FEMS Microbiology Letters, 365, 2018, fny199

doi: 10.1093/femsle/fny199
Advance Access Publication Date: 13 August 2018
Minireview

MINIREVIEW – Pathogens & Pathogenicity

Lpp, the Braun lipoprotein, turns 50—major achievements and remaining issues

Abir T. Asmar[1,2] and Jean-François Collet[1,2,*]

[1]WELBIO, Université catholique de Louvain, avenue Hippocrate 75, Brussels 1200, Belgium and [2]de Duve Institute, Université catholique de Louvain, Avenue Hippocrate 75, Brussels 1200, Belgium

*Corresponding author: Institut de Duve, Avenue Hippocrate 75 - B1.75.08, B-1200 Bruxelles. Tel: +32 2 764 75 62; Fax: +32 2 764 75 98; E-mail: jfcollet@uclouvain.be

One sentence summary: We review the knowledge gained on Lpp since its discovery until the recent finding that it functions as a major size determinant in the cell envelope and discuss remaining questions.

Editor: Lily Karamanou

ABSTRACT

The discovery of *Escherichia coli* Lpp as the first protein with three acyl groups covalently attached to its N-terminal cysteine residue defined a new class of bacterial proteins, the lipoproteins. Lipoproteins are extracytoplasmic, globular proteins that are anchored to a membrane by a lipid moiety. Being anchored to the outer membrane, Lpp, which is also known as the Braun lipoprotein, is small (5.8 kDa) and folds into a trimeric helical structure. It is also the numerically most abundant protein in *E. coli*. A unique feature of Lpp is that its C-terminal lysine residue is covalently attached to the peptidoglycan, providing the only covalent connection between the outer membrane and the cell wall. Here, we review the knowledge gained on Lpp since its discovery in 1969 until the recent finding that Lpp functions as a major size determinant in the bacterial cell envelope. We also discuss the role played by Lpp in virulence and highlight the major questions that remain to be solved.

Keywords: lipoprotein; Braun lipoprotein (Lpp); Gram-negative bacteria; periplasmic size; outer membrane—peptidoglycan connection

INTRODUCTION

The envelope that characterizes Gram-negative bacterial cells has attracted the attention of microbiologists since it was first visualized by electron microscopy in the sixties (Claus and Roth 1964; Ushijima 1967). The Gram-negative cell envelope is a complex macromolecular structure that features three distinct layers: the cytoplasmic membrane, also called inner membrane (IM), the peptidoglycan sacculus (PG; also called murein) and the outer membrane (OM), which constitutes the interface with the environment. An aqueous, viscous compartment, called periplasm, lies between the OM and the IM, thus containing the PG (Silhavy, Kahne and Walker 2010). In the model bacterium *Escherichia coli*, the OM is covalently attached to the PG via the lipoprotein Lpp, which is the focus of this review. Lpp, also known as the Braun lipoprotein, is a small α-helical lipoprotein that is the numerically most abundant protein in *E. coli*. Since its discovery almost 5 decades ago, Lpp has been the subject of intense scrutiny, researchers exploring its structural features, biosynthesis, maturation, physiological function and role during infection. Herein, we summarize the current knowledge on this important envelope protein, discussing both the experiments that led to its discovery and initial characterization and the most recent achievements. We also highlight the fascinating questions that remain unsolved. We dedicate this review to Prof. V. Braun, the discoverer of Lpp, on the occasion of his 80th birthday.

Received: 4 June 2018; **Accepted:** 10 August 2018

MAJOR PROPERTIES OF Lpp

Lpp, the first discovered lipoprotein

PG is a polymer consisting of linear glycan chains made of alternating N-acetylglucosamine and N-acetylmuramic acid units. The glycan strands are cross-linked together via short stem peptides. In 1969, Braun and Rehn reported that when purified PG sacculi were treated with trypsin, a lysine residue remained covalently attached to about 1 out of every 10 repeating units (Braun and Rehn 1969). This led them to conclude that this lysine originated from a protein that was covalently bound to the PG but had been released by the proteolytic treatment. In addition, they also found that a lipid could be extracted from the proteolytic digest when it was treated with organic solvents, thus suggesting that the protein that was linked to the PG by a lysine was also anchored to a membrane by a lipid (Braun and Rehn 1969). In 1972, the sequence of that protein was reported (Braun and Bosch 1972b), revealing a highly repetitive nature and the presence of a C-terminal lysine residue bound via its ε-amino group to the PG (Fig. 1A and B). A year later, in 1973, it was shown that the protein was anchored to the OM via three acyl groups covalently attached to an N-terminal cysteine residue (Hantke and Braun 1973) (Fig. 1A). Because this protein was the first found to exhibit these unusual features, it was first referred to as 'the lipoprotein' (Braun and Rehn 1969) and only later as Lpp (Inouye, Shaw and Shen 1977). In fact, Lpp turned out to be the first identified member of a large family of bacterial envelope proteins that are attached to a membrane by a lipid moiety (Hayashi and Wu 1990). A bacterium like *E. coli* expresses about 100 different lipoproteins, which are targeted either to the IM or to the OM, and that carry out important functions in cellular processes such as envelope biogenesis, signal transduction, stress sensing, virulence and cell division (Szewczyk and Collet 2016). Lpp has remained a unique member among the lipoprotein family in that it is so far the only one found to be covalently attached to the PG.

Soon after Lpp was identified in *E. coli*, the existence of homologous PG-bound lipoproteins was reported in other γ-proteobacteria, including *Pseudomonas* and *Salmonella* (Braun, Rehn and Wolff 1970) (Fig. S1, Supporting Information). In this latter bacterium, two *lpp* genes (*lppA* and *lppB*) are encoded in tandem (Sha *et al.* 2004; Kroger *et al.* 2013), but only one of them (LppA) is expressed under laboratory conditions. As of today, Lpp has been identified in many γ-proteobacteria species, including Enterobacteriaceae, Vibrionaceae, Shewanellaceae and Pseudomonadaceae (Fig. S1, Supporting Information). From now on, unless specified otherwise, we will focus on the Lpp protein from *E. coli*.

The PG-bound C-terminal lysine residue is highly conserved among Lpp homologues and often present in an YRK motif (Fig. S1, Supporting Information) (Zhang and Wu 1992). Whereas it is not surprising that mutating the C-terminal lysine results in the loss of PG crosslinking, it is remarkable that replacing the tyrosine or arginine with β-turn inducing amino acids within the α-helix (Tyr76 replaced by Pro, Ser, Gly or Arg or Arg77 replaced by Leu) or negatively charged residues (Asp, Glu), while keeping the C-terminal lysine, also results in a reduced attachment of Lpp to the PG (Zhang and Wu 1992). Finally, it is interesting to note that some bacteria, including *E. coli*, *Shigella flexneri*, *Shigella boydii*, *Escherichia fergusonii*, *Shigella sonnei*, *Shigella dysenteriae* and *Salmonella enterica* encode in addition to *lpp*, an *lpp* paralog, *yqhH*, in which the C-terminal lysine residue is replaced by a negatively charged glutamate. The function of this protein, which is not constitutively expressed in *E. coli* (Li *et al.* 2014), remains elusive.

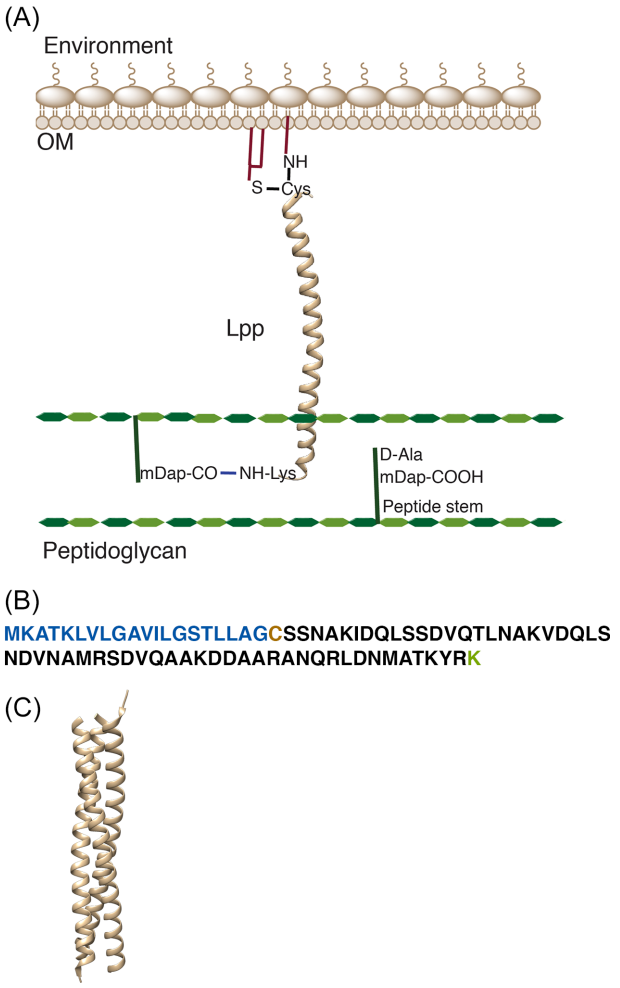

Figure 1. Structural features of Lpp. (**A**) The lipoprotein Lpp (drawn as a monomer) is anchored to the OM via a lipid moiety composed of three acyl chains (red lines) that are attached to the N-terminal cysteine residue of the mature protein. Lpp is also bound to mDap residues in the peptide stems of the peptidoglycan via its C-terminal lysine residue. (**B**) Sequence of *E. coli* Lpp, highlighting the signal sequence in blue, the lipidated cysteine residue in brown and the C-terminal lysine in green. (**C**) Crystal structure of *E. coli* Lpp, showing a trimeric α-helical coiled coil (PDB: 1EQ7). D-Ala, D-alanine; Cys, cysteine; Lys, lysine; OM, outer-membrane.

Lpp is a massively abundant protein

Lpp is massively expressed; being present at around 1 million copies per cell (Li *et al.* 2014). Consistently, the Lpp mRNA represents up to ~10% of all cellular mRNAs and ~8% of all translation events in standard conditions (Li *et al.* 2014). A couple of interesting features can be highlighted here to explain how such high expression levels can be reached. First, the promoter region of the *lpp* gene has a high AT content, not only in *E. coli* (Nakamura and Inouye 1979), but also in *Serratia marcescens* (Nakamura and Inouye 1980) and *Erwinia amylovora* (Yamagata, Nakamura and Inouye 1981). Because high AT content is thought to facilitate strand unwinding by the RNA polymerase for transcription initiation, this feature likely favors the high transcription efficiency of the *lpp* gene (Nakamura and Inouye 1979). A second feature that can also contribute to the high expression levels of Lpp is the fact that the Lpp mRNA is extraordinarily stable, having a half-life of 12 min (Hirashima and Inouye 1973),

which is substantially more than most other *E. coli* mRNAs (average half-life of 1.3 min [Ingraham *et al.* 1983]).

Lpp is so abundant that it is not surprising that cells turn down its synthesis when problems occur in the assembly of the bacterial cell envelope. Indeed, it was recently shown that Lpp synthesis is under control of sigmaE, an essential sigma factor that induces a stress response when problems occur in β-barrel folding or LPS targeting to the OM (Lima *et al.* 2013). This control is exerted at the post-transcriptional level by *micL*, an sRNA that is induced by sigmaE (Guo *et al.* 2014) and that represses Lpp translation by blocking ribosome initiation. Given the high stability of the Lpp mRNA, blocking its translation appears to be a more efficient mechanism to rapidly lower the flux of Lpp molecules to the cell envelope than transcriptional repression.

How is Lpp crosslinked to the PG?

The binding of Lpp to the PG is catalyzed by L,D-transpeptidases that covalently link the ε-amino group of the C-terminal lysine of Lpp to the carboxyl group of the meso-diaminopimelic acid (mDAP) residues present in the PG peptide stems (Fig. 1A). As mentioned above, experiments carried out by Braun and colleagues using purified, trypsin-digested PG sacculi established that binding between Lpp and the PG occurs every 10th to 12th mDAP residue (Braun and Rehn 1969). In addition, using immunoelectron microscopy, it was also deduced by visual inspection that the distribution of bound Lpp was homogeneous over the entire sacculus (Hiemstra *et al.* 1987), although this deduction still awaits confirmation using modern approaches (see section Conclusions and Perspectives).

In *E. coli*, three L,D-transpeptidases, ErfK, YbiS and YcfS (renamed LdtA, LdtB and LdtC, respectively), are able to catalyze the crosslinking of Lpp to the PG (Magnet *et al.* 2007). All three enzymes are homologous and present a conserved cysteine residue in their catalytic site. This residue, which is prone to oxidation by reactive oxygen species, is protected from irreversible inactivation by the oxidoreductase DsbG (Depuydt *et al.* 2009). DsbG functions in a periplasmic reductive pathway fueled by electrons originating from the cytoplasmic pool of NADPH (Depuydt *et al.* 2011). LdtB appears to be the most important L,D-transpeptidase in exponential phase for at least two reasons. First, it is substantially more abundant than LdtA and LdtC, being present at around 5000 copies per cell, in comparison to 500 copies for LdtC and 70 for LdtA (Li *et al.* 2014). Second, Magnet *et al.* showed that the formation of Lpp-PG crosslinks is almost abolished in cells lacking this enzyme (Magnet *et al.* 2007). Why cells express three different L,D-transpeptidases remains unclear and suggests that these enzymes may have specific roles to play under certain conditions.

A fraction of the Lpp pool is not attached to the PG

Shortly after the discovery of Lpp, Inouye and colleagues reported that a fraction of the Lpp pool was not covalently bound to the PG (Inouye, Shaw and Shen 1972), remaining free in the envelope. Interestingly, the free form of Lpp seems to keep the ability to interact with the PG non-covalently (Hancock *et al.* 1981; Choi *et al.* 1986; Clark and Bavoil 2002), but the molecular details of the interaction remain unknown. In cells grown under normal conditions, the free and bound forms of Lpp co-exist in an approximate 2:1 ratio, respectively (Inouye, Shaw and Shen 1972). Interestingly, this ratio varies depending on the growth stage, the binding of Lpp to PG increasing by 70% when cells enter stationary phase (Glauner, Holtje and Schwarz 1988). In addition, changes in the structure of the PG also appear to impact the abundance of Lpp-PG crosslinks. For instance, deleting *ynhG* and *ycbB*, two genes responsible for the formation of mDAP-mDAP crosslinks, increases the amount of PG-bound Lpp by 2-fold, perhaps because more mDAP residues are available for crosslinking with Lpp (Schwechheimer, Kulp and Kuehn 2014). Intriguingly, the *lpp* gene is encoded right next, although in the opposite orientation, to *ynhG*, further suggesting a functional link between these two genes (Schwechheimer, Kulp and Kuehn 2014). In the same line, PG-Lpp crosslinking levels decrease by 40% when cells lack NlpI, a lipoprotein that was proposed to control the activity of the PG endopeptidase Spr (Schwechheimer, Rodriguez and Kuehn 2015), but double when cells are treated with mecillinam, a β-lactam that targets PBP2, a transpeptidase involved in PG assembly (Braun and Wolff 1975). Thus, altogether, these results suggest a link between Lpp crosslinking to PG and PG synthesis, but further studies are required to clarify this.

Interestingly, Cowles and co-workers recently reported that the C-terminal moiety of the free form of Lpp could be labeled by NHS-LCLC-biotin, an amine-reactive, biotinylated probe that is OM impermeable (Cowles *et al.* 2011). Thus, in contrast to the general view that *E. coli* OM lipoproteins face the periplasm (Szewczyk and Collet 2016), these results indicated that free Lpp is exposed on the surface. Consistently, the amount of surface-exposed Lpp was increased in cells expressing the Lpp mutant lacking the C-terminal lysine residue, whereas no labeling of Lpp was detected in cells expressing $lpp_{\Delta 17-37}$, an Lpp variant that only exists as PG-bound (Cowles *et al.* 2011). How Lpp crosses the OM to reach the surface remains completely unknown.

THE STRUCTURE OF Lpp

The features of the primary sequence of Lpp (58 residues in the *E. coli* mature protein) suggested that the protein was α-helical (Braun and Bosch 1972a), which was confirmed by circular dichroism (Braun *et al.* 1976; Lee, Cheng and Inouye 1977). In addition, the presence of a seven amino acid repeat (known as the heptad repeat) in which positions 1 and 4 are occupied by a hydrophobic residue was consistent with the formation of an α-helical coiled coil. These initial predictions were confirmed when the crystal structure of *E. coli* Lpp was finally solved (Shu *et al.* 2000; Liu, Cao and Lu 2002), revealing that the protein folds into an α-helical trimeric coiled-coil (Fig. 1C). The structure of Lpp presents three domains: an N-terminal capping motif, a long (residues 5–53) coiled-coil domain consisting of three parallel α helices and a hydrophobic helix-termination motif.

In vitro analysis of the melting profile of Lpp showed that formation of the trimeric structure is a very slow process since it requires the alignment of the three strands in a helical conformation (Dragan *et al.* 2004). As soon as it is achieved, however, all three strands cooperatively collapse into a three-stranded coiled coil (Dragan *et al.* 2004). Unfolding of the Lpp rigid trimeric structure is even slower, since it requires the simultaneous breaking of all van der Waals contacts and salt bridges between the strands (Dragan *et al.* 2004).

FUNCTIONS OF Lpp

PG-bound Lpp maintains envelope integrity

Perhaps surprisingly, given the abundance of Lpp and its role in attaching the OM to the PG, *E. coli* cells lacking Lpp are viable and have no major growth defect under laboratory conditions. They

exhibit, however, several phenotypes, including an increased sensitivity to hydrophobic antibiotics, chelating agents and toxic compounds such as SDS, EDTA, deoxycholate, dibucain and vancomycin (Hirota *et al.* 1977; Yem and Wu 1978; Cascales *et al.* 2002; Nichols *et al.* 2011). They also release periplasmic proteins to the extracellular medium (Hirota *et al.* 1977; Yem and Wu 1978). In addition, Δ*lpp* mutants also form OM blebs (Hoekstra *et al.* 1976; Suzuki *et al.* 1978; Schwechheimer, Kulp and Kuehn 2014; Cohen *et al.* 2017), thus suggesting that Lpp functions as a tether for the OM under normal growth conditions, preventing this membrane to tear apart from the cell. Interestingly, cells expressing an Lpp variant lacking the C-terminal lysine ($lpp_{\Delta K58}$) or deleted for the three L,D-transpeptidases that crosslink Lpp to the PG share most of the phenotypes of the Δ*lpp* mutant (Sanders and Pavelka 2013; Asmar *et al.* 2017), which further highlights the important structural role played by Lpp in providing the only covalent attachment between the OM and the PG layer.

We would like to highlight here the role of Pal and OmpA, two proteins that also contribute to envelope integrity by connecting the OM to the PG, although non-covalently. Pal is an abundant OM lipoprotein (60 000 copies per cell [Li *et al.* 2014]) that is part of the transenvelope Tol-Pal system. Tol-Pal is thought to play a role in membrane constriction (Gerding *et al.* 2007) during cell division and in phospholipid retrograde transport (Shrivastava, Jiang and Chng 2017). OmpA is an abundant (207 000 copies per cell [Li *et al.* 2014]), widely conserved OM protein, which adopts a two-domain structure with an N-terminal eight-stranded β-barrel inserted in the OM (Pautsch and Schulz 1998) and a C-terminal, globular domain present in the periplasm. Although Pal and the periplasmic domain of OmpA have low sequence similarity, they both fold into a homologous structure that is able to bind non-covalently to the PG (recently reviewed in [Egan 2018]), thereby cooperating with Lpp in connecting the two outermost envelope layers. Consistently, Pal overexpression was shown to complement the sensitivity of Δ*lpp* mutants to SDS and vancomycin and to decrease bleb formation (Cascales *et al.* 2002). It is tempting to speculate that by evolving different proteins capable to connect the OM to the PG either covalently or non-covalently, bacteria are able to modulate the type of PG-OM connection along the cell axis to optimize cellular processes.

PG-bound Lpp is a distance keeper

It was intuitive for the scientific community that the distance between the OM and the PG was dependent on the length of Lpp (Braun and Wu 1994). However, it is only recently that the function of Lpp as a key determinant of the size of the envelope was experimentally demonstrated. In studies performed in *S. enterica* (Cohen *et al.* 2017) and *E. coli* (Asmar *et al.* 2017), it was indeed shown that the distance between the IM and the OM was increased in cells expressing longer Lpp variants from the *lpp* locus (Asmar *et al.* 2017; Cohen *et al.* 2017) (Fig. 2). Remarkably, the increase was proportional to the length of Lpp. Insertions of 14 (Lpp_{+14}) or 21 residues (Lpp_{+21}) augmented the IM-to-OM distance by 3 and 4 nm, respectively, as measured by cryo-electron microscopy (cryo-EM), a technique in which cells are preserved in a frozen-hydrated, near-native state (Asmar *et al.* 2017). It was therefore concluded that Lpp dictates the size of the periplasm.

In addition, the use of these Lpp length variants also brought important new insights into the cell envelope architecture. First, Cohen *et al.* showed that controlling the IM-to-OM distance is required for the OM to exert physical pressure on structures spanning the intermembrane space. In particular, they studied the length of the rod, a portion of the flagellum that forms a drive shaft transmitting torque generated by the cytoplasmic motor to the external filament. The authors found that the defined length of the rod (25 nm) is determined by the width of the space between the IM and the OM. Increasing the intermembrane distance alleviated the physical pressure of the OM on the rod allowing it to extend beyond 25 nm (Fig. 2) (Cohen *et al.* 2017). Second, we showed that strict control over the IM-to-OM distance is required for effective envelope surveillance and protection during exponential growth. Indeed, we found that increasing the length of Lpp resulted in the inability of the OM stress sensor lipoprotein RcsF to transfer stress signals to IgaA, its downstream partner in the IM (Cho *et al.* 2014), thus preventing the activation of the Regulation of Capsule Synthesis (Rcs) stress response system when peptidoglycan damage occurs (Asmar *et al.* 2017). Remarkably, efficient signaling could be restored by increasing the length of the intrinsically disordered linker (Asmar *et al.* 2017) that RcsF displays at its N-terminus (Leverrier *et al.* 2011; Rogov *et al.* 2011). This study demonstrated the physiological importance of the size of the envelope, suggesting that cellular architecture and the structure of transenvelope protein complexes have been evolutionarily co-optimized for correct function.

In this context, it is interesting to note that the length of Lpp is highly conserved among species with only small variations ranging from 1 to 8 amino acids (Fig. S1, Supporting Information). Intriguingly, *Baumania cicadellinicola*, a γ-proteobacterium, has an exceptionally long Lpp protein with 34 additional amino acids (Uniprot accession number: AKZ65694.1), suggesting that the size of its envelope might be larger. However, this has never been investigated so far.

Functions of the free form of Lpp

In contrast to the bound form of Lpp, whose structural and functional importance has been demonstrated, the role of the free form remains unclear. Soon after its discovery, it was first proposed that it could create diffusion pores through the OM (Inouye 1974). However, the fact that Δ*lpp* mutants are not altered for permeability to hydrophilic molecules (Nakae 1976) and that Lpp did not produce pores in an *in vitro* reconstitution assay quickly argued against this hypothesis. An alternative hypothesis that remains worth exploring is that cells maintain a pool of free Lpp molecules, available for cross-linking, to enable them to modulate the level of covalent attachment between the PG and the OM during the cell cycle or along the cell axis. Supporting this idea, bacterial cells increase the number of covalent connections between the OM and the PG when they enter stationary phase (see above). In addition, it is also possible that cells respond to specific envelope stress conditions by increasing the number of Lpp-PG crosslinks, which would require a pool of readily available Lpp molecules. Further experimental work is required to test this hypothesis and to elucidate the function of free Lpp. The recent identification of the free form of Lpp on the cell surface (Cowles *et al.* 2011) only makes this task more challenging.

Function of Lpp in virulence

We also would like to briefly highlight here that Lpp does not only play a structural role in the cell envelope, but is also important for bacterial pathogenesis, either by contributing directly or indirectly to the virulence of the pathogen or by modulating the immune response of the host. For instance, in the pathogenic *E.*

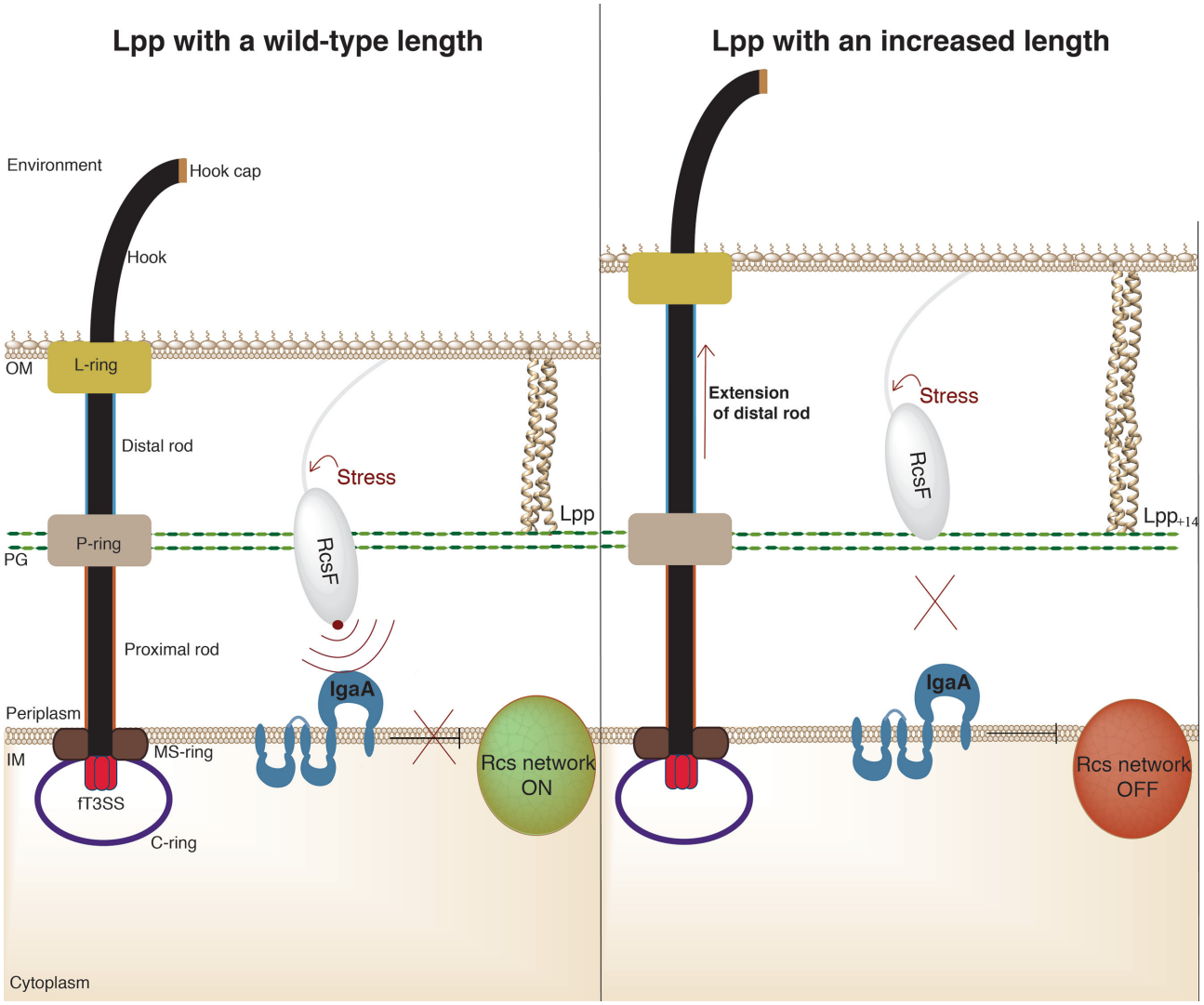

Figure 2. Peptidoglycan-bound Lpp controls the size of the envelope, which is important for flagellum assembly and intermembrane communication. Lpp was recently shown to be a major determinant of the size of the envelope: increasing the length of Lpp (Lpp_{+14}) increases the distance between the inner and the OM. Because the length of the rod of the flagellum (25 nm) is limited by the intermembrane distance, increasing the size of the envelope allows rod length to extend beyond this value (Cohen *et al.* 2017). In addition, increasing the intermembrane distance also prevents the outer membrane lipoprotein stress sensor RcsF to interact with IgaA, its downstream partner in the IM in conditions of envelope stress, thus preventing the activation of the Rcs signaling cascade (Asmar *et al.* 2017). C-ring, cytoplasmic ring; ft3ss, flagellar type III secretion system; IM, inner-membrane; L-ring, Lipopolysaccharides ring; MS-ring, membrane-supramembrane ring; OM, outer-membrane; PG, peptidoglycan; P-ring, peptidoglycan ring.

coli strain O157:H7, *lpp* deletion has been shown to induce the Cpx stress response system (Uhlich *et al.* 2009), which in turn reduces cell invasiveness by turning down biofilm formation. Similarly, in *Salmonella* Typhimurium, the absence of Lpp resulted in reduced virulence and host cell invasion (Sha *et al.* 2004), probably caused by the downregulation of a series of important factors including components of the type III secretion system and of the flagellar apparatus (Fadl *et al.* 2006). More recently, in the uropathogenic *E. coli* strain CFT073, the Δ*lpp* deletion mutant was shown to be sensitive to serum killing *in vitro* and to be impaired in its ability to produce capsular polysaccharides, a major virulence determinant (Diao *et al.* 2017). Interestingly, the authors showed a correlation between impairment of the Lpp-PG attachment and the correct localization of the capsule polysaccharide translocon KpsD, which could explain the observed defect in capsule production.

From the host perspective, *E. coli* Lpp has been shown to contribute to the activation of an inflammatory response in epithelial cells (Neilsen, Zimmerman and McIntyre 2001) and to the septic and inflammatory responses in mice (Lakshmikanth *et al.* 2016). Lpp appears to act through TLR2 (Aliprantis *et al.* 1999; Lakshmikanth *et al.* 2016), a toll-like receptor (TLR) that recognizes the broadest range of ligands activating macrophages during infection (Jiménez-Dalmaroni, Gerswhin and Adamopoulos 2016). The ability of TLR2 and other TLRs to recognize lipoproteins like Lpp is now being taken advantage of by synthetic lipopeptides that are used for vaccination without additional adjuvants (Zaman and Toth 2013).

Lpp AND BIOTECHNOLOGICAL APPLICATIONS

The unique structural features of Lpp, its cellular abundance and high stability have inspired many biological applications. For instance, due to their increased OM permeability, *E. coli* cells deleted for *lpp* exhibit an improved ability to secrete recombinant proteins directly into the culture medium (Shin and

Chen 2008), thus facilitating subsequent detection and purification. The secretion of recombinant proteins in the external environment without cell lysis is also interesting for the production of enzymes involved in the degradation of toxic pollutants. Additionally, because *lpp* deletion increases OM permeability without substantially affecting cellular growth, it can also be used to facilitate the entry of substrates of recombinant enzymes produced by the cell (Ni, Reye and Chen 2007; Chen *et al.* 2014). Finally, while cells deleted for *lpp* were proposed as attenuated live vaccine candidates against *Salmonella* (Liu *et al.* 2008; Erova *et al.* 2016) and *Yersinia* (Sha *et al.* 2008), recombinant OprI (Lpp in *Pseudomonas aeruginosa*) was tested as a vaccine and found to protect mice against *P. aeruginosa* (Finke *et al.* 1990).

CONCLUSIONS AND PERSPECTIVES

Lpp, the first ever discovered lipoprotein, has been studied since the late sixties from different perspectives. At first, it served as a paradigm for lipoprotein biosynthesis and maturation, before interest aroused not only for its involvement in virulence and infection but also for its structural role in the bacterial cell envelope. In particular, the important role played by the bound form of Lpp in tethering the OM to the PG and in maintaining periplasmic size has been recently unraveled. In that respect, future research should address to which extent envelope-spanning systems in *E. coli* and other bacteria are dependent on strict control over the IM-to-OM distance by Lpp. It will also be interesting to elucidate how the OM-PG connection is maintained in bacteria that do not express Lpp. It is possible that these bacteria rely on proteins like Pal and OmpA to tether the OM to the PG and control the size of the envelope via non-covalent interactions. Alternatively, it is also possible that other envelope proteins, lipoproteins or β-barrels, will be found to be covalently cross-linked to the PG in the future.

Importantly, despite the impressive wealth of knowledge gained on Lpp, crucial questions remain unsolved regarding this protein. For instance, it remains to be carefully investigated where and when the crosslinking of Lpp to the PG occurs, if the distribution of bound Lpp is homogeneous along the cell axis and at the pole and how cells control the ratio between the bound and free forms of this protein. In the same line, further work will reveal why bacteria like *E. coli* express different L,D-transpeptidases, such a redundancy supporting the hypothesis that these enzymes carry out specific yet complementary functions. Finally, how the different pathways involved in OM and PG biogenesis depend on the attachment of Lpp to the PG for coordination and how the free form of Lpp reaches the cell surface are additional aspects that are worth exploring. Although Lpp has been studied for 5 decades, this small, yet fascinating protein keeps several well-guarded secrets. To reveal them, scientists will benefit from recent technological advances, in particular in microscopy and labeling approaches that should facilitate the exploration of Lpp function at the spatio-temporal level.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

ACKNOWLEDGEMENTS

We thank Raphaël Helaers, Michael Deghelt, Pauline Leverrier and Alexandra Gennaris for critical reading of the manuscript and for providing helpful comments. AA is Aspirant and J-FC is Directeur de Recherche of the FRS-FNRS. We apologize to authors whose work was not cited directly due to word count limitations.

FUNDING

This work was supported by grants from the Fonds de la Recherche Scientifique (FRS-FNRS) and Walloon Excellence in Life Sciences and Biotechnology (WELBIO) to J-F.C.

Conflict of interest. None declared.

REFERENCES

Aliprantis AO, Yang RB, Mark MR *et al.* Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Biochim Biophys Acta* 1999;**285**:650–6.

Asmar AT, Ferreira JL, Cohen EJ *et al.* Communication across the bacterial cell envelope depends on the size of the periplasm. *PLoS Biol* 2017;**15**:e2004303.

Braun V, Bosch V. Repetitive sequences in the Murein-Lipoprotein of the cell wall of *Escherichia coli*. *Proc Natl Acad Sci USA* 1972a;**69**:970–4.

Braun V, Bosch V. Sequence of the murein-lipoprotein and the attachment site of the lipid. *Eur J Biochem* 1972b;**28**:51–69.

Braun V, Rehn K. Chemical characterization, spatial distribution and function of a lipoprotein (murein-lipoprotein) of the *E. coli* cell wall. The specific effect of trypsin on the membrane structure. *Eur J Biochem* 1969;**10**:426–38.

Braun V, Rehn K, Wolff H. Supramolecular structure of the rigid layer of the cell wall of *Salmonella*, *Serratia*, *Proteus*, and *Pseudomonas fluorescens*. Number of lipoprotein molecules in a membrane layer. *Biochemistry* 1970;**9**:5041–9.

Braun V, Rotering H, Ohms JP *et al.* Conformational studies on Murein-Lipoprotein from the outer membrane of *Escherichia coli*. *Eur J Biochem* 1976;**70**:601–10.

Braun V, Wolff H. Attachment of lipoprotein to murein (peptidoglycan) of *Escherichia coli* in the presence and absence of penicillin FL 1060. *J Bacteriol* 1975;**123**:888–97.

Braun V, Wu HC. Chapter 14 Lipoproteins, structure, function, biosynthesis and model for protein export. In: Ghuysen JM, Hakenbeck R (eds). *New Comprehensive Biochemistry*, vol. 27. the Netherlands: Elsevier, 1994, 319–41.

Cascales E, Bernadac A, Gavioli M *et al.* Pal lipoprotein of *Escherichia coli* plays a major role in outer membrane integrity. *J Bacteriol* 2002;**184**:754–9.

Chen Z-Y, Cao J, Xie L *et al.* Construction of leaky strains and extracellular production of exogenous proteins in recombinant *Escherichia coli*. *Microb Biotechnol* 2014;**7**:360–70.

Cho SH, Szewczyk J, Pesavento C *et al.* Detecting envelope stress by monitoring beta-barrel assembly. *Cell* 2014;**159**:1652–64.

Choi DS, Yamada H, Mizuno T *et al.* Trimeric structure and localization of the major lipoprotein in the cell surface of *Escherichia coli*. *J Biol Chem* 1986;**261**:8953–7.

Clark VL, Bavoil PM. *Bacterial Pathogenesis, Part C: Identification, Regulation and Function of Virulence Factors*. USA: Elsevier Science, 2002.

Claus GW, Roth LE. Fine structure of the gram-negative bacterium *Acetobacter suboxydans*. *J Cell Biol* 1964;**20**:217–33.

Cohen EJ, Ferreira JL, Ladinsky MS *et al.* Nanoscale-length control of the flagellar driveshaft requires hitting the tethered outer membrane. *Science* 2017;**356**:197–200.

Cowles CE, Li Y, Semmelhack MF *et al*. The free and bound forms of Lpp occupy distinct subcellular locations in *Escherichia coli*. *Mol Microbiol* 2011;**79**:1168–81.

Depuydt M, Leonard SE, Vertommen D *et al*. A periplasmic reducing system protects single cysteine residues from oxidation. *Science* 2009;**326**:1109–11.

Depuydt M, Messens J, Collet JF. How proteins form disulfide bonds. *Antioxid Redox Signal* 2011;**15**:49–66.

Diao J, Bouwman C, Yan D *et al*. Peptidoglycan association of murein lipoprotein is required for KpsD-Dependent group 2 capsular polysaccharide expression and serum resistance in a uropathogenic *Escherichia coli* isolate. *mBio* 2017;**8**: e00603–17.

Dragan AI, Potekhin SA, Sivolob A *et al*. Kinetics and thermodynamics of the unfolding and refolding of the three-stranded alpha-helical coiled coil, Lpp-56. *Biochemistry* 2004;**43**:14891–900.

Egan AJF. Bacterial outer membrane constriction. *Mol Microbiol* 2018;**107**:676–87.

Erova TE, Kirtley ML, Fitts EC *et al*. Protective immunity elicited by oral immunization of mice with salmonella enterica serovar typhimurium braun lipoprotein (Lpp) and acetyltransferase (MsbB) mutants. *Front Cell Infect Microbiol* 2016;**6**:676–87.

Fadl AA, Galindo CL, Sha J *et al*. Global gene expression of a murein (Braun) lipoprotein mutant of Salmonella enterica serovar Typhimurium by microarray analysis. *Gene* 2006;**374**:121–7.

Finke M, Duchene M, Eckhardt A *et al*. Protection against experimental *Pseudomonas aeruginosa* infection by recombinant *P. aeruginosa* lipoprotein I expressed in *Escherichia coli*. *Infect Immun* 1990;**58**:2241–4.

Gerding MA, Ogata Y, Pecora ND *et al*. The trans-envelope Tol–Pal complex is part of the cell division machinery and required for proper outer-membrane invagination during cell constriction in *E. coli*. *Mol Microbiol* 2007;**63**:1008–25.

Glauner B, Holtje JV, Schwarz U. The composition of the murein of *Escherichia coli*. *J Biol Chem* 1988;**263**:10088–95.

Guo MS, Updegrove TB, Gogol EB *et al*. MicL, a new E-dependent sRNA, combats envelope stress by repressing synthesis of Lpp, the major outer membrane lipoprotein. *Genes Dev* 2014;**28**:1620–34.

Hancock RE, Irvin RT, Costerton JW *et al*. Pseudomonas aeruginosa outer membrane: peptidoglycan-associated proteins. *J Bacteriol* 1981;**145**:628–31.

Hantke K, Braun V. Covalent binding of lipid to protein. *Eur J Biochem* 1973;**34**:284–96.

Hayashi S, Wu HC. Lipoproteins in bacteria. *J Bioenerg Biomembr* 1990;**22**:451–71.

Hiemstra H, Nanninga N, Woldringh CL *et al*. Distribution of newly synthesized lipoprotein over the outer membrane and the peptidoglycan sacculus of an *Escherichia coli* lac-lpp strain. *J Bacteriol* 1987;**169**:5434–44.

Hirashima A, Inouye M. Specific biosynthesis of an envelope protein of *Escherichia coli*. *Nature* 1973;**242**:405–7.

Hirota Y, Suzuki H, Nishimura Y *et al*. On the process of cellular division in *Escherichia coli*: a mutant of *E. coli* lacking a murein-lipoprotein. *Proc Natl Acad Sci* 1977;**74**:1417–20.

Hoekstra D, van der Laan JW, de Leij L *et al*. Release of outer membrane fragments from normally growing *Escherichia coli*. *Biochim Biophys Acta* 1976;**455**:889–99.

Ingraham JL, Maaløe O, Neidhardt FC. *Growth of the Bacterial Cell*. Sinauer Associates, 1983.

Inouye M. A three-dimensional molecular assembly model of a lipoprotein from the *Escherichia coli* outer membrane. *Proc Natl Acad Sci USA* 1974;**71**:2396–400.

Inouye M, Shaw J, Shen C. The assembly of a structural lipoprotein in the envelope of *Escherichia coli*. *J Biol Chem* 1972;**247**:8154–9.

Inouye S, Wang S, Sekizawa J *et al*. Amino acid sequence for the peptide extension on the prolipoprotein of the *Escherichia coli* outer membrane. *Proc Natl Acad Sci USA* 1977;**74**: 1004–8.

Jiménez-Dalmaroni MJ, Gerswhin ME, Adamopoulos IE. The critical role of toll-like receptors - From microbial recognition to autoimmunity: a comprehensive review. *Autoimmun Rev* 2016;**15**:1–8.

Kroger C, Colgan A, Srikumar S *et al*. An infection-relevant transcriptomic compendium for Salmonella enterica Serovar Typhimurium. *Cell Host Microbe* 2013;**14**:683–95.

Lakshmikanth CL, Jacob SP, Kudva AK *et al*. *Escherichia coli* Braun Lipoprotein (BLP) exhibits endotoxemia - like pathology in Swiss albino mice. *Sci Rep* 2016;**6**:34666.

Lee N, Cheng E, Inouye M. Optical properties of an outer membrane lipoprotein from *Escherichia coli*. *Biochim Biophys Acta* 1977;**465**:650–6.

Leverrier P, Declercq JP, Denoncin K *et al*. Crystal structure of the outer membrane protein RcsF, a new substrate for the periplasmic protein-disulfide isomerase DsbC. *J Biol Chem* 2011;**286**:16734–42.

Li GW, Burkhardt D, Gross C *et al*. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* 2014;**157**:624–35.

Lima S, Guo MS, Chaba R *et al*. Dual molecular signals mediate the bacterial response to outer-membrane stress. *Science* 2013;**340**:837–41.

Liu J, Cao W, Lu M. Core side-chain packing and backbone conformation in Lpp-56 coiled-coil mutants. *J Mol Biol* 2002;**318**:877–88.

Liu T, Konig R, Sha J *et al*. Immunological responses against Salmonella enterica serovar Typhimurium Braun lipoprotein and lipid A mutant strains in Swiss-Webster mice: potential use as live-attenuated vaccines. *Microb Pathog* 2008;**44**:224–37.

Magnet S, Bellais S, Dubost L *et al*. Identification of the L,D-transpeptidases responsible for attachment of the Braun lipoprotein to *Escherichia coli* peptidoglycan. *J Bacteriol* 2007;**189**:3927–31.

Nakae T. Outer membrane of Salmonella. Isolation of protein complex that produces transmembrane channels. *J Biol Chem* 1976;**251**:2176–8.

Nakamura K, Inouye M. DNA sequence of the gene for the outer membrane lipoprotein of *E. coli*: an extremely AT-rich promoter. *Cell* 1979;**18**:1109–17.

Nakamura K, Inouye M. DNA sequence of the *Serratia marcescens* lipoprotein gene. *Proc Natl Acad Sci USA* 1980;**77**:1369–73.

Neilsen PO, Zimmerman GA, McIntyre TM. *Escherichia coli* Braun lipoprotein induces a lipopolysaccharide-like endotoxic response from primary human endothelial cells. *J Immunol* 2001;**167**:231–5239.

Ni Y, Reye J, Chen RR. lpp deletion as a permeabilization method. *Biotechnol Bioeng* 2007;**97**:1347–56.

Nichols RJ, Sen S, Choo YJ *et al*. Phenotypic landscape of a bacterial cell. *Cell* 2011;**144**:143–56.

Pautsch A, Schulz GE. Structure of the outer membrane protein A transmembrane domain. *Nat Struct Mol Biol* 1998;**5**: 1013–7.

Rogov VV, Rogova NY, Bernhard F *et al*. A disulfide bridge network within the soluble periplasmic domain determines structure and function of the outer membrane protein RCSF. *J Biol Chem* 2011;**286**:18775–83.

Sanders AN, Pavelka MS. Phenotypic analysis of *Escherichia coli* mutants lacking l,d-transpeptidases. *Microbiology* 2013;**159**:1842–52.

Schwechheimer C, Kulp A, Kuehn MJ. Modulation of bacterial outer membrane vesicle production by envelope structure and content. *BMC Microbiol* 2014;**14**:324.

Schwechheimer C, Rodriguez DL, Kuehn MJ. NlpI-mediated modulation of outer membrane vesicle production through peptidoglycan dynamics in *Escherichia coli*. *Microbiologyopen* 2015;**4**:375–89.

Sha J, Agar SL, Baze WB *et al*. Braun lipoprotein (Lpp) contributes to virulence of yersiniae: potential role of Lpp in inducing bubonic and pneumonic plague. *Infect Immun* 2008;**76**:1390–409.

Sha J, Fadl AA, Klimpel GR *et al*. The two murein lipoproteins of Salmonella enterica serovar Typhimurium contribute to the virulence of the organism. *Infect Immun* 2004;**72**:3987–4003.

Shin HD, Chen RR. Extracellular recombinant protein production from an *Escherichia coli* lpp deletion mutant. *Biotechnol Bioeng* 2008;**101**:1288–96.

Shrivastava R, Jiang XE, Chng SS. Outer membrane lipid homeostasis via retrograde phospholipid transport in *Escherichia coli*. *Mol Microbiol* 2017;**106**:395–408.

Shu W, Liu J, Ji H *et al*. Core structure of the outer membrane lipoprotein from *Escherichia coli* at 1.9 A resolution. *J Mol Biol* 2000;**299**: 1101–12.

Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2010;**2**:a000414.

Suzuki H, Nishimura Y, Yasuda S *et al*. Murein-lipoprotein of *Escherichia coli*: a protein involved in the stabilization of bacterial cell envelope. *Mol Gen Genet* 1978;**167**: 1–9.

Szewczyk J, Collet JF. The journey of lipoproteins through the cell: one birthplace, multiple destinations. *Adv Microb Physiol* 2016;**69**:1–50.

Uhlich GA, Gunther NW, Bayles DO *et al*. The CsgA and Lpp proteins of an *Escherichia coli* O157:H7 strain affect HEp-2 cell invasion, motility, and biofilm formation. *Infect Immun* 2009;**77**:1543–52.

Ushijima T. Fine structure of strictly anaerobic, non-spore-forming, Gram-Negative bacilli. *Jpn J Microbiol* 1967;**11**: 275–87.

Yamagata H, Nakamura K, Inouye M. Comparison of the lipoprotein gene among the Enterobacteriaceae. DNA sequence of *Erwinia amylovora* lipoprotein gene. *J Biol Chem* 1981;**256**: 2194–8.

Yem DW, Wu HC. Physiological characterization of an *Escherichia coli* mutant altered in the structure of murein lipoprotein. *J Bacteriol* 1978;**133**:1419–26.

Zaman M, Toth I. Immunostimulation by synthetic lipopeptide-based vaccine candidates: structure-activity relationships. *Front Immunol* 2013;**4**:318.

Zhang WY, Wu HC. Alterations of the carboxyl-terminal amino acid residues of *Escherichia coli* lipoprotein affect the formation of murein-bound lipoprotein. *J Biol Chem* 1992;**267**:19560–4.