein translocases in organelles derived by endosymbiosis

There are two eukaryotic counterparts for BAM and these are found in plastids and mitochondria, organelles derived from bacterial endosymbionts.

Mitochondria evolved from Alphaproteobacteria and phylogenetics has shown that the mitochondrial outer membrane protein Sam50 evolved from the alphaproteobacterial BamA [59]. In addition, biochemical assays have demonstrated that Sam50 functions in the assembly of β-barrel proteins into the mitochondrial outer membrane [59–61]. Despite the relationship between Sam50 and BamA, the mitochondrial Sam50 operates in conjunction with two partner proteins, Sam35 and Sam37, that bear no resemblance to the lipoprotein partners in bacteria, but perhaps have some common topological features [23]. This scenario highlights that the conserved function of BAM complexes can be driven by non-homologous protein subunits docked to the Omp85 core, BamA in bacteria and Sam50 in eukaryotes.

Plastids are organelles derived from a cyanobacterial ancestor. They too have a BamA-related protein called

Box 3. Outstanding questions

- What is the atomic structure of BamA, and how do the POTRA and β-barrel move relative to each other?
- What is the architectural arrangement of the subunits in the BAM complex in *E. coli* and other phyla?
- What is the specific biochemical function of each of the lipoprotein components of the BAM complex?
- What is the mechanism by which a β-barrel precursor protein folds into a closed barrel and inserts into the plane of the membrane?
- Does the observed oligomerization of BamA have a physiological relevance, and can BamA:TamA oligomers form in the course of assembly of complicated β-barrels such as autotransporters into the bacterial outer membrane?

Oep80 (Toc75-V in *Arabidopsis*) that functions in protein assembly into the plastid outer membrane, and molecular phylogenetics demonstrates a clear scenario for its evolution from the BamA found in Cyanobacteria [62]. The first evolutionary link drawn between bacterial and eukaryotic Omp85 family members came from such an example [63]. Plastids have a second member of the Omp85 protein family, a functionally distinct protein called Toc75 (Toc75-III *Arabidopsis*) that is responsible for protein translocation across the plastid outer membrane [64,65]. Thus, just as bacteria have exploited Omp85 proteins for protein translocation across outer membranes as an adjunct to their key role in β-barrel protein assembly into membranes (i.e., the TPSS), evolution has also followed this route for the outer membranes of eukaryotic cell organelles.

Concluding remarks

The mechanism governing β-barrel protein assembly into a membrane remains elusive. Unlike transmembrane helices, which are inherently hydrophobic, β-barrels only adopt a hydrophobic surface once the β-strands interact and form their tertiary fold. Several studies have demonstrated that precursor β-barrel proteins can fold merely in the presence of a phospholipid bilayer [66]. In this way, integration into the membrane is directly associated with polypeptide folding. However, in a cellular context, this process is dependent on a protein machinery essential for function and hence cellular integrity. One of the major questions driving interest in the BAM complex is how it achieves this conversion of an amphipathic polypeptide into a barrel with a uniformly hydrophobic outer face. Coming to grips with the mechanistic detail has been made possible by new strategies using both *in vitro* and *in vivo* systems, although this is just the beginning [29,39]. Also needed are an understanding of the architectural arrangement of the subunits of the BAM complex and information on the specific biochemical function of each BAM complex subunit. Comparative analysis of the BAM complexes of distinct subunit ensembles offers an interesting avenue to determine the core activities needed to fold a β-barrel.

Of great interest is the protein–lipid interface surrounding BamA. Tantalizing suggestions that BamA oligomers can form, particularly when partner proteins are removed, lead to current hypotheses of a dynamic interstitial space in between the membrane-inserted domains of multiple copies of BamA, in which newly formed substrate β-barrels might move as their hydrophobic character is realized on folding. Given the intimate roles that BAM and TAM play in the assembly of autotransporters, whose enormous extracellular domains make them one of the most challenging of β-barrel proteins to assemble into the outer membrane, studies on oligomerization of BamA are promising a better general understanding of the process of β-barrel assembly into outer membranes.

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