The versatile β-barrel membrane protein

William C Wimley

The β-barrel membrane protein is found in the outer membranes of bacteria, mitochondria and chloroplasts. Approximately 2–3% of the genes in Gram-negative bacterial genomes encode β-barrels. Whereas there are fewer than 20 known three-dimensional β-barrel structures, genomic databases currently contain thousands of β-barrels belonging to dozens of families. New research is revealing the variety of β-barrel structures and the variety of functions performed by these versatile proteins.

Addresses
Department of Biochemistry SL43, Tulane University Health Sciences Center, New Orleans, LA 70112-2699, USA
e-mail: wwimley@tulane.edu

Abbreviations
OM   outer membrane
OMP   outer membrane protein
PDB   Protein Data Bank
TM   transmembrane

Introduction

Recent studies in genomics, proteomics, structural biology and genetics have begun to reveal the versatility and ubiquity of the β-barrel membrane protein. Although unified by many common structural features, β-barrel membrane proteins carry out diverse functions in diverse organisms. Their distribution extends from many families of bacteria to eukaryotes, all of which have β-barrel membrane proteins in their genomes. The variety of known functions performed by β-barrels is expanding and now includes all the functional categories listed in Table 1. The common structural features of the few β-barrels of known structure have been reviewed in detail elsewhere (e.g. [1,2]). Here, the focus will be on the diversity of distribution, function and architecture of β-barrel membrane proteins to draw attention to the many β-barrels whose three-dimensional structures are yet to be determined.

Structure and architecture

The number of possible designs for stable transmembrane (TM) β-sheets is limited by the physical constraints imposed by the nonpolar core of the bilayer membrane [3]. All TM proteins must fold into structures that are roughly cylindrical across the bilayer and that expose predominantly nonpolar sidechains to the membrane. Most importantly, any TM structure must involve all membrane-buried backbone polar groups in hydrogen bonds [3]. The only TM β-sheet structure known to satisfy these constraints is the β-barrel, in which the TM β-strands are laterally hydrogen bonded in a circular pattern. In a β-barrel membrane protein, these interstrand hydrogen bonds are the dominant stabilizing interaction [4] because they occur in the nonpolar bilayer milieu. The extensive nonlocal backbone hydrogen bonds between strands also greatly rigidify the core of the barrel structure [5•]. The contribution of each hydrogen bond to stabilization has been estimated to be as high as 0.5 kcal/mol/residue [3,6,7], which is very significant because even the smallest β-barrels have at least 80 amino acids in the membrane. As a result of these hydrogen-bonding interactions, β-barrels make very stable structures that do not readily unfold in membranes [8,9].

Currently, fewer than 20 unique TM β-barrel structures have been solved. A gallery of β-barrels is shown in Figure 1. Schulz [10] outlined a set of simple rules that describe the structural features of all β-barrels of known structure. In brief, all are cylindrical, closed barrels with an even number of TM β-strands that are connected in a β-meander topology with alternating tight turns and longer loops. This architecture suggests that the β-hairpin is the basic structural subunit of a TM β-barrel. Typically, one side of the membrane has only tight turns, as shown in Figure 1. The hydrophobic, lipid-exposed surface of a β-barrel is approximately 27 Å thick and includes two bands of aromatic residues that are found in the interfacial region of the bilayer, ± 10 Å from the bilayer mid-plane [11••]. Membranes are spanned by β-strands of 9–11 residues with a tilt of 20–45° out of the TM axis. The smallest known barrels contain 8 TM strands; this may be the minimum possible size [12] because of packing constraints in the barrel interior. The largest barrels of known structure, the TonB-dependent importers, contain 22 strands each. However, there is good evidence for the existence of β-barrels that are much larger [13•].

The membrane-interacting surfaces of β-barrels are cryptically encoded in their amino acid sequences, with a dyad repeat pattern of alternating residues oriented either toward the lipid bilayer or toward the barrel interior. As expected, the composition of the lipid-exposed surfaces of β-barrels is very similar to that of the lipid-exposed surfaces of helical membrane proteins, see Figure 2, with an abundance of phenylalanine, tyrosine,

Table 1

A variety of functions that are provided by β-barrel membrane proteins*.

Functional category	One example	Structure (PDB accession)
Nonspecific porin	OmpF	2OMF
Facilitated transporter	Maltoporin	1MPR
Energy-dependent transporter-efflux	TolC	1QJ8
Energy-dependent transporter-influx	FepA	1FEP
Protein secretion pore	PulD	NA
OM usher pore	PapC	NA
Adhesin	OmpX	1QJ8
Lipase	OmpLA	1QD6
Protease	OmpT	1I78
Mitochondria protein import pore	Tom40	NA
Protein pore-forming toxin	α-Hemolysin	7AHL

*For each category, I give one example for which some detailed information is known. These are broad functional categories. Some of them include multiple distinct families of proteins with hundreds of known members [25••]. A PDB accession number is given for those examples for which a three-dimensional structure is known. NA, not available.

tryptophan, valine and leucine, and the strong exclusion of polar and charged residues [11••,14,15]. The abundance of aromatic residues is especially striking at the bilayer interfaces, where they constitute about 40% of the lipid-exposed amino acids [11••]. Despite the fact that many β-barrels contain a water-filled channel, or a mobile hatch or plug domain, the abundant interior residues are predominantly the small or polar amino acids glycine, threonine, serine, asparagine and glutamine. Tyrosine is also relatively abundant, whereas the other hydrophobes are scarce [11••].

Although Schulz's rules [10] describe β-barrels of known structure very well, the sparse sampling of β-barrels to date means that many families of β-barrels with different architectures and topologies are yet to be described. Some examples of these other families are discussed below.

The bacterial 'OMPome'
Gram-negative bacteria have two bilayer membranes, an inner phospholipid membrane containing only α-helical membrane proteins and an outer phospholipid/lipopolysaccharide membrane containing only β-barrel membrane proteins. How many outer membrane (OM) β-barrel genes are in the genomes of Gram-negative bacteria? In the *Escherichia coli* genome, there are about 60 proteins, or 1.5% of the total, annotated as known, 'probable' or 'putative' outer membrane proteins (OMPs). This characterization is based mainly on sequence similarities to known proteins. Recognizing other TM β-barrels can be done using physical principles, but this is a more difficult task because the lipid-exposed surfaces of β-barrels are cryptic, obscured in the inside-outside dyad repeat

pattern of the TM β-strands (Figure 2). Several groups have recently developed algorithms to identify β-barrels using either composition/architecture-based methods [11••,16••] or neural networks [17••]. See Figure 3 for an example of one analysis. For Gram-negative bacterial genomes, all of these algorithms find an asymmetric distribution of β-barrel scores or ranking from which identification can be made with moderate accuracy. Wimley [11••] found that the highest scoring 2.5% of the genes of *E. coli*, about 105 proteins, encoded almost all known β-barrels and only a few false positives. Similarly, Zhai and Saier [16••] found 118 proteins (2.8% of the genome) that scored high enough to be considered putative β-barrels. The overlap between these two putative β-barrel sets is greater than 90%. Using a neural network analysis, Casadio and co-workers [17••] identified about 75 proteins, or 1.8% of the *E. coli* genome, as strong candidate β-barrels. All three groups found that one-third or more of the potential β-barrels in the *E. coli* genome are unknown or hypothetical proteins. Application of the same selection criteria to the genomes of less well annotated Gram-negative bacteria produces very similar β-barrel abundances, from about 2.1% of the approximately 5500 genes of *Pseudomonas aeruginosa* [11••] to 3.5% of the 3900 genes of *Yersinia pestis*. *Hemophilus influenza*, one of the smallest Gram-negative genomes (with ∼1700 genes), has approximately 40 proteins (or 2.5% of the total genome) that are potential β-barrel membrane proteins (WC Wimley, unpublished data). In summary, multiple independent analyses indicate that 2–3% of the genes in all Gram-negative bacteria encode β-barrel OMPs.

Recent proteomic analyses have begun to measure the number of OMPs that are actually expressed in Gram-negative bacteria. Molloy *et al.* [18] analyzed the OM proteome of *E. coli* using 2D gels and mass spectrometry, and identified about 20 proteins, or 0.5% of the genome, that are highly abundant OMPs. Maddock, Andrews and colleagues [19] have been analyzing the OM proteome of another Gram-negative bacterium, *Caulobacter crescentus*. By improving methodology and extending the pI range of separation, they were able to identify about 50 proteins (1.5% of the genome) in their OM preparations that are thought to be OM β-barrels [20••]. Importantly, as many as one-third of these observed OMPs are annotated as hypothetical proteins or are not annotated with any known function. Nonetheless, most of these are predicted by computational means (e.g. [11••]) to be OM β-barrels.

Folding and insertion
Tamm and colleagues [21] have shown that the insertion and folding of OmpA, an eight-stranded β-barrel, into phospholipid vesicles is a spontaneous and cooperative process. A similarly cooperative assembly of β-sheets has been noted for small, hydrophobic model peptides [6] and for pore-forming toxins [13•,22]. An all-or-none insertion process makes sense for β-barrels in light of their network

Figure 1
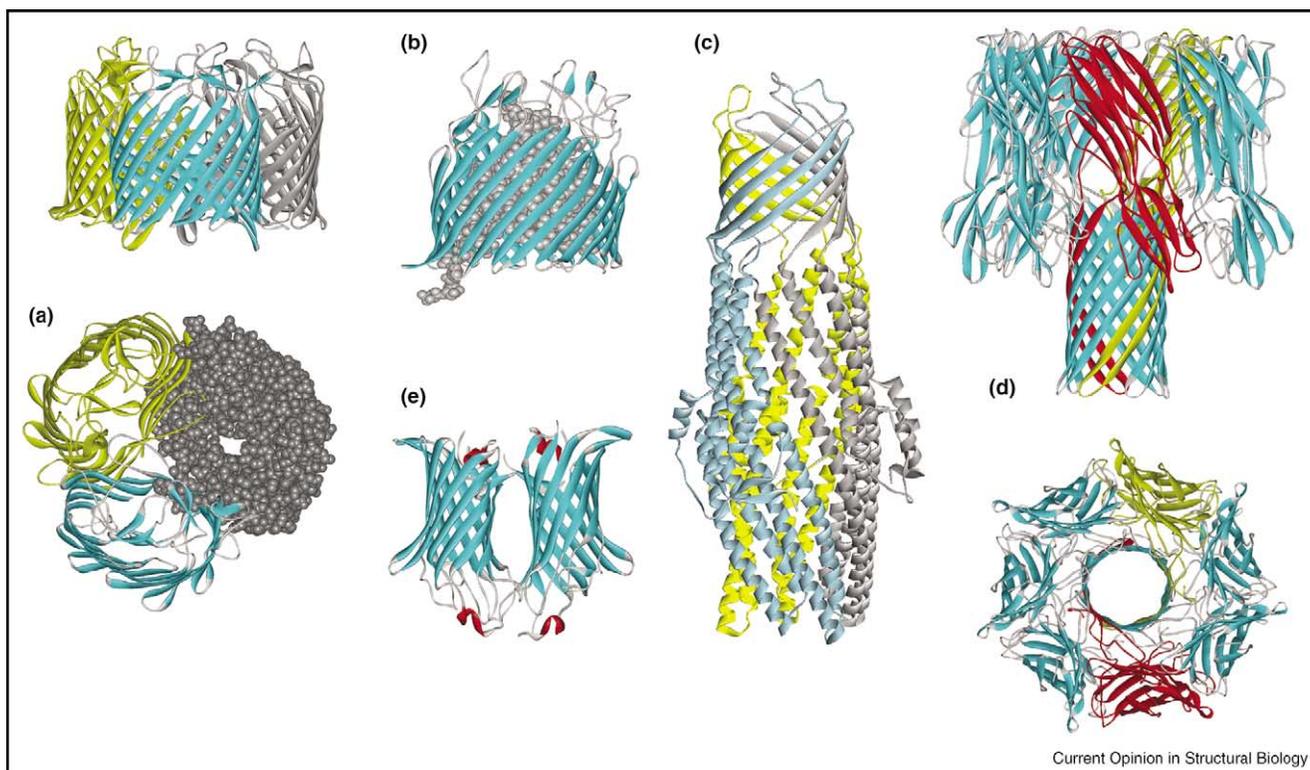
A gallery of β-barrel membrane protein structures. **(a)** Maltoporin, side view (upper) and bottom view (lower). Maltoporin is an example of the trimeric β-barrel porins, which are either nonspecific or facilitated transporters in the OMs of Gram-negative bacteria [51]. Maltoporin is a maltose transporter. Known trimeric porins have 16 or 18 TM strands, which comprise three separate barrels in the trimer. Each of the barrels contains a narrow, central channel. **(b)** FepA, a TonB-dependent Fe-siderophore transporter [52]. The four TonB-dependent receptors of known structure all have 22 membrane-spanning β-strands comprising a C-terminal β-barrel domain. They also have an N-terminal 'hatch' domain, shown here in gray space-filling representation, that plugs the barrel and regulates transport. TonB-dependent transporters use metabolic energy through interactions with inner membrane proteins [52,53]. **(c)** The protein TolC is a small-molecule exporter belonging to the OM factor family [25••,54]. TolC is involved in multidrug resistance of bacteria. It derives metabolic energy from its interaction with the inner bacterial membrane. TolC is unique among known β-barrel structures, comprising a trimeric, 12-stranded single barrel [55] in which each monomer contributes four strands to the barrel. The pore of the β-barrel is connected to the pore of a hollow α-helical bundle that extends across the periplasmic space to the inner membrane. **(d)** The protein toxin α-hemolysin from *S. aureus* [22,49]. This toxin is secreted as a monomeric protein that subsequently assembles into a heptameric 'prepore' on the membrane, followed by the cooperative insertion of the β-barrel across the membrane. Each monomer contributes one β-hairpin to the 14-stranded barrel. The central pore is an ungated path through the membrane that leads to osmotic cytolysis. The upper image is a side view and the lower image is a bottom view of the heptameric pore. **(e)** OmpLA, an OM phospholipase [56]. OmpLA is a dimer of 12-stranded barrels. The role of OmpLA may be to hydrolyze phospholipids that have migrated to the outer surface of the OM, where they are normally not present [56].

of nonlocal hydrogen bonds. The manner in which β-barrels are inserted into bacterial OMs is not well understood. Spontaneous insertion of β-barrels into membranes is unlikely to be the major pathway for several reasons. First, β-barrels must transit the inner membrane without inserting into it to be correctly inserted into the OM. Second, although it is easy to visualize a β-barrel inserting a set of tight β-hairpins across a membrane, β-barrels in bacteria often have the opposite topology, with their long loops on the outside of the OM and the tight turns of the β-hairpins on the periplasmic surface. OM β-barrels are first secreted across the inner membrane into the periplasmic space by virtue of an N-terminal signal sequence that targets the protein to the Sec translocation machinery

[23]. In the periplasmic space, there are chaperones, such as the SurA and skp proteins of *E. coli*, that stabilize unfolded OMPs and are essential for proper insertion into the OM [21]. There is also at least one OMP, called Omp85 in *Neisseria*, that is conserved in all Gram-negative bacteria and has been shown to be essential for OM biogenesis, perhaps through a role in the insertion and folding of OMPs [24]. This folding pathway offers a possible explanation for the strict segregation of β-barrel and α-helical membrane proteins in Gram-negative bacteria. Proteins destined for either membrane can use the Sec translocation machinery in the inner membrane because they use distinct pathways for subsequent membrane targeting and insertion. Segregation is by structural

Figure 2
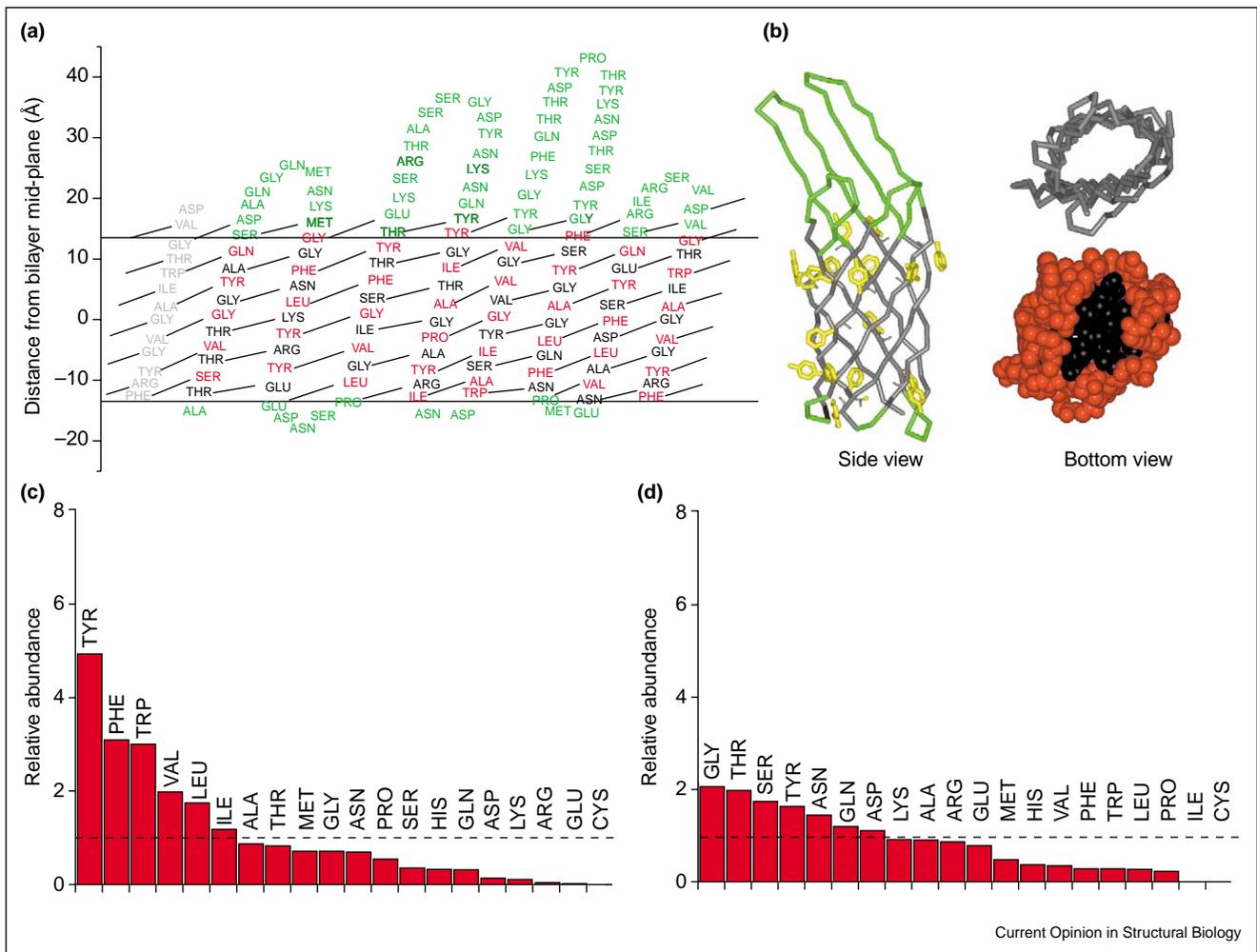
Architecture of a β-barrel membrane protein. **(a)** Exposed and buried residues of the *E. coli* β-barrel protein OmpX. Residues in the membrane-spanning part of this eight-stranded β-barrel are either red (lipid exposed) or black (barrel interior). Extramembranous loops are green. The y-axis shows the protein's dimensions across the membrane in angstroms, with the origin at the bilayer mid-plane [11••]. The horizontal lines at 13.5 Å denote the limits of the hydrophobic part of the membrane [11••]. The network of hydrogen bonds that stabilizes the barrel in the membrane is also shown by black lines. Each hydrogen bond can contribute as much as 0.5 kcal/mol/residue to stability, as suggested by peptide studies [3,6,7]. Thus, hydrogen bonding can easily account for the high stability of β-barrel membrane proteins. The eighth, C-terminal TM β-strand is repeated in gray at the far left. **(b)** Structure of OmpX. The side view shows the β-strands on the same y-scale as in (a), with the membrane-spanning strands in gray. Notice the abundance of aromatic residues on the lipid-exposed surface, in yellow. The bottom view shows the disposition of the sidechains in the barrel using the same color scheme as in (a): red, lipid exposed; black, barrel interior. Extramembranous loops are not shown. **(c,d)** Abundance of amino acids in β-barrels compared to the overall genomic abundance [11••]. External surfaces (c) are dominated by aromatic residues, whereas internal residues (d) are dominated by small and polar amino acids.

class, such that α-helical membrane proteins, with their long contiguous hydrophobic helices, move laterally from the translocation machinery directly into the inner membrane lipids [23], whereas β-barrel OMPs, with their cryptically encoded hydrophobic surfaces, are translocated through the inner membrane into the periplasmic space and then subsequently integrated into the OM.

Large oligomeric β-barrel pores

Among the β-barrels for which there currently are no three-dimensional structures, there is a broad assortment that are multimeric OM pores required for the secretion of proteins across the OM, or the secretion and assembly of filamentous structures such as pili. In any species of Gram-negative bacteria, there are dozens of such secretion pathways [25••], many of which are important for pathogenicity. The proteins required for filamentous structures are often found in a plasmid-encoded operon that also contains a specific OM usher protein. As an example, the *E. coli* OM usher PapC is required for P pilus assembly [26]. A typical OM usher protein [25••,27], PapC is approximately 840 amino acids long and is predicted to span the OM

Figure 3
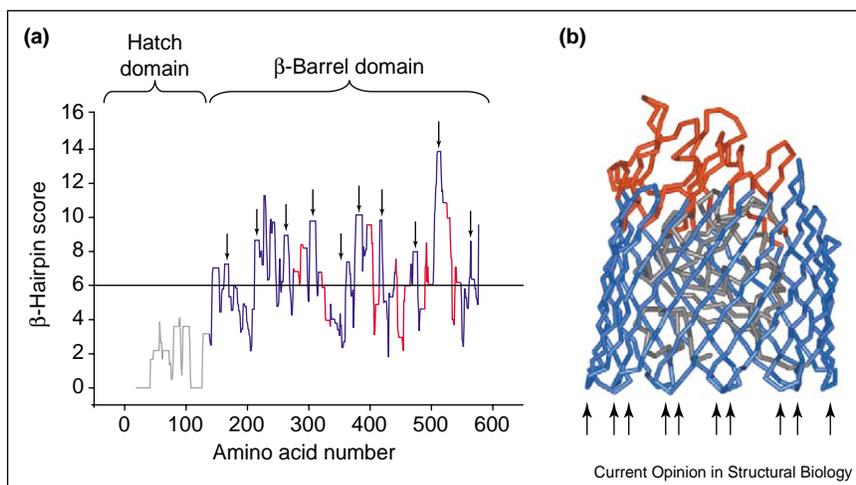
Identification of the β-hairpins of a typical β-barrel. Using one of the methods described in the text [11**], the predicted and actual membrane-spanning β-hairpins of the TonB-dependent transporter BtuB are shown. BtuB is involved in the transport of cobalamin (vitamin B_{12}). **(a)** A calculated, sliding-window score for β-hairpin propensity, color coded to match the backbone protein structure shown in **(b)**. Actual membrane-spanning hairpins are shown in blue. The longer extramembranous loops are shown in red, whereas the N-terminal 'hatch' domain is shown in gray. This structure, solved recently by Wiener and colleagues [57*], is a classical β-barrel with every TM β-strand participating in at least one tight β-hairpin. The positions of the tight periplasmic turns are indicated with arrows. The horizontal line at a β-hairpin score of six is an empirical cutoff that was selected because it correctly identifies most actual TM β-hairpins [11**]. This transporter β-barrel shares many architectural features in common with other families of β-barrel transporters [25**]. Most of the residues in the C-terminal barrel domain participate in the membrane-spanning barrel, whereas the N-terminal domain is a globular domain involved in regulating transport across the barrel. A web-based calculator of these β-barrel scores can be found at http://blanco.biomol.uci.edu/mpex.

with a large C-terminal β-barrel domain of between 18 and 24 TM β-strands [25**]. The tight turns of the β-hairpins are on the external surface of the OM [26]. Their N-terminal domains are periplasmic 'plugs' that regulate transport through the pore. PapC monomers assemble in the OM into multimeric structures containing 10–12 subunits and a 20–30 Å central pore [26].

Also identified are 'autotransporter' proteins and 'two-partner secretion' pores [25**], which have a similar architecture, including a C-terminal β-barrel domain that assembles into an oligomeric OM pore [28,29] and an N-terminal periplasmic domain that regulates transport. Like the usher proteins, these pores allow the specific transport of a single protein species. The transported protein is usually encoded in the N-terminal part of the pore sequence, if it is an autotransported protein, or as an upstream gene in an operon. Electron microscopy revealed a 20 Å pore with sixfold symmetry in the structure of the *Neisseria* IgA protease autotransporter [28]. Transport of the C-terminal protease domain was shown to occur through a common, central channel [28]. Interestingly, the C-terminal β-barrel of the two-partner secretion pore FhaC most likely spans the membrane with an odd number of β-strands, probably 17 or 19 [30]. Although this feature has not yet been observed in the few known β-barrel structures, it could be present in other β-barrels or families of barrels.

These families of multimeric β-barrel pores are abundant in bacteria [25**,31] and are important in bacterial pathogenicity. Homologs of two-partner secretion pores are even found in eukaryotes [25**]. Currently, the best structural information we have for them is from electron microscopy [26,27,29], which has shown that all of these multimeric β-barrel pores are roughly symmetrical, often have 6- or 12-fold symmetry, and have a single, central pore. It is not currently known if these multimeric β-barrel pores are formed by rings of closed monomeric β-barrels or if they consist of a single large β-barrel made from the TM β-hairpins of the subunits.

Architectural nonconformists

The secretins comprise another distinct family of multimeric OMP secretion pores. They function as one of the terminal OM pores of the general secretion pathway [25**,27]. Secretin pores are observed to be large in electron microscopy images, from 50 to 100 Å in diameter [27,32,33]. A dodecameric pore structure has been proposed for the meningococcal secretin PilQ [32] and for the C-terminal domain of the secretin protein PulD [34]. Also, electron microscopy analysis has revealed a two-domain architecture for the secretin complexes, with a membrane-spanning ring domain and a periplasmic 'cup' domain [32]. Despite the large pore, the periplasmic domains keep secretins closed to nonspecific solute flow. Most importantly for this discussion, the architecture

of secretin pores is very different from all other known β-barrels. Their TM β-strands are not found in the obvious strand-turn-strand β-hairpin structures that are always present in the other known β-barrels. Instead, the TM β-strands of secretins are isolated single strands in the sequence bracketed by longer loops that are presumably on both sides of the membrane [25••]. Thus, the secretins are not identified as β-barrels using algorithms that are based on known β-barrel structures (e.g. [11••,16••,17••]). Based on their unusual architecture, it remains possible that the secretin pores are not β-barrels at all.

Mitochondria and chloroplasts

It has been hypothesized that some, perhaps most, of the integral OMPs of mitochondria and chloroplasts are β-barrels because these membranes are relics of the organelles' evolutionary history as symbiotic intracellular Gram-negative bacteria. Based on structure predictions and experimental studies of individual proteins, it is now clear that the OMs of these organelles contain both α-helical and β-barrel membrane proteins. For example, the abundant mitochondrial voltage-dependent anion channel (VDAC) has long been thought to be a β-barrel [35] with about 14–16 TM β-strands [36,37], as has the functionally similar chloroplast protein OEP24 [38]. The detailed work of Pfanner and others [39,40•,41••] has shown that the protein translocase of the outer mitochondrial membrane (TOM) is a complex of membrane proteins with a central pore composed of oligomers of the β-barrel protein Tom40 [39]. However, the Tom40 β-barrel pore is closely associated with several integral membrane proteins that are anchored to the membrane by membrane-spanning α-helices [40•]. Similarly, Toc75, the pore of the chloroplast OM translocase, is a β-barrel [42], whereas the other subunits of the translocase are anchored in the membrane by TM α-helices [42].

Schleiff *et al.* [43••] have recently performed a genomic and proteomic analysis of the chloroplast OM. By using a combination of structure prediction, target sequence detection, pI calculation and manual screening, they identified a list of approximately 900 proteins in *Arabidopsis* (3.1% of the genome) that are potentially chloroplast β-barrels. They also performed a preliminary proteomic analysis of the chloroplast OM, which identified a small set of proteins including both β-barrel and α-helical membrane proteins. Preliminary application of similar multistage screening to the genome of the yeast *Saccharomyces cerevisiae* revealed at least 100 proteins, or about 1.5% of the genome, that are potentially mitochondrial β-barrels (WC Wimley, unpublished data).

The image that emerges from these analyses is that the OMs of mitochondria and chloroplasts do contain β-barrel membrane proteins, but, during their long evolutionary history as intracellular organelles, some α-helical membrane proteins have also been targeted to these membranes. Despite recent advances in mitochondrial proteomics [44], the exact proportion of α-helical and β-barrel proteins in mitochondrial OMs is currently not known.

Other bacteria

The single membranes of classical Gram-positive bacteria and archaea apparently do not contain β-barrel membrane proteins [25••]. However, some other bacteria that are not proteobacteria, (i.e. not classical Gram-negatives) do contain β-barrels. In a recent review of bacterial OM transporters, Saier and colleagues [25••] showed that members of some β-barrel transporter families are found in nonproteobacterial families with inner and outer membranes. These families include *Deinococcus*, *Chlamydia* and *Cyanobacteria*. Also, the cell walls of some 'acid-fast' bacteria, including those belonging to the *Treponema* and *Mycobacteria* families, are coated with a mycolic-acid-rich lipid layer attached to the cell wall that functions as a protective barrier. These waxy OMs can be 50–100 Å thick, compared to about 30 Å for a phospholipid bilayer, due to the very long chains of the mycolic acid groups. In all of these dual-membrane organisms, there are large protein pores of approximately 20 Å diameter that span the OM [45–47]. It has been proposed that these protein pores, such as major surface protein A (MspA) from *Mycobacterium smegmatis* and the major outer membrane proteins (MOMPs) of *Chlamydia*, are β-barrels [45–47]. This idea is supported by the fact that these pores span the outer lipid layer without long hydrophobic α-helical sequences and that they have a high potential for TM β-sheet formation [11••]. However, the exact nature of the pore structure remains to be elucidated.

Pore-forming toxins

Some secreted bacterial toxins exert their cytotoxic effects by assembling into membrane-spanning β-barrel pores [48]. The list includes α-hemolysin of *Staphylococcus aureus* [49], which assembles into the heptameric 14-stranded β-barrel pore that is shown in Figure 1. The large family of cholesterol-dependent cytolysins (e.g. perfringolysin [13•]) has also been shown to assemble into very large β-barrel pores, as much as 300 Å in diameter [13•]. In these proteins, each monomer contributes two β-hairpins to the pore's single barrel [13•]. Several multipart protein toxins, such as the anthrax toxin [50], also have a multimeric, β-barrel pore-forming subunit through which the other protein toxin subunits cross the membrane. An interesting structural theme that unifies the β-barrel toxins is that the TM β-barrel is formed from sequences that are present as long loops that are normally folded back into the monomeric or inactive protein. After assembly of the multimeric 'prepore' structure, the loop sequences undergo a large concerted conformational switch in which the tip of the

loop inserts into the membrane to become one of the β-hairpins of the barrel [2,13•]. The formation of the hydrogen-bonded network of the β-barrel makes insertion essentially irreversible.

Conclusions

The β-barrel membrane protein is found in the OMs of bacteria, mitochondria and chloroplasts, and in the TM pores of some secreted protein toxins. Here I have reviewed the diversity of their function, architecture and distribution. It is hoped that future studies will bring to light the diversity of their three-dimensional structure as well.

Acknowledgements

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Schulz GE: **The structure of bacterial outer membrane proteins**. *Biochim Biophys Acta* 2002, **1565**:308-317.

2. Montoya M, Gouaux E: **β-barrel membrane protein folding and structure viewed through the lens of α-hemolysin**. *Biochim Biophys Acta* 2003, **1609**:19-27.

3. White SH, Wimley WC: **Membrane protein folding and stability: physical principles**. *Annu Rev Biophys Biomol Struct* 1999, **28**:319-365.

4. Bishop CM, Walkenhorst WF, Wimley WC: **Folding of β-sheet membrane proteins: specificity and promiscuity in peptide model systems**. *J Mol Biol* 2001, **309**:975-988.

5. Arora A, Abildgaard F, Bushweller JH, Tamm LK: **Structure of**
•   **outer membrane protein A transmembrane domain by NMR spectroscopy**. *Nat Struct Biol* 2001, **8**:334-338.
The determination of a β-barrel membrane protein structure by NMR spectroscopy is an important accomplishment in structural biology. This study provided critical information about the dynamics of the β-barrel motions in the bilayer, and showed that the barrel is most rigid in the bilayer center and most flexible near the bilayer interfaces.

6. Wimley WC, Hristova K, Ladokhin AS, Silvestro L, Axelsen PH, White SH: **Folding of β-sheet membrane proteins: a hydrophobic hexapeptide model**. *J Mol Biol* 1998, **277**:1091-1110.

7. Ladokhin AS, White SH: **Folding of amphipathic α-helices on membranes: energetics of helix formation by melittin**. *J Mol Biol* 1999, **285**:1363-1369.

8. Haltia T, Freire E: **Forces and factors that contribute to the structural stability of membrane proteins**. *Biochim Biophys Acta* 1995, **1241**:295-322.

9. Rosenbusch JP: **Stability of membrane proteins: relevance for the selection of appropriate methods for high-resolution structure determinations**. *J Struct Biol* 2001, **136**:144-157.

10. Schulz GE: **β-Barrel membrane proteins**. *Curr Opin Struct Biol* 2000, **10**:443-447.

11. Wimley WC: **Toward genomic identification of β-barrel**
•• **membrane proteins: composition and architecture of known structures**. *Protein Sci* 2002, **11**:301-312.
This paper describes one of several algorithms developed recently to identify β-barrel membrane proteins. Taken together, these algorithms have established that the OM β-barrel proteome of Gram-negative bacteria encompasses about 2–3% of the genome.

12. Sansom MSP, Kerr ID: **Transbilayer pores formed by β-barrels: molecular modeling of pore structures and properties**. *Biophys J* 1995, **69**:1334-1343.

13. Ramachandran R, Heuck AP, Tweten RK, Johnson AE: **Structural**
•   **insights into the membrane-anchoring mechanism of a cholesterol-dependent cytolysin**. *Nat Struct Biol* 2002, **9**:823-827.
A very nice biophysical study of pore formation in the protein perfringolysin. Multiple independent techniques were used to show that two separate segments of the protein each contribute to the multimeric pore. The same techniques demonstrated that these segments span a bilayer membrane as β-hairpins.

14. Seshadri K, Garemyr R, Wallin E, von Heijne G, Elofsson A: **Architecture of β-barrel membrane proteins: analysis of trimeric porins**. *Protein Sci* 1998, **7**:2026-2032.

15. Ulmschneider MB, Sansom MS: **Amino acid distributions in integral membrane protein structures**. *Biochim Biophys Acta* 2001, **1512**:1-14.

16. Zhai Y, Saier MH Jr: **The β-barrel finder (BBF) program, allowing**
•• **identification of outer membrane β-barrel proteins encoded within prokaryotic genomes**. *Protein Sci* 2002, **11**:2196-2207.
This paper describes one of several algorithms developed recently to identify β-barrel membrane proteins. Taken together, these algorithms have established that the OM β-barrel proteome of Gram-negative bacteria encompasses about 2–3% of the genome.

17. Martelli PL, Fariselli P, Krogh A, Casadio R: **A sequence-profile-**
•• **based HMM for predicting and discriminating β-barrel membrane proteins**. *Bioinformatics* 2002, **18(suppl 1)**:S46-S53.
This paper describes one of several algorithms developed recently to identify β-barrel membrane proteins. Taken together, these algorithms have established that the OM β-barrel proteome of Gram-negative bacteria encompasses about 2–3% of the genome.

18. Molloy MP, Herbert BR, Slade MB, Rabilloud T, Nouwens AS, Williams KL, Gooley AA: **Proteomic analysis of the *Escherichia coli* outer membrane**. *Eur J Biochem* 2000, **267**:2871-2881.

19. Phadke ND, Molloy MP, Steinhoff SA, Ulintz PJ, Andrews PC, Maddock JR: **Analysis of the outer membrane proteome of *Caulobacter crescentus* by two-dimensional electrophoresis and mass spectrometry**. *Proteomics* 2001, **1**:705-720.

20. Molloy MP, Phadke ND, Chen H, Tyldesley R, Garfin DE, Maddock
•• JR, Andrews PC: **Profiling the alkaline membrane proteome of *Caulobacter crescentus* with two-dimensional electrophoresis and mass spectrometry**. *Proteomics* 2002, **2**:899-910.
Technical proteomics work that is beginning to reveal the true complexity of the OMs of Gram-negative bacteria. This work, taken together with [19], has shown that the OM of this Gram-negative bacterium contains many β-barrels of unknown function.

21. Kleinschmidt JH, Tamm LK: **Secondary and tertiary structure formation of the β-barrel membrane protein OmpA is synchronized and depends on membrane thickness**. *J Mol Biol* 2002, **324**:319-330.

22. Bayley H: **Toxin structure: part of a hole?** *Curr Biol* 1997, **7**:R763-R767.

23. Mori H, Ito K: **The Sec protein-translocation pathway**. *Trends Microbiol* 2001, **9**:494-500.

24. Voulhoux R, Bos MP, Geurtsen J, Mols M, Tommassen J: **Role of a highly conserved bacterial protein in outer membrane protein assembly**. *Science* 2003, **299**:262-265.

25. Yen MR, Peabody CR, Partovi SM, Zhai Y, Tseng YH, Saier MH:
•• **Protein-translocating outer membrane porins of Gram-negative bacteria**. *Biochim Biophys Acta* 2002, **1562**:6-31.
This paper contains a wealth of information about the genomics, phylogenetics and distributions of the major families of OM β-barrel protein transporters. The protein families covered are the OM factor proteins, the OM ushers, secretins, autotransporters and two-partner secretion pores.

26. Thanassi DG, Saulino ET, Lombardo MJ, Roth R, Heuser J, Hultgren SJ: **The PapC usher forms an oligomeric channel: implications for pilus biogenesis across the outer membrane**. *Proc Natl Acad Sci USA* 1998, **95**:3146-3151.

27. Thanassi DG: **Ushers and secretins: channels for the secretion of folded proteins across the bacterial outer membrane**. *J Mol Microbiol Biotechnol* 2002, **4**:11-20.

28. Turner DP, Wooldridge KG, Ala'Aldeen DA: **Autotransported serine protease A of *Neisseria meningitidis*: an immunogenic, surface-exposed outer membrane, and secreted protein.** *Infect Immun* 2002, **70**:4447-4461.

29. Veiga E, Sugawara E, Nikaido H, de LV, Fernandez LA: **Export of autotransported proteins proceeds through an oligomeric ring shaped by C-terminal domains.** *EMBO J* 2002, **21**:2122-2131.

30. Guedin S, Willery E, Tommassen J, Fort E, Drobecq H, Locht C, Jacob-Dubuisson F: **Novel topological features of FhaC, the outer membrane transporter involved in the secretion of the *Bordetella pertussis* filamentous hemagglutinin.** *J Biol Chem* 2000, **275**:30202-30210.

31. Ma Q, Zhai Y, Schneider JC, Ramseier TM, Saier MH: **Protein secretion systems of *Pseudomonas aeruginosa* and *P. fluorescens*.** *Biochim Biophys Acta* 2003, **1611**:223-233.

32. Collins RF, Davidsen L, Derrick JP, Ford RC, Tonjum T: **Analysis of the PilQ secretin from *Neisseria meningitidis* by transmission electron microscopy reveals a dodecameric quaternary structure.** *J Bacteriol* 2001, **183**:3825-3832.

33. Brok R, Van Gelder P, Winterhalter M, Ziese U, Koster AJ, de Cock H, Koster M, Tommassen J, Bitter W: **The C-terminal domain of the *Pseudomonas* secretin XcpQ forms oligomeric rings with pore activity.** *J Mol Biol* 1999, **294**:1169-1179.

34. Nouwen N, Stahlberg H, Pugsley AP, Engel A: **Domain structure of secretin PulD revealed by limited proteolysis and electron microscopy.** *EMBO J* 2000, **19**:2229-2236.

35. Bay DC, Court DA: **Origami in the outer membrane: the transmembrane arrangement of mitochondrial porins.** *Biochem Cell Biol* 2002, **80**:551-562.

36. Song J, Midson C, Blachly-Dyson E, Forte M, Colombini M: **The topology of VDAC as probed by biotin modification.** *J Biol Chem* 1998, **273**:24406-24413.

37. Casadio R, Jacoboni I, Messina A, De PV: **A 3D model of the voltage-dependent anion channel (VDAC).** *FEBS Lett* 2002, **520**:1-7.

38. Rohl T, Motzkus M, Soll J: **The outer envelope protein OEP24 from pea chloroplasts can functionally replace the mitochondrial VDAC in yeast.** *FEBS Lett* 1999, **460**:491-494.

39. Hill K, Model K, Ryan MT, Dietmeier K, Martin F, Wagner R, Pfanner N: **Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins.** *Nature* 1998, **395**:516-521.

40. Pfanner N, Wiedemann N: **Mitochondrial protein import:**
• **two membranes, three translocases.** *Curr Opin Cell Biol* 2002, **14**:400-411.
A rich review of the fast moving field of mitochondrial translocase research.

41. Model K, Prinz T, Ruiz T, Radermacher M, Krimmer T, Kuhlbrandt
•• W, Pfanner N, Meisinger C: **Protein translocase of the outer mitochondrial membrane: role of import receptors in the structural organization of the TOM complex.** *J Mol Biol* 2002, **316**:657-666.
This paper describes the formation of the mitochondrial translocase pore and how pore formation is modulated by some of the other members of the TOM complex. This paper nicely illustrates the interplay between the β-barrel pore protein Tom40 and its associated α-helical membrane protein partners.

42. Schleiff E, Soll J, Kuchler M, Kuhlbrandt W, Harrer R: **Characterization of the translocon of the outer envelope of chloroplasts.** *J Cell Biol* 2003, **160**:541-551.

43. Schleiff E, Eichacker LA, Eckart K, Becker T, Mirus O, Stahl T, Soll J:
•• **Prediction of the plant β-barrel proteome: a case study of the chloroplast outer envelope.** *Protein Sci* 2003, **12**:748-759.
This paper is a bold attempt to identify potential β-barrel chloroplast OMPs from the genome of *Arabidopsis*. Using a hierarchical selection process, about 900 proteins, or 3.1% of the genome, were found to encode proteins that could potentially be β-barrels.

44. Taylor SW, Warnock DE, Glenn GM, Zhang B, Fahy E, Gaucher SP, Capaldi RA, Gibson BW, Ghosh SS: **An alternative strategy to determine the mitochondrial proteome using sucrose gradient fractionation and 1D PAGE on highly purified human heart mitochondria.** *J Proteome Res* 2002, **1**:451-458.

45. Hughes ES, Shaw KM, Ashley RH: **Mutagenesis and functional reconstitution of chlamydial major outer membrane proteins: VS4 domains are not required for pore formation but modify channel function.** *Infect Immun* 2001, **69**:1671-1678.

46. Engelhardt H, Heinz C, Niederweis M: **A tetrameric porin limits the cell wall permeability of *Mycobacterium smegmatis*.** *J Biol Chem* 2002, **277**:37567-37572.

47. Heinz C, Engelhardt H, Niederweis M: **The core of the tetrameric mycobacterial porin MspA is an extremely stable β-sheet domain.** *J Biol Chem* 2003, **278**:8678-8685.

48. Olson R, Gouaux E: ***Vibrio cholerae* cytolysin is composed of an α-hemolysin-like core.** *Protein Sci* 2003, **12**:379-383.

49. Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE: **Structure of staphylococcal α-hemolysin, a heptameric transmembrane pore.** *Science* 1996, **274**:1859-1866.

50. Lacy DB, Collier RJ: **Structure and function of anthrax toxin.** *Curr Top Microbiol Immunol* 2002, **271**:61-85.

51. Schulz GE: **Porins: general to specific, native to engineered passive pores.** *Curr Opin Struct Biol* 1996, **6**:485-490.

52. Faraldo-Gomez JD, Sansom MS: **Acquisition of siderophores in gram-negative bacteria.** *Nat Rev Mol Cell Biol* 2003, **4**:105-116.

53. Postle K: **Active transport by customized β-barrels.** *Nat Struct Biol* 1999, **6**:3-6.

54. Wiener MC: **Bacterial export takes its Tol.** *Structure Fold Des* 2000, **8**:R171-R175.

55. Koronakis V, Sharff A, Koronakis E, Luisi B, Hughes C: **Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export.** *Nature* 2000, **405**:914-919.

56. Snijder HJ, Dijkstra BW: **Bacterial phospholipase A: structure and function of an integral membrane phospholipase.** *Biochim Biophys Acta* 2000, **1488**:91-101.

57. Chimento DP, Mohanty AK, Kadner RJ, Wiener MC: **Substrate-**
• **induced transmembrane signaling in the cobalamin transporter BtuB.** *Nat Struct Biol* 2003, **10**:394-401.
A recent structural study of BtuB, a TonB-dependent cobalamin (vitamin B_{12}) importer. Gram-negative bacteria have many TonB-dependent importers. All are OM β-barrels that are energetically coupled to the inner membrane so that they can drive the import of rare cofactors. This study has revealed some important details of the structural transitions that are involved in transporter activity.