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Review

Structural biology of membrane-intrinsic β-barrel enzymes: Sentinels of the bacterial outer membrane

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Abstract

The outer membranes of Gram-negative bacteria are replete with integral membrane proteins that exhibit antiparallel β-barrel structures, but very few of these proteins function as enzymes. In *Escherichia coli*, only three β-barrel enzymes are known to exist in the outer membrane; these are the phospholipase OMPLA, the protease OmpT, and the phospholipid: lipid A palmitoyltransferase PagP, all of which have been characterized at the structural level. Structural details have also emerged for the outer membrane β-barrel enzyme PagL, a lipid A 3-*O*-deacylase from *Pseudomonas aeruginosa*. Lipid A can be further modified in the outer membrane by two β-barrel enzymes of unknown structure; namely, the *Salmonella enterica* 3′-acyloxyacyl hydrolase LpxR, and the *Rhizobium leguminosarum* oxidase LpxQ, which employs O_2 to convert the proximal glucosamine unit of lipid A into 2-aminogluconate. Structural biology now indicates how β-barrel enzymes can function as sentinels that remain dormant when the outer membrane permeability barrier is intact. Host immune defenses and antibiotics that perturb this barrier can directly trigger β-barrel enzymes in the outer membrane. The ensuing adaptive responses occur instantaneously and rapidly outpace other signal transduction mechanisms that similarly function to restore the outer membrane permeability barrier.

Keywords: OMPLA; OmpT; PagP; PagL; LpxR; LpxQ

Contents

1. Introduction

Structural biology is now indicating how membrane-intrinsic β-barrel enzymes can function as sentinels that

Abbreviations: L-Ara4N, 4-amino-4-deoxy-L-arabinose; GroPEtn, glycerophosphoethanolamine; GPL, glycerophospholipid; O_{Ag}, O-antigen; PtdEtn, phosphatidylethanolamine

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respond rapidly to perturbations in the outer membrane permeability barrier of Gram-negative bacteria. More generally, transmembrane signal transduction pathways control most adaptive responses toward environmental assaults on the integrity of the cell. An extracellular sensory transducer detects the environmental stimulus, which, upon binding, induces a conformational change that is communicated across the cytoplasmic membrane. The cytoplasmic components of the signal transduction pathway are thereby engaged to control gene expression and/or enzymatic activities that, ultimately, lead to an appropriate adaptive response. In the case of assaults on the integrity of the outer membrane, this general signal transduction mechanism can be troublesome when it is coupled to the expression of proteins that are themselves outer membrane components; these proteins are targeted for secretion through the cytoplasmic membrane and depend on extracytoplasmic chaperones and docking proteins in the outer membrane for their proper assembly.

Clearly, the outer membrane is first in line for external attack, but it is also last to receive any support from cellular utilities, which makes it vulnerable to potentially irreversible damage that might take effect before general signal transduction pathways can usher an adaptive response. Emerging evidence indicates that most β-barrel enzymes were selected to function in their own right as specialized sensory transducers, which are poised to respond instantaneously to environmental assaults on the bacterial outer membrane itself. General signal transduction pathways can respond gradually to looming danger by modulating the expression levels of these β-barrel enzymes. However, β-barrel enzymes can further act like air marshals at the cellular periphery by maintaining a low profile until the ultimate incursion on the outer membrane finally springs them into action. By expressing a small subset of β-barrel enzymes, the cell acquires extra protection for a membrane system at the furthest extremes of the cell's centralized communication networks.

Here we discuss the structural biology of four β-barrel enzymes, each illustrating how their activity can be triggered to provide an appropriate adaptive response for the outer membrane in which they reside. The phospholipase OMPLA [1], the protease OmpT [2], the phospholipid∷lipid A palmitoyltransferase PagP [3], and the lipid A 3-*O*-deacylase PagL [4] each represent β-barrel enzymes with established structure–function relationships that lend support to an emerging sentinel paradigm in outer membrane enzymology. In order to effectively illustrate their proposed roles as outer membrane sentinels, it is necessary to first discuss some basic principles of outer membrane biology.

2. Principles of outer membrane biology

2.1. Outer membrane β-barrel structure and assembly

The Gram-negative cell envelope of *Escherichia coli* includes inner (cytoplasmic) and outer membrane systems, which are separated by the periplasmic space (Fig. 1). Whereas the cytoplasmic membrane is an energy-transducing glycerophospholipid bilayer structure that harbors transmembrane α-helical proteins, the outer membrane is a highly asymmetric bilayer with glycerophospholipids in the inner leaflet and lipopolysaccharides in the outer leaflet [5]. The lipopolysaccharides are tripartite molecules consisting of the hydrophobic and highly immunogenic anchor lipid A (endotoxin), the core oligosaccharide, which can be divided into the inner and outer core regions, and the O-antigen polysaccharide [6]. The peptidoglycan or murein provides a highly reticulated exoskeleton that determines cell shape and protects the cytoplasmic membrane from high internal osmotic pressure [7]. Bacterial lipoproteins can be anchored to the inner leaflet of the outer membrane by a lipid-modified amino terminal cysteine residue in order to provide a physical connection between the murein and the outer membrane [8–10]. The membrane-intrinsic proteins of the outer membrane are distinguished from their counterparts in the cytoplasmic membrane by an antiparallel β-barrel architecture [11,12].

β-barrel membrane proteins are not normally found in the cytoplasmic membrane, although there is no physical reason why they could not assemble in that location. The β barrel structure is very effective at creating pores that would dissipate the proton motive force, suggesting that natural selection acts against their inner membrane localization. Indeed, several protein toxins act by forming β-barrel structures within energy-transducing membrane systems [13]. Compared to soluble globular domains, β-barrel membrane proteins appear to be inside out because they have a hydrophobic surface in contact with the lipid milieu of the membrane, and a more polar interior region. The relatively large extracellular loops and short periplasmic turns constitute the remaining hydrophilic surfaces [14]. The antiparallel β-barrel structure satisfies the requirement of providing continuous hydrogen bonding between the amide nitrogen proton and carbonyl oxygen of the peptide bonds, which are otherwise too polar to interact stably with the hydrocarbon chains in a lipid bilayer membrane [15].

Roughly 8 amino acids are minimally sufficient to span the membrane in the extended β-conformation, and half of these residues project into the polar interior region. These amphipathic β-strands can be much longer when they are significantly tilted in the membrane. Only those residues whose registration projects them into the lipid milieu of the membrane need to possess hydrophobic side-chains [11]. Consequently, β-barrel membrane proteins typically display an overall hydrophobicity that is intermediate between soluble globular proteins and transmembrane α-helical proteins. It is the outward exposure of their hydrophobic surfaces that calls for the use of detergents to keep properly folded β-barrel membrane proteins in solution, but detergents also promote folding by inducing polar groups to shield themselves internally. Many integral membrane β-barrel proteins can be purified in an unfolded state in guanidine or urea and refolded at high protein concentrations by dilution into detergent micelles, which greatly facilitates biochemical analysis [16].

β-barrel membrane proteins are normally transported through the inner membrane and, after cleavage of their amino-terminal secretory signal peptides, through the periplasmic space. A

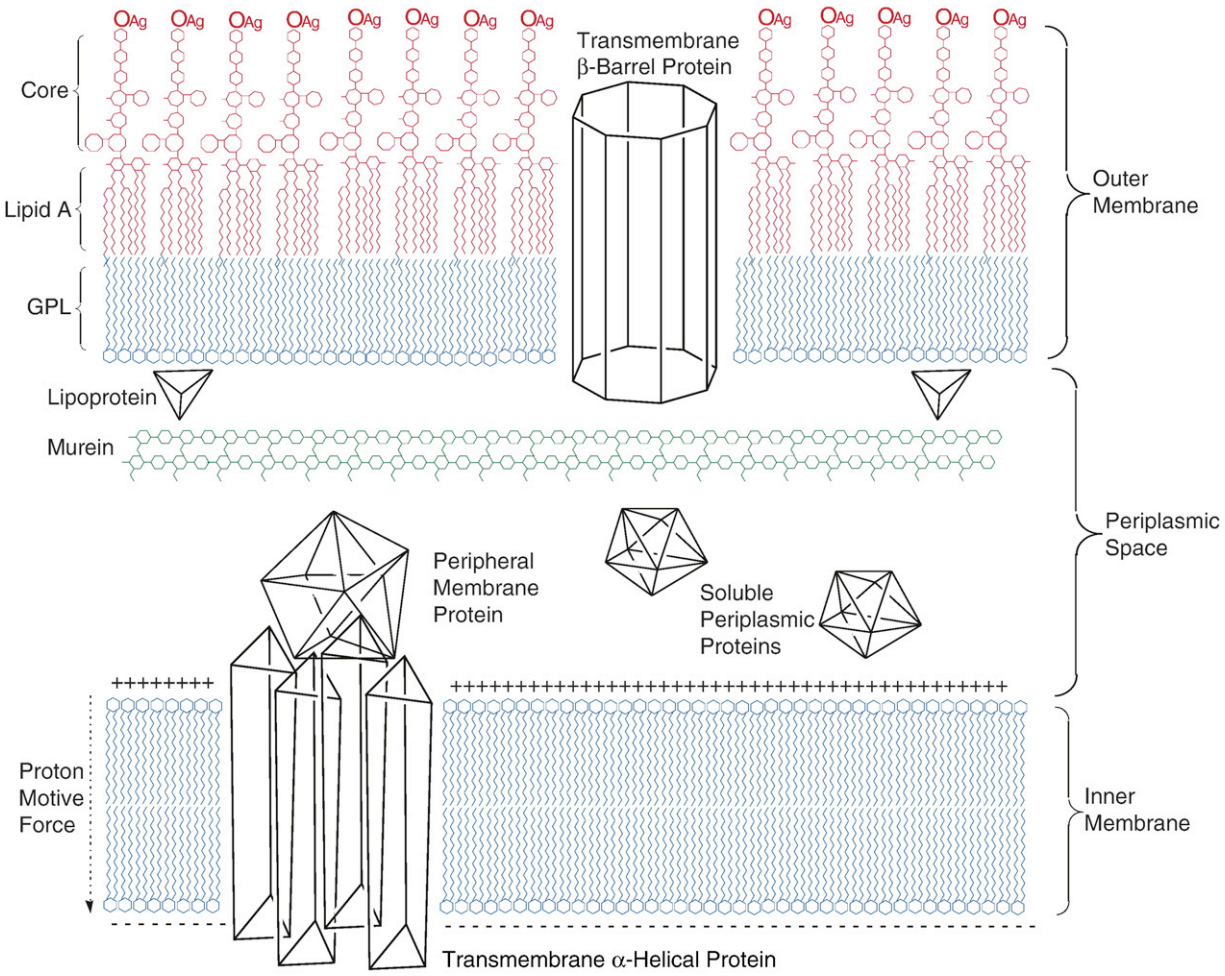

Fig. 1. Molecular organization of the *Escherichia coli* cell envelope. The outer membrane is an asymmetric bilayer with an inner leaflet of glycerophospholipids (GPL), and an outer leaflet of lipopolysaccharide, which can be divided into the lipid A, core oligosaccharide, and O-antigen (O_{Ag}) polysaccharide regions. The membrane-intrinsic proteins of the outer membrane are transmembrane β-barrels, while lipoproteins, anchored to the outer membrane inner leaflet, can provide a link with the murein exoskeleton. The energy-transducing inner membrane is a glycerophospholipid bilayer that supports the proton motive force and contains transmembrane α-helical proteins. The periplasmic space is the region between the inner and outer membranes and contains numerous globular proteins.

system of periplasmic chaperones and folding factors (SurA/Skp and DsbABCD) and an outer membrane docking complex (Omp85 β-barrel and YfgL/NlpB/YfiO/SmpA lipoproteins) are needed to assemble outer membrane β-barrel proteins in Gram-negative bacteria [17–23], and similar machinery appears to be needed to assemble β-barrel proteins in the outer membranes of mitochondria and plastids [24]. The extracytoplasmic accumulation of misfolded β-barrel proteins can be lethal in the absence of a key periplasmic protein DegP (HtrA), which functions both as a chaperone and a protease [25]. An extracellular function sigma factor σ^E directs RNA polymerase to transcribe genes like *degP* that are needed to respond to cell envelope stress [26]. The conserved carboxyl-terminal peptide sequences of misfolded β-barrel proteins can trigger the σ^E response by activating the periplasmic DegS protease, which initiates cleavage of the transmembrane protein RseA in order to release σ^E from inhibition at the cytoplasmic surface of the inner membrane [27]. This signal transduction pathway represents part of the so-called periplasmic stress response.

The periplasmic stress response also receives input from the CpxA/CpxR transmembrane phosphorylation cascade, which responds to misassembled subunits of a cell surface adhesive appendage known as the P-pilus [28,29]. CpxA/CpxR is representative of the two-component signal transduction pathways that depend on a membrane bound sensor kinase and cognate response regulator of transcription. The flux of phosphate between a phosphohistidine residue in the sensor kinase and a phosphoaspartate residue in the response regulator can be modulated by environmental conditions to control adaptive responses. Another example is provided by the EnvZ/OmpR transmembrane phosphorylation cascade, which is activated by osmotic stress exerted on the cell envelope, and modulates expression of a specific set of outer membrane channel proteins [30].

The majority of β-barrel membrane proteins function as channels or transporters of various substrates and usually

possess large open pores within their interiors. These include non-selective channels known as porins, substrate-selective channels, and active transporters that derive energy from the inner membrane protein TonB [31]; the latter pores are plugged by residues contributed from an amino terminal domain that forms part of the ligand-binding site. Channels and transporters are all relatively large in possessing between 12 and 22 β-strands in order to create the space needed for transbilayer passage of substrates. However, several smaller outer membrane proteins possessing between 8 and 12 β-strands tend to have their interiors occluded by a network of mostly polar amino acid side-chains and solvent molecules. These proteins behave more like plugs in the outer membrane and tend to fulfill functions unrelated to transport, but including enzymatic functions, cell adhesion, and the binding of ligands such as detergents [32–35]. An intriguing exception is the 8-stranded β-barrel protein OmpW, which is implicated in the transport of small hydrophobic molecules [36].

All solved structures of β-barrel membrane proteins so far reveal an even number of antiparallel β-strands with the amino and carboxyl termini placed on the periplasmic side of the outer membrane. A notable exception that does not really violate this topological rule is seen in the autotransporter proteins, which display their amino terminal domains on the cell surface after threading them through the carboxyl terminal domains that form the β-barrel proper [37]. Another generally observed feature of β-barrel membrane proteins is the presence of aromatic belts composed of Trp, Tyr and Phe residues that demarcate the interfacial boundaries between the hydrophobic and aqueous domains on both sides of the outer membrane [11].

Outer membrane β-barrel proteins are most often formed from single polypeptide chains, which can undergo various levels of oligomerization, or the pore itself can sometimes be formed in segments supplied by multiple subunits. For example, many porins are 16-stranded β-barrels that assemble into trimers of three pore-forming subunits [38], whereas the TolC channel spans the outer membrane as a trimeric 12-stranded β-barrel in which each subunit contributes 4 β-strands to a single pore [39]. The fact that some 12-stranded β-barrels can form pores while others form plugs is a reflection of the role of β-strand tilt or shear angle in determining pore size [40,41]. Topological arguments indicate that the 8-stranded antiparallel β-barrel is likely the smallest design to be capable of spanning the outer membrane [42].

Nature's other solution to provide continuous polypeptide hydrogen bonding is the transmembrane α-helix, which demands ~20 sequentially hydrophobic amino acid side-chains in order to span the membrane [15]. The greater hydrophobicity of transmembrane α-helical proteins dictates that their targeting to the inner membrane is essentially irreversible, a fact that explains why transmembrane α-helical proteins are not found in the outer membrane. One near-exception to this rule is seen in the structure of the Wza protein involved in the secretion of extracellular polysaccharides. Wza is an octamer that spans the outer membrane by assembling a single amphipathic α-helix in a segmental fashion to form an eight-fold symmetric pore structure [43]. Cationic amphipathic α-helices that function as antimicrobial peptides might form transient pores of similar structure as they cross the outer membrane *en route* to their target — the energy transducing inner membrane (see below). To date, no typically hydrophobic transmembrane α-helices have been described that remain stably integrated in the outer membrane.

2.2. Lipid trafficking to the outer membrane

The peptidoglycan or murein exoskeleton is sandwiched between the inner and outer membranes of Gram-negative bacteria and is believed to effectively block wholesale membrane exchange by vesicular trafficking [7]. Consequently, outer membrane biogenesis likely depends on soluble periplasmic lipid-transfer proteins and/or localized contact bridges between inner and outer membranes [19]. A well-characterized example is provided by the bacterial lipoproteins, which can serve to link the outer membrane with the peptidoglycan wall and are secreted with a distinctly lipidated amino terminus [8]. Nearly 100 lipoproteins are encoded in the *E. coli* genome and serve a multitude of functions in the biogenesis of the cell envelope. Roughly 90% of lipoproteins have their lipid anchors targeted to the inner leaflet of the outer membrane. An ATP-binding cassette transporter (LolCDE) disengages from the inner membrane external leaflet those lipoproteins lacking an inner membrane retention signal and delivers them into the jaws of a periplasmic chaperone (LolA). The LolA–lipoprotein complex is carried through the periplasmic space to an outer membrane docking protein (LolB), which itself is a lipoprotein and facilitates release of LolA concomitantly with integration of the lipoprotein payload into the outer membrane inner leaflet.

In contrast to lipoproteins, the precise mechanism by which other lipids are transported to the outer membrane is poorly understood [44]. Glycerophospholipids are known to freely exchange between the inner and outer membranes while the lipopolysaccharides are exported in a unidirectional manner [45]. The enzymes of the Kennedy pathway for glycerophospholipid biosynthesis require cytoplasmic cofactors and act on the cytoplasmic leaflet of the inner membrane [46]. Although various integral membrane proteins are reported to promote the rapid flip-flop of glycerophospholipids between the cytoplasmic and periplasmic leaflets of the inner membrane [47,48], the precise mechanism for reversibly transporting them between the inner and outer membranes remains a longstanding mystery. The ATP-binding cassette transporter MsbA is responsible for translocating the lipid A-core to the periplasmic surface of the inner membrane (see below), but its activity is also a required first step for the intermembrane exchange of glycerophospholipids in *E. coli* [49,50]. In *Neisseria meningitidis*, however, MsbA translocates lipid A-core independently of glycerophospholipid transport [51]. MsbA might be a dedicated flippase for lipid A-core that only secondarily affects glycerophospholipid transport in *E. coli*.

The lipopolysaccharide structure is assembled within three distinct subcellular compartments; namely, the cytoplasmic membrane, the periplasmic space, and the outer membrane. Like the Kennedy pathway enzymes, the primary lipopolysaccharide biosynthetic reactions require energy-rich precursors and are

catalyzed by enzymes that reside within the cytoplasm or at the cytoplasmic surface of the inner membrane [6]. The Raetz pathway for lipid A biosynthesis was worked out primarily in *E. coli* and includes nine Lpx enzymes. The *E. coli* lipid A molecule is a β-1′,6-linked disaccharide of glucosamine, which is phosphorylated at positions 1 and 4′, and acylated with *R*-3-hydroxymyristate in ester linkage at positions 3 and 3′ and in amide linkage at positions 2 and 2′; the molecule is further acylated with lauroyl and myristoyl groups attached in acyloxyacyl linkage on the distal glucosamine unit (Fig. 2). The *waa* (*rfa*) operons encode the glycosyl transferase enzymes needed for step-wise assembly on lipid A of the core oligosaccharide, for which five distinct types can occur in *E. coli* [52]. Diverse operons control assembly of the various O-antigen polysaccharides. Although ~170 distinct O-antigen serotypes have been identified in *E. coli* alone, they are all assembled on the lipid carrier analog of dolichol known as bactoprenol [6]. The so-called rough lipopolysaccharide, or lipooligosaccharide, includes only the lipid A-core and is usually distinguished from the smooth lipopolysaccharide that additionally includes the polymerized O-antigen.

Translocation of the lipid A-core and bactoprenol phosphate-O-antigen to the periplasmic surface of the inner membrane can be followed by polymerization of the O-antigen polysaccharide

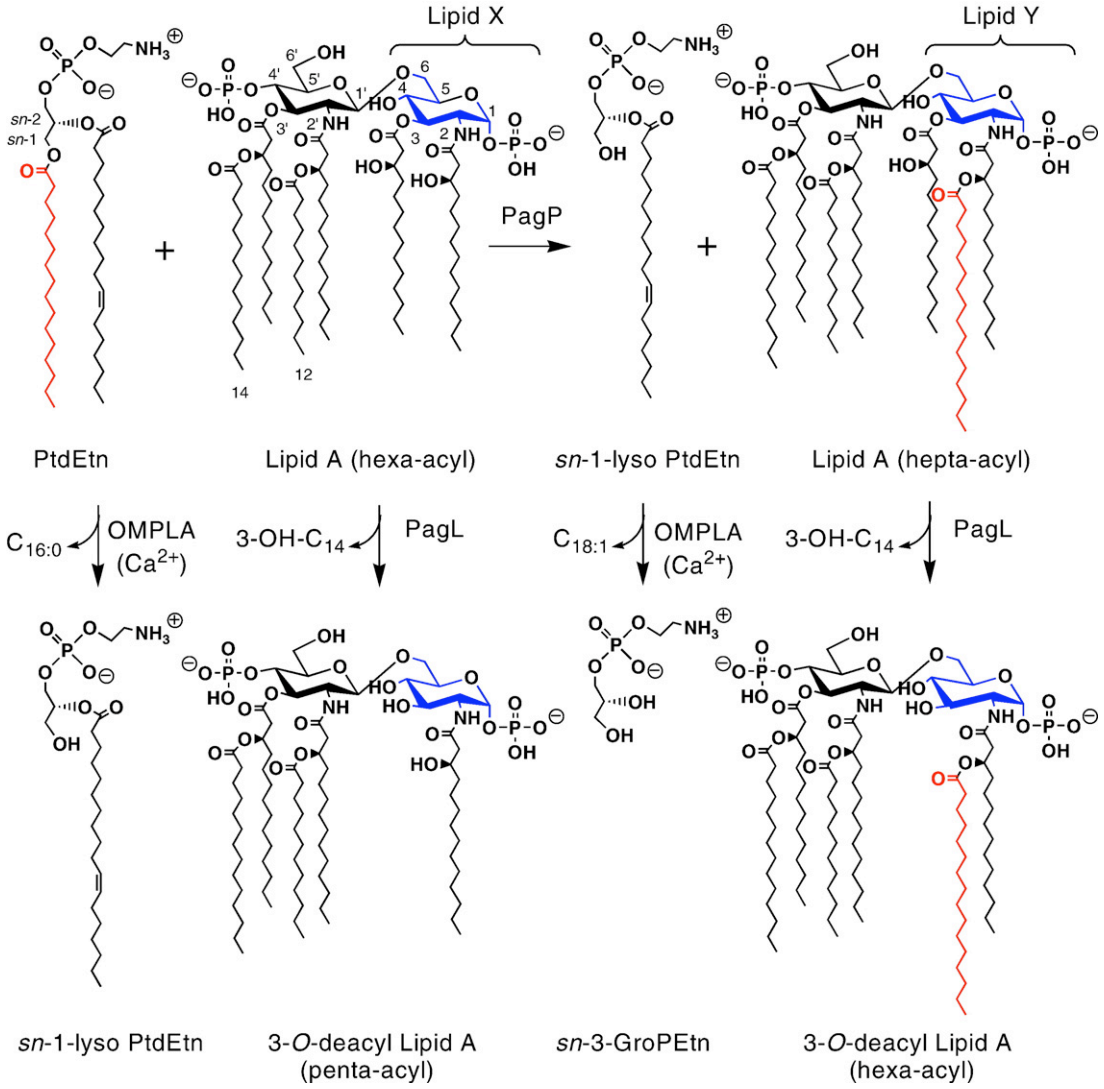

Fig. 2. Outer membrane transformations of glycerophospholipids and lipid A. PagP catalyzes transfer of a palmitate chain ($C_{16:0}$) from the *sn*-1 position of phosphatidylethanolamine (PtdEtn) to lipid A (endotoxin). *Escherichia coli* lipid A is a β-1′,6-linked disaccharide of glucosamine that is acylated with *R*-3-hydroxymyristate chains at the 2, 3, 2′, and 3′ positions, and phosphorylated at the 1 and 4′ positions. Acyloxyacyl linkages with laurate and myristate chains at the 2′ and 3′-positions, respectively, provide the constitutive hexa-acylated lipid A, which is a potent endotoxin. A regulated proportion of lipid A in *E. coli* contains a palmitate chain in acyloxyacyl linkage at position 2, which yields a hepta-acylated molecule that both is attenuated as an endotoxin and provides resistance to cationic antimicrobial peptides. PagL makes similar contributions by removing the *R*-3-hydroxymyristate chain at position 3 from either hexa-acyl or hepta-acyl lipid A. PagP can acylate the cytoplasmic monosaccharide lipid A precursor lipid X to produce lipid Y *in vitro*, and PagL can similarly deacylate lipids X and Y *in vitro*. The *sn*-1-lysophospholipid byproduct of the PagP reaction can be generated directly by the phospholipase activity of OMPLA when Ca^{2+} ions are available. OMPLA also displays lysophospholipase activity *in vitro*, which can release the *sn*-3-glycerophosphoethanolamine (*sn*-3-GroPEtn) polar head group from the remaining unsaturated fatty acyl chain ($C_{18:1}$).

with its subsequent *en-bloc* ligation to the outer core. Although MsbA translocates lipid A-core, at least three distinct mechanisms for bactoprenol phosphate-O-antigen translocation have been reported [6]. At this periplasmic stage, several regulated partial modifications can occur on both lipid A and the inner core sugars [53]. Unlike the constitutive and cytoplasmic lipid A-core biosynthetic enzymes, which are widely conserved among Gram-negative bacteria, the regulated modification enzymes tend to be localized to the extracytoplasmic compartments and are distributed among particular groups of organisms. Modification enzymes located at the periplasmic face of the inner membrane largely control the addition or removal of polar groups, whereas those located in the outer membrane tend to control the addition or removal of fatty acyl-chains.

The recently discovered LptA/LptB proteins are implicated in transporting lipopolysaccharide across the periplasmic space for delivery to the inner leaflet of the outer membrane [54]. It is still undecided whether lipopolysaccharide transport is mediated by a soluble carrier or by localized contact bridges between the inner and outer membranes [19,55]. The lipopolysaccharide in the outer membrane next encounters the Imp β-barrel/RlpB lipoprotein complex, which is needed to display the lipopolysaccharide on the bacterial cell surface [56,57]. The *E. coli* outer membrane is an asymmetric lipid bilayer in which lipopolysaccharide exclusively lines the external leaflet while glycerophospholipids line the inner leaflet [5,31]. The asymmetric lipid organization provides a permeability barrier to hydrophobic antibiotics and detergents encountered in the natural and host environments. Divalent cations, especially Mg^{2+} and, to a lesser extent, Ca^{2+}, are needed to maintain outer membrane lipid asymmetry by neutralizing negative charge repulsions that would otherwise occur between neighboring lipopolysaccharide molecules in the outer leaflet [58,59].

2.3. The outer membrane permeability barrier

The bridging of negatively charged groups by divalent cations between neighboring lipopolysaccharide molecules represents a double-edged sword for Gram-negative bacteria. Although the glycerophospholipid bilayer of the cytoplasmic membrane is intrinsically permeable to hydrophobic antibiotics and detergents, these same compounds are repelled on encountering Mg^{2+} ions bridged between the negatively charged inner core and lipid A sugars [5]. In the gastrointestinal tracts of mammals, enteric Gram-negative bacteria like *E. coli* and *Salmonella* encounter bile salts whose detergent action prevents their growth when the outer membrane structure is compromised [60]. While the majority of β-barrel proteins in the outer membrane allow exchange of essential nutrients and waste products, the asymmetric lipid organization helps to protect the cells from bile salts [31]. The outer membranes of these Gram-negative bacteria represent a major store for the divalent cations that are needed to maintain the permeability barrier.

This dependence on divalent cations can also be an Achilles' heel for the bacterium. Under Mg^{2+}-limited growth conditions, negatively charged groups begin to repel the neighboring lipopolysaccharide molecules and create space for glycerophospholipids that migrate from the outer membrane inner leaflet. Rafts of glycerophospholipid bilayers now mix among the lipopolysaccharides in the external leaflet. These locally symmetric bilayer rafts are freely permeable to hydrophobic antibiotics and detergents, which can now gain access to the periplasm while the outer membrane continues to retain the more polar periplasmic contents [5]. This model is based largely on permeability studies of *E. coli* cells treated briefly with EDTA [61], and likely explains the increased membrane permeability of *imp* mutants [57]. Some details might not apply in *Pseudomonas aeruginosa*, which is highly sensitive to EDTA [62], or in *Neisseria* species, where lipooligosaccharide is non-essential under certain conditions [63] and normal outer membrane lipid organization is not strictly asymmetric to begin with [64], but cell surface net negative charge appears to be a general property of Gram-negative outer membranes.

Nature exploits the outer membrane's dependence on divalent cations, as is evidenced in the design principles for cationic antimicrobial peptides. Although capable of assuming an impressive diversity of structures, two common features are apparent in all antimicrobial peptides. Firstly, antimicrobial peptides take on a net positive charge and, secondly, they are capable of assuming an amphipathic structure that segregates polar and non-polar amino acid residues to opposite faces of the molecule [65,66]. In aqueous solution, antimicrobial peptides tend to remain unstructured, but their net positive charge electrostatically attracts them toward the negatively charged groups on the bacterial cell surface. As the dielectric constant of the medium gradually decreases, water molecules are stripped from the peptide bonds, which drives formation of hydrogen bonded secondary structures. At this stage, positively charged groups in the peptide likely displace some divalent cations from the outer membrane surface. The non-polar face of the induced amphipathic structure can now interact favorably with the hydrocarbon chains of the lipid bilayer, which facilitates the transit of the antimicrobial peptide into the periplasmic space. This model is aptly named the self-promoted uptake pathway [67].

The antimicrobial peptides are thought to kill bacteria primarily by disrupting the proton motive force in the cytoplasmic membrane, but it has recently emerged that some antimicrobial peptides likely target key cytoplasmic proteins as well [68]. In either case, antimicrobial peptides probably approach the cytoplasmic membrane in much the same manner as they approach the outer membrane because anionic glycerophospholipids are displayed on the external surface of bacterial cytoplasmic membranes. By contrast, eukaryotic cells actively sequester their anionic glycerophospholipids on the cytoplasmic surface of the plasma membrane. This lipid asymmetry, combined with the hydrophobic and van der Waals forces uniquely associated with the presence of cholesterol in eukaryotic membranes, has probably allowed the antimicrobial peptides to acquire selective toxicity towards bacteria [69]. In the host, antimicrobial peptides are not always benign as some can modulate the immune response [70].

Faced with cationic antimicrobial peptides in nature, it is no surprise that bacteria have acquired defensive measures to resist

the threat [71]. In Gram-negative bacteria, the PhoP/PhoQ signal transduction pathway controls many important antimicrobial peptide resistance mechanisms [72]. The PhoQ protein is a transmembrane sensory transducer, which directs a patch of acidic amino acid residues in its periplasmic domain toward the anionic glycerophospholipids located at the external surface of the cytoplasmic membrane [73]. Mg^{2+} ions are thought to bridge the anionic lipid polar head groups with the acidic patch on the sensory transducer to maintain it in a repressed state [74]. Cationic antimicrobial peptides likely displace the bound Mg^{2+} ions and thus trigger a phosphorylation cascade that terminates in the activation of the PhoP transcription factor [75]. Mg^{2+} limitation similarly triggers the PhoP/PhoQ system in the absence of any challenge from antimicrobial peptides [76]. In *Salmonella*, PhoP/PhoQ activation triggers the PmrA/PmrB phosphorylation cascade via a posttranslational mechanism using an effector known as PmrD [77], but the *pmrD* gene is nonfunctional in *E. coli* [78]. PmrA/PmrB can also respond directly, as can PhoP/PhoQ [79], to mildly acidic growth conditions encountered in macrophage phagolysosomes [80], an environment that is already replete in both Mg^{2+} ions and antimicrobial peptides [81].

Among the consequences of PhoP/PhoQ activation is the expression of several lipid A modification enzymes. One PhoP/PhoQ-activated gene encodes the outer membrane β-barrel enzyme PagP, which contributes directly and indirectly to antimicrobial peptide resistance [82]. PagP introduces a palmitate chain into the lipid A molecule, which, by altering the hydrophobic and van der Waals forces in the outer membrane, is thought to block interactions with the non-polar faces of amphipathic antimicrobial peptides [83]. PagP also reduces the endotoxicity of lipid A, which otherwise contributes to the production of antimicrobial peptides by triggering the key host innate immune receptor TLR4 [82,84]. Another PhoP/PhoQ-activated gene encodes the outer membrane β-barrel enzyme PagL, which similarly contributes directly and indirectly to antimicrobial peptide resistance. PagL removes the *R*-3-hydroxymyristate chain at position 3 in lipid A [85], which can provide resistance to the antimicrobial peptide polymyxin B under certain conditions [86], and it also reduces lipid A signaling mediated by TLR4 [84].

PmrA/PmrB-dependent enzymes include those that incorporate phosphoethanolamine, derived from phosphatidylethanolamine [87,88], and 4-amino-4-deoxy-L-arabinose (L-Ara4N), derived from bactoprenol phosphate-L-Ara4N [89,90], into the lipid A phosphate groups presented at the periplasmic surface of the inner membrane. By incorporating positively charged groups into lipid A, it is thought that these modifications block the initial cell surface electrostatic interactions with antimicrobial peptides such as polymyxin B. Other strategies to alter cell surface charge include phosphate group removal [91,92], which can simultaneously provide polymyxin B resistance and attenuated lipid A endotoxicity [93,94], and the addition of a bactoprenol phosphate-activated galactosamine sugar [95]. Although the enzymes for these lipid A modifications might be subjected to different patterns of regulation, they similarly act at the periplasmic surface of the inner membrane [96]. Lipid A modification systems clearly endow bacterial cells with a coordinated response to antimicrobial peptides that not only blocks key steps in the self-promoted uptake pathway, but also attenuates antimicrobial peptide production by the host.

3. Sentinels of the bacterial outer membrane

Given the elaborate periplasmic stress response and PhoP/PhoQ signal transduction mechanisms, it would appear that the bacterial cell is well equipped to respond to environmental attacks on the integrity of its cell envelope. However, the structural biology of outer membrane β-barrel enzymes now suggests that a more rapid response can be achieved by directly triggering enzymatic activity in response to outer membrane lipid reorganization. In this regard, the signal transduction pathways can be seen to modulate the expression levels of effectors that are themselves designed to provide a complementary, but instantaneous, signal-response coupling mechanism.

3.1. The phospholipase OMPLA

The first outer membrane β-barrel enzyme to be characterized was the Ca^{2+}-dependent phospholipase OMPLA. The activity was uncovered in the late 1960's and its purification and association with the outer membrane fraction of *E. coli* was first reported in 1971 [97,98]. The association of a phospholipase with membranes was highly enigmatic because research at the time had focused its attention on snake venom phospholipases, which were known to be soluble toxins that attached at membrane surfaces to catalyze their dissolution. What business did the cell have in targeting a phospholipase to its own membrane systems? It should be remembered that this period coincided with the introduction of the fluid mosaic model of phospholipid bilayer membranes [99] and predated any knowledge of the correct structure for lipid A or the asymmetric lipid organization of the outer membrane [100–102].

Awareness of the presence of OMPLA in the outer membrane provided plausible explanations for a number of phenomena that had been uncovered during fledgling studies of Gram-negative cell envelope biogenesis. Several *E. coli* mutants with defective outer membrane permeability barriers were found to exhibit elevated OMPLA activity [103,104]. Additionally, the secretion of colicin antibiotic proteins across the outer membrane was shown to depend on small bacteriolytic lipoproteins that were able to trigger OMPLA activity [105]. Colicins are encoded by extrachromosomal plasmid DNA molecules, which also encode a colicin immunity determinant [106]. Any cells in the population that jettison the colicin plasmid will likely be killed by the secreted colicin, which provides a kind of plasmid maintenance mechanism. Other exogenous assaults on the integrity of the outer membrane such as transfection of phage DNA [107], phage-induced lysis [108], spheroplast formation [109], heat shock [110], EDTA-treatment [111], and the action of antimicrobial peptides/proteins [112,113] have all been identified to trigger OMPLA.

It is difficult at first to envision how phospholipase activity provides a selective advantage towards most of the documented

OMPLA triggers. As noted by Dekker, the constitutive expression of the enzyme suggested more of a housekeeping function [114]. The elucidation of the OMPLA crystal structure in 1999 served to localize the active site to the external leaflet of the outer membrane and indicated how activity could be modulated by a reversible dimerization mechanism [1]. When outer membrane lipid asymmetry is intact, OMPLA is proposed to assume an inactive monomeric structure. Events that promote the migration of glycerophospholipids into the external leaflet induce OMPLA dimerization, which leads to the formation of two active sites at the subunit interface where Ca^{2+} ions needed for catalysis are able to bind [115]. Perturbations of outer membrane lipid asymmetry that occur in the presence of divalent cations are most likely to trigger OMPLA, which would provide an appropriate adaptive response – restoration of the permeability barrier – by removing phospholipids from the external leaflet [114]. Although the fatty acid and lysophospholipid products have detergent-like properties that would be expected to destabilize the outer membrane, mechanisms for the active uptake and recycling of these compounds have been described [116–120].

The crystal structure of *E. coli* OMPLA shows how it hydrolyzes glycerophospholipids in the outer membrane (Fig. 3A). OMPLA consists of 12 antiparallel β-strands that fold into a transmembrane β-barrel. An intricate hydrogen-bonding network occludes the central cavity of OMPLA. The three active site residues, Asn156, His142, and Ser144, are organized on the exterior of the β-barrel in a catalytic triad that normally associates with the inert glucosamine backbone of lipid A in the outer leaflet [121,122]. The nucleophilic Ser144 of OMPLA can be irreversibly sulfonylated by the palmitate analog hexadecanesulfonyl fluoride. The crystal structure of sulfonylated OMPLA shows it to be a homodimer with Ca^{2+} contributing to the oxyanion holes that stabilize the tetrahedral intermediate during acylenzyme ester formation and hydrolysis [1,123–125]. Molecular dynamics investigations also suggest that increased stability around the active site in dimeric OMPLA is a consequence of the local ordering of water near the bound Ca^{2+} ions [126].

OMPLA first selects a fatty acyl-chain at the *sn*-1 position of its glycerophospholipid substrate [127], but it can also hydrolyze the *sn*-2 acyl chain and will continue to hydrolyze acyl-chains from the lysophospholipid byproducts *in vitro* (Fig. 2). Snake venom phospholipases typically display positional selectivity in the glycerol backbone of their phospholipid substrates, but they are hard-pressed to reach into the bilayer and select particular fatty acyl-chain types. OMPLA is largely unspecific for phospholipid polar head groups, and recent thermodynamic studies of OMPLA dimerization have revealed that the formation of acyl-chain binding pockets at the subunit interface endows the enzyme with the ability to select fatty acyl-chains of 14 carbons or more in length [128,129].

The presence of OMPLA in *Neisseria* species is problematic because the outer membranes of these facultative intracellular pathogens exhibit considerable permeability toward hydrophobic compounds and likely display phospholipids on the cell surface [64]. Recent studies show that *Neisseria* OMPLA can induce bacteriolysis after prolonged growth. By releasing the cellular contents of a moribund subpopulation, it is proposed that this behavior altruistically sustains the remaining healthy cells in the population [130]. Although OMPLA activity is evident in most strains that cause gonorrhoeae and meningitis, some of the latter strains could be shown to contain premature stop codons in their OMPLA-encoding *pldA* genes. OMPLA activity is known to be associated with changes in cell surface structure that promote host–pathogen interactions in *Campylobacter* and *Helicobacter* infections [131–134], but its inactivation might also confer a selective advantage under certain pathogenic conditions in *N. meningitidis*.

3.2. The protease OmpT

An outer membrane protein of *E. coli* that was initially identified in polyacrylamide gels as protein a or 3B was later renamed OmpT because its expression was significantly reduced during growth at low temperature [135]. Proteolytic activity was first attributed to OmpT when outer membrane cleavage of the ferric enterobactin receptor protein was demonstrated in 1979 [136]. OmpT was subsequently recognized to be a nuisance for the purification of many recombinant proteins expressed in *E. coli* [137]. First purified in 1988, a striking feature of OmpT is its specificity for cleavage between paired basic amino acid residues [138]. The early identification of OmpT-deficient strains, such as *E. coli* BL21, provided a host strain to limit proteolysis during the expression and purification of recombinant proteins that is widely used today [137]. In most *E. coli* strains, the *ompT* gene is encoded by a cryptic prophage [139], but a homolog, *ompP*, can also be encoded by a conjugal F-plasmid that is sometimes present [140–142].

OmpT expression in *E. coli* can be regulated by the EnvZ/OmpR transmembrane phosphorylation cascade [135,143], which acts through the expression of two small RNA molecules OmrA/OmrB [144]. Control of OmpT is also exerted by PhoP/PhoQ, which, in *E. coli*, receives upstream signals from another transmembrane phosphorylation cascade EvgA/EvgS [145]. When rationalizing the teleonomic significance of regulatory signals acting on a gene, one should probably focus on the selective advantages conferred to the genetic element that encodes the gene [146]. In the case of a cryptic prophage, it is unclear if selection acted previously in favor of the phage or more recently in favor of the cell. Fortunately, a *bona fide* chromosomal gene encodes a *Salmonella* homolog of OmpT, known as PgtE, which is similarly governed by PhoP/PhoQ [147]. A logical function for PgtE, therefore, might be to confer antimicrobial peptide resistance on *Salmonella*. Indeed, the proteolytic specificity in OmpT homologs for paired basic residues appears to be ideally suited for the recognition of cationic antimicrobial peptides. OmpT provides resistance against protamine [148], a polycationic antimicrobial peptide known to disorganize outer membrane lipids, and PgtE is required for resistance to certain α-helical cationic antimicrobial peptides [147].

The crystal structure of *E. coli* OmpT shows how it degrades antimicrobial peptides presented at the bacterial cell surface

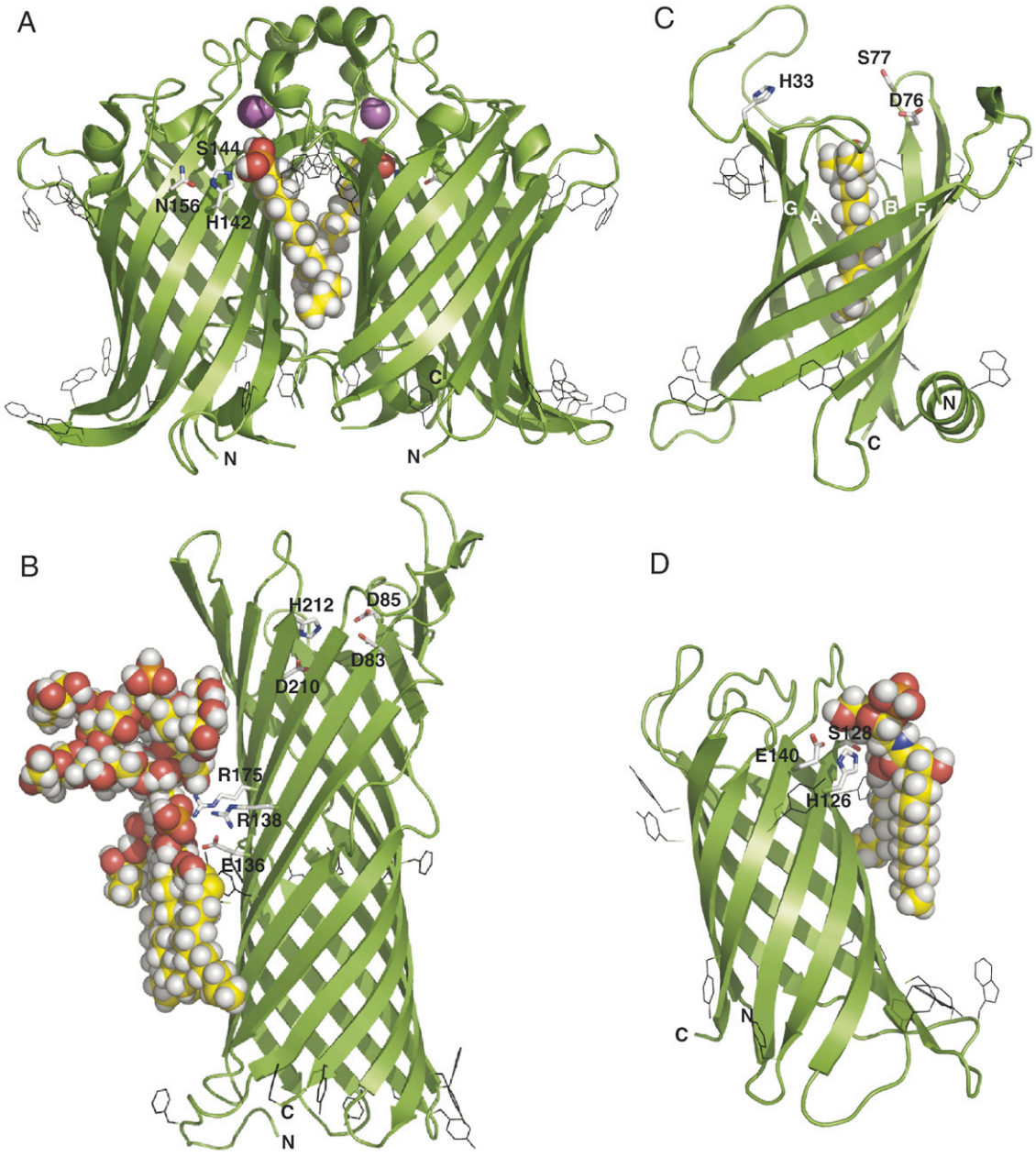

Fig. 3. Structures of outer membrane β-barrel enzymes. (A) The outer membrane phospholipase OMPLA from *E. coli* with bound Ca^{2+} (purple) and hexadecanesulfonyl group (pdb: 1QD6). (B) The outer membrane protease OmpT from *E. coli* (pdb: 1I78); coordinates with bound lipooligosaccharide provided by Piet Gros and Lucy Rutten (Utrecht University). (C) The phospholipid: :lipid A palmitoyltransferase PagP from *E. coli* with bound lauroyldimethylamine-*N*-oxide (pdb: 1THQ); coordinates with L1 loop introduced between β-strands A and B provided by Chris Neale and Régis Pomès (University of Toronto). (D) The outer membrane lipid A 3-*O*-deacylase from *Pseudomonas aeruginosa* (pdb 2ERV); coordinates with bound lipid X provided by Jan Tommassen and Lucy Rutten (Utrecht University).

(Fig. 3B). The enzyme is a 10-stranded antiparallel β-barrel that extends well above the external surface of the bilayer [2]. The active site is displayed within the external crevice formed by the OmpT surface-exposed loops. The catalytic groups include two pairings of Asp85 and Asp83 on one face, and His 212 and Asp210 on the other face. Although the X-ray structure was solved at insufficient resolution to visualize water molecules, arguments based on residue geometry and the enzyme's pH optimum (pK_a=6.2) suggested that a water molecule bridging His212 and Asp83 is delivered directly to the scissile peptide bond in the antimicrobial peptide substrate, and this model has since been substantiated by molecular dynamics simulations [149].

The OmpT active site organization appears to be stabilized by interactions on the β-barrel exterior with lipopolysaccharide, which is a required cofactor [150–152]. A motif of charged amino acids on the β-barrel exterior includes Arg175, Arg138, and Glu136, which are implicated in providing electrostatic

binding interactions with charged groups in lipid A and the inner core [2,153]. A molecule of lipid A-core from its complex with the FhuA ferrichrome receptor [154] has been modeled onto the exterior of OmpT (Fig. 3B). This lipooligosaccharide molecule reaches toward, but not beyond, the active site cleft. The presence of the O-antigen polysaccharide in wild-type bacteria is expected to extend far beyond the surface of the OmpT active site and likely shields most proteins from OmpT-mediated digestion [155]. Those cationic peptides that interact with negatively charged groups in lipopolysaccharide are expected to penetrate through the O-antigen layer and suffer proteolytic degradation once they encounter OmpT [147]. The OmpT protein thereby captures only those cationic antimicrobial peptides that reveal its activity in the process of self-promoted uptake.

Shigella flexneri is a close relative of *E. coli* that has acquired additional genetic determinants to enable actin-based intracellular motility during shigellosis. Part of this machinery includes an autotransporter protein VirG (IcsA), which displays a cell surface domain needed for actin polymerization at one bacterial pole. OmpT efficiently cleaves the VirG extracellular domain and thus limits intracellular motility [139]. It appears that *ompT* gene absence was a prerequisite in the acquisition of *Shigella* virulence from its *E. coli* ancestor [156]. Nevertheless, a *Shigella* virulence plasmid encodes another OmpT homolog, SopA (IcsP), which assists in the polar localization of the VirG protein by selectively removing it from other regions of the cell surface [157,158]. In *Yersina pestis*, the O-antigen-deficient causative agent of bubonic plague, a plasmid-encoded OmpT homolog, Pla, proteolytically activates the human proenzyme plasminogen and inactivates the antiprotease α2-antiplasmin in order to enhance bacterial migration through tissue barriers [159,160]. Although these different OmpT homologs clearly fulfill distinct physiological functions, their active site architectures are conserved; the so-called omptins are now widely recognized as a distinct proteolytic enzyme family [161].

3.3. The phospholipid: :lipid A palmitoyltransferase PagP

PagP is only the third enzyme shown to be an integral outer membrane protein in *E. coli* [162]. PagP transfers a palmitate chain from the *sn*-1 position of a phospholipid to the hydroxyl group on the *R*-3-hydroxymyristate chain at position 2 of lipid A in the outer membrane (Fig. 2). The enzymology of palmitate addition to lipid A can be traced to the discovery of cytoplasmic monosaccharide lipid A precursors in 1983. Lipid X was shown to be a diacylglucosamine 1-phosphate bearing *R*-3-hydroxymyristoyl groups at positions 2 and 3, and lipid Y only differed from lipid X by the presence of a palmitoyl group in acyloxyacyl linkage at position 2 [163,164]. The discovery of a membrane-bound enzyme from *E. coli* that could generate lipid Y by transferring a palmitoyl group from the *sn*-1 position of a glycerophospholipid to lipid X was first reported in 1987 [165]. In 1997, several regulated covalent modifications of lipid A, including palmitoylation, were shown to be controlled by PhoP/PhoQ [166]. The gene *pagP* was then identified in a *Salmonella* mutant that exhibited both a deficiency in lipid A palmitoylation and a hypersensitivity to vertebrate cationic antimicrobial peptides [83]. A homolog in *E. coli*, known as *crcA*, had been identified previously among a cluster of genes involved in chromosome condensation and resistance to camphor vapors [167]. In the year 2000, the *E. coli* PagP (CrcA) protein was purified and established to be an outer membrane glycerophospholipid: :lipid A palmitoyltransferase [162], thus demonstrating that the lipid A moiety of fully assembled lipopolysaccharide is the correct physiological substrate.

The structure and dynamics of *E. coli* PagP have been determined by both X-ray crystallography and nuclear magnetic resonance spectroscopy [3,168]. PagP is an 8-stranded antiparallel β-barrel with a short α-helix at its *N*-terminus and it sits in the membrane with the barrel axis tilted by roughly 25 degrees (Fig. 3C) [169]. The PagP palmitate-recognition pocket, known as the hydrocarbon ruler, resides within the interior of the β-barrel and is localized in the outer lipopolysaccharide-exposed region of the protein. A single molecule of the detergent lauroyldimethylamine-*N*-oxide serves to identify the position of the hydrocarbon ruler within the β-barrel interior. PagP is remarkable among lipid acyltransferases in its ability to select a single 16-carbon acyl-chain in its phospholipid donor. A cluster of buried aromatic amino acid side-chains that line the floor of the hydrocarbon ruler can undergo a sensitive exciton interaction, which has been exploited to probe PagP's internal structure by circular dichroism spectroscopy [170]. Mutation and chemical modification at residue 88 can modulate acyl-chain selection with methylene unit precision provided that the exciton is not perturbed.

Two discontinuities in β-strand hydrogen bonding within the lipopolysaccharide-exposed region provide obvious routes for lateral access of lipids into the hydrocarbon ruler. The presence of conserved proline residues at key positions between β-strands A–B and F–G prevents the local formation of interstrand hydrogen bonds. Acting like crenellations in the turret of a medieval castle, lipids in the outer membrane external leaflet probably migrate laterally through these openings [3]. Although His33, Asp76, and Ser77 have been implicated in catalysis, no evidence supports an acylenzyme mechanism [168]. Catalysis most likely proceeds when both the lipid A and phospholipid substrates engage in a ternary complex with the enzyme [82]. PagP exists in two dynamically distinct states, but the solved structure represents an R-state that is inhibited by the bound detergent [171]. The catalytically competent T-state is largely determined by an ordering of residues in and around the L1 loop that connects β-strands A–B. Given that PagP displays slow phospholipase in addition to rapid lipid A palmitoyltransferase activities *in vitro* [172], and that L1 loop mutations can specifically block the lipid A palmitoyltransferase activity without affecting the phospholipase activity [82], it is likely that lipopolysaccharide approaches the hydrocarbon ruler between β-strands A–B; by default, the phospholipid approach is expected between β-strands F–G.

The implication that PagP depends on the aberrant migration of phospholipids into the external leaflet creates an important topological problem for the enzyme. PagP activity is insensitive to divalent cations and, conceivably, provides a specific

response to those perturbations in outer membrane lipid asymmetry induced by Mg^{2+} limitation [162,165]. Under these conditions, transacylation to lipid A might provide a superior adaptive response compared with phospholipid hydrolysis, which is otherwise catalyzed exclusively by OMPLA, albeit inefficiently, in response to brief EDTA treatment [111]. By contrast, brief treatment of *E. coli* cells with EDTA induces lipid A palmitoylation instantaneously through a process that far outpaces induction of *pagP* gene expression, and occurs independently of *de novo* protein synthesis [173]. Lipid A palmitoylation induced by EDTA *in vivo* requires functional MsbA, which probably replenishes phospholipids in the outer membrane inner leaflet after they translocate into the external leaflet. *E. coli imp* mutants respond similarly by increasing lipid A palmitoylation [57] (unpublished observations). In *E. coli* O157:H7, a mutant deficient in lipid A myristoylation was reported recently to trigger palmitoylation of lipid A in the outer membrane [53]; palmitate addition could correct permeability defects associated with the absence of myristate and, surprisingly, exerted control over cytoplasmic enzymes involved in assembly of the core oligosaccharide. Emerging evidence has indicated that palmitoylated lipid A in the outer membrane can trigger the periplasmic stress response governed by σ^E [174]. It seems reasonable that palmitoylated lipid A subtypes and/or lysophospholipids, accumulated in the outer membrane in response to breaches in the permeability barrier, might adversely influence β-barrel membrane protein assembly and, thereby, trigger the periplasmic stress response.

As previously discussed, lipid A palmitoylation at position-2 attenuates endotoxin signaling through the host TLR4 pathway [84]. In more divergent organisms, where palmitate is attached on the distal glucosamine unit at position-3′, lipid A palmitoylation has been reported to accentuate the endotoxicity of lipid A [175,176], but this might reflect differences in the overall extent of acylation in addition to the different positional specificity. Combined with its contributions to antimicrobial peptide resistance [83], palmitoylated lipid A likely functions to help pathogenic bacteria evade the host immune response [82]. PagP homologues are now known to be necessary for disease causation in the respiratory pathogens *Legionella pneumophila* and *Bordetella bronchiseptica* [177,178]; in the latter organism, the *pagP* gene is needed to provide resistance to antibody-mediated lysis executed by the membrane-attack complex of the serum complement cascade [179]. Other PagP homologues are narrowly distributed among a group of pathogenic Gram-negative bacteria that often regulate *pagP* gene expression in order to meet the specific needs of their infectious lifestyles [82]. Mutational inactivation of PagP is observed in *Y. pestis* and *B. pertussis*, which, together with the aforementioned bacterium-specific genetic silencing of OMPLA and OmpT [130,139], reveals a theme that outer membrane β-barrel enzymes might be incompatible with certain pathogenic states.

3.4. The lipid A 3-O-deacylase PagL

The discovery of lipid A palmitoyltransferase activity in membranes from PhoP-constitutive mutants of *Salmonella* fortuitously revealed the activity of another enzyme that simultaneously removed an acyl chain from lipid A precursors (Fig. 2) [162]. The PhoP/PhoQ-activated gene *pagL* was subsequently established to encode the responsible enzyme [85]. PagL functions as a lipid A 3-*O*-deacylase and is localized to the outer membrane, but it shows no discernible activity under normal conditions. The presence of L-Ara4N in lipid A was shown to inhibit PagL and its activity in outer membranes only occurs when L-Ara4N is absent from lipid A [180]. Activated PagL confers polymyxin B resistance, which appears to compensate for sensitivity associated with the absence of L-Ara4N [86]. Lipid A 3-*O*-deacylation also attenuates lipid A endotoxicity and likely contributes to the evasion of immune defenses [84]. PagL is absent from *E. coli*, but, compared to PagP, its homologues are more widely distributed and not primarily restricted to pathogenic organisms [175,181]. Interestingly, loss of PagL function in *P. aeruginosa* has been observed during long-term adaptation to the cystic fibrosis airway [182].

The crystal structure of PagL from *P. aeruginosa* reveals an overall fold that is similar to PagP, and its active site likewise faces the outer surface of the outer membrane (Fig. 3D). PagL is similarly an 8-stranded antiparallel β-barrel and is strikingly tilted in the outer membrane [4]. However, PagL differs in the presentation of its lipid substrate to a distinct catalytic triad formed by Glu140, His126, and Ser128 on the β-barrel exterior. Energy minimization with the model substrate lipid X reveals that acyl-chains likely bind into hydrophobic grooves on the PagL β-barrel exterior. Although the enzyme normally encounters *R*-3-hydroxydecanoyl chains in its substrates *in vivo*, it also utilizes *R*-3-hydroxymyristoyl chains *in vitro*, which indicates that acyl-chains encountered on the β-barrel exterior cannot be measured with the same precision as performed by PagP [182]. The potential of PagL to dimerize at an interface between active sites suggests a possible mechanism to inhibit activity in the outer membrane when L-Ara4N is present. Whereas OMPLA is activated by Ca^{2+}-dependent dimerization, a Ca^{2+}-independent dimerization process is proposed to inactivate PagL [4].

4. β-Barrel enzymes of unknown structure

At least two other β-barrel enzymes of unknown structure have been identified. LpxR removes the 3′-acyloxyacyl unit in lipid A and was recently identified in outer membranes of *Salmonella* and other important enteric pathogens [183]. The enzyme requires Ca^{2+} as a cofactor and is not regulated by PhoP/PhoQ. The function of LpxR is unknown, but alteration of the lipid A acylation pattern probably affects lipid A endotoxicity and/or antimicrobial peptide resistance. The activity of LpxR cannot be detected under normal growth conditions, indicating that its activity is latent. It seems likely that LpxR will function together with OMPLA in response to membrane perturbations when divalent cations are replete. Another lipid A modification that requires Ca^{2+} as a cofactor is the EptB-catalyzed incorporation of phosphoethanolamine into a key inner core sugar at the periplasmic surface of the inner membrane [88]. EptB also requires Ca^{2+} in the growth medium for expres-

sion [184], but LpxR expression, like that of OMPLA, appears to be constitutive.

Rhizobium species establish a symbiotic relationship with the root cells of certain plants and are responsible for nitrogen fixation. These Gram-negative bacteria fail to trigger the host innate immune response partly because they express a detoxified lipid A. The conserved Raetz pathway enzymes are largely intact, but additional extracytoplasmic enzymes serve to remodel the lipid A structure [53]. Among the observed structural variations is a two-step modification of the proximal glucosamine sugar. First, the phosphate group is removed by the lipid A 1-phosphatase LpxE, which acts at the periplasmic surface of the inner membrane [91,96]. Second, oxidation of the glucosamine aldehyde yields the open chain sugar 2-aminogluconate. The responsible oxidase is the EDTA-sensitive and O_2-dependent outer membrane enzyme LpxQ [185,186]. Based on the foregoing discussion, it might be tempting to speculate that LpxQ behaves, like the other outer membrane β-barrel enzymes, as a sentinel triggered by perturbations to the outer membrane structure. However, the LpxQ reaction proceeds efficiently under normal conditions when it encounters 1-dephosphorylated lipid A in the outer membrane. It is unknown if the 2-aminogluconate moiety is compatible with the transport of lipid A across the periplasmic compartment, but LpxQ might be a terminal biosynthetic enzyme that is restricted to the outer membrane simply because its activity is incompatible with lipid A transport. While the majority of outer membrane β-barrel enzymes are most easily comprehended in terms of the sentinel hypothesis, LpxQ underscores the fact that it need not necessarily apply to them all. It will certainly be interesting to learn how LpxQ uses O_2 to catalyze lipid A oxidation in the outer membrane environment.

5. Conclusions

The structure and function of β-barrel enzymes indicates that they have been adapted to serve as sentinels in the outer membrane. By functioning as sensory transducers whose activity is triggered by perturbations to outer membrane lipid organization, β-barrel enzymes provide a first line of defense against external attack. Centralized signal transduction networks that function to maintain the outer membrane permeability barrier can modulate the expression levels of these outer membrane sentinels, but the expansive outer membrane assembly pathway places huge demands for speed in responding to external assaults. The sentinels likely provide the instantaneous responses needed to outpace the centralized signal transduction pathways. Additionally, the discovery of outer membrane β-barrel enzymes has provided useful probes to monitor the biogenesis of the Gram-negative cell envelope. By following the modification of cell surface lipids catalyzed by β-barrel enzymes, insights into outer membrane assembly and function that were not previously possible are now dramatically changing our perspective on Gram-negative cell envelope structure. The importance of β-barrel enzymes in bacterial pathogenesis testifies that knowledge of their structure-function relationships might also lead to novel treatments for bacterial infections.

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