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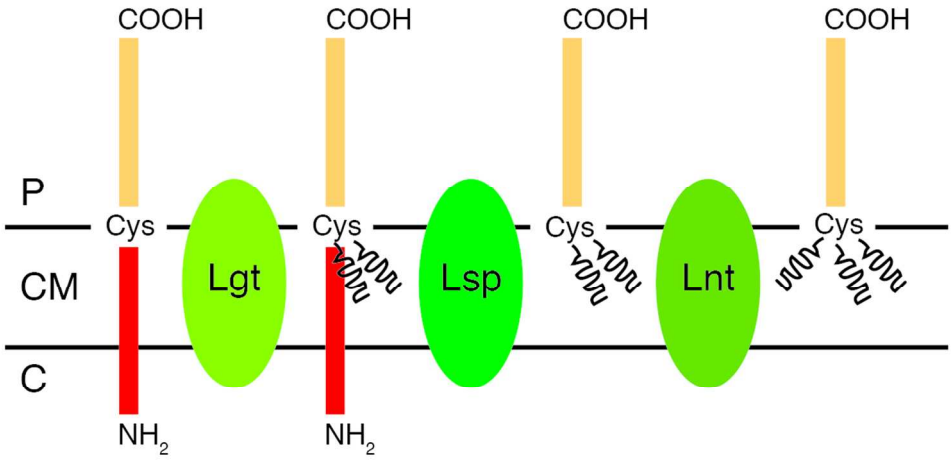
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The molecular mechanism of bacterial lipoprotein modification – how, when and why?

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Abstract

Posttranslational modification of proteins by lipidation is a common process in biological systems. Lipids provide protein stability, interaction with other membrane components, and in some cases, due to reversibility of the process, a mechanism for regulating protein localization and function. Bacterial lipoproteins possess fatty acids at their amino-termini that are derived from phospholipids and this lipid moiety anchors the proteins into the membrane. These lipids, as is the case for lipopolysaccharides and lipoteichoic acids, play an important role in signaling of the innate immune system through the interaction with Toll-like receptors. Over the past three years tremendous progress has been made in understanding the mechanism by which lipoproteins become lipidated. Advanced methodology in mass spectrometry, proteomics and genome-wide analyses allowed precise characterization of lipoprotein modifications and the identification of the enzymes catalyzing the reactions in diverse bacterial species. This review will highlight new findings on bacterial lipoprotein modification with focus on the reaction mechanisms and the role of lipoproteins in cell envelope homeostasis.

Introduction

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Bacterial lipoproteins are exported proteins and often exposed on the bacterial cell surface and include an important class of virulence factors expressed by many pathogenic species (Kovacs-Simon *et al.*, 2010). Prolipoprotein is synthesized in the cytoplasm and translocated via Sec or Tat secretion machineries (Inouye *et al.*, 1977, Hayashi & Wu, 1985, Gibbons *et al.*, 2007, Siboo *et al.*, 2008, Shruthi *et al.*, 2010, Thompson *et al.*, 2010). Lipoprotein modification occurs in the cytoplasmic (inner) membrane by the sequential action of three membrane-bound enzymes (Fig. 1) (Wu *et al.*, 1982). Lipoproteins possess a conserved sequence, so-called lipobox, which allows specific recognition by the modification machinery. The invariant cysteine residue in the lipobox becomes lipidated and the first amino acid after complete maturation of lipoprotein. Phosphatidylglycerol:prolipoprotein diacylglyceryl transferase (Lgt) adds an *sn*-1,2-diacylglyceryl group from phosphatidylglycerol (PG) to the sulfhydryl group of cysteine via a thioether bond. Prolipoprotein signal peptidase (Lsp) cleaves the signal peptide thereby liberating the α-amino group of diacylglyceryl-cysteine. The third and last step is *N*-acylation of diacylglyceride-cysteine by Apolipoprotein *N*-acyltransferase (Lnt), resulting in mature *N*-acyl-*S*-diacylglyceryl-cysteine linked proteins. Lgt and Lsp are conserved in all bacterial species, while Lnt has only been identified in proteobacteria and actinomycetes. Recent findings suggest that a non-canonical lipidation pathway exist by the demonstration that lipoproteins of firmicutes and terenicutes (that include mollicutes), whose genome lack a gene encoding Lnt, exist in a triacylated form (Fig. 1) (Jan *et al.*, 1995, Kurokawa, 2009, Asanuma *et al.*, 2010, Serebryakova *et al.*, 2011). Various N-terminal modifications were observed: *N*-acyl-*S*-diacylglyceryl-cysteine, but also *N*-acyl-*S*-monoacyl-cysteine, *N*-acetyl-*S*-diacylglyceryl-cysteine and *N*-peptidyl-*S*-diacylglyceryl-cysteine (Nakayama *et al.*, 2012). Correct lipoprotein modification and consequently, the enzymes that carry them out, are involved in a process essential for bacterial viability in proteobacteria and some

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actinomycetes (Table 1). Understanding the molecular details of their catalytic mechanism and function should provide detailed knowledge on bacterial envelope homeostasis and facilitate the discovery of new antibacterial agents targeting this essential pathway. Most enzymatic and functional studies have been performed with the lipoprotein modification enzymes of *Escherichia coli* that are discussed in this review. It is stated in the text when enzymes from other bacterial species are described.

Phospholipids are substrates for acyltransferases involved in lipoprotein modification

The lipid moiety of lipoproteins is derived from membrane phospholipids, linking the lipoprotein modification pathway to phospholipid biogenesis (Lai *et al.*, 1980). Two different systems exist in bacteria to transfer fatty acids from acyl-acyl carrier proteins (acyl-ACP), the end products of the fatty acid synthase II pathway (FASII), to glycerol-3-phosphate. The PlsX/Y/C is a widespread pathway while the PlsB/C system is found predominantly in γ-proteobacteria, but both systems result in the synthesis of phosphatidic acid, the building block of phospholipids. Phosphatidic acid is subsequently converted into the key intermediate CDP-diacylglycerol. Two pathways then lead to the synthesis of phophatidylethanolamine (PE) (via phosphatidylserine) and phosphatidylglycerol (PG) and cariolipin (CL), respectively (for a recent review see (Parsons & Rock, 2013)). Movement of lipids, including PE and lipopolysaccharides (LPS), across the cytoplasmic membrane is dependent on MsbA (Zhou *et al.*, 1998, Doerrler & Raetz, 2002). The ABC transporter MsbA functions as a dimer in which each monomer is composed of six transmembrane segments (TMS) and a nucleotide-binding domain (Ward *et al.*, 2007). The dimer hydrolyses ATP and then undergoes large conformational changes, which is thought to lead to the trans-bilayer movement of lipids across the membrane (Doerrler *et al.*, 2001, Doerrler *et al.*, 2004, Doshi & van Veen, 2013). The main phospholipids of *E. coli* and *Salmonella enterica* serovar *Thyphimurium* are

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phosphatidylethanolamine (PE), phosphatidylglycerol (PG), cardiolipin (CL) and minor phospholipids phosphatidylserine (PS) and phosphatidic acid (PA) (Ames, 1968). The fatty acids of phospholipids of a typical *E. coli* strain contain about 45% palmitic acid (C16:0), 2% myristic acid (C14:0), 35% palmitoleic acid (C16:1) and 18% *cis*-vaccenic acid (C18:1*cis*-11) (Cronan & Rock, 1996). The main fatty acids of major lipoprotein Lpp (murein-lipoprotein or Braun's lipoprotein) of *E. coli* are palmitic acid (53%), *cis*-vaccenic acid (20.7%), 9,10-methylene-hexadecanoic acid (C17:0) (10.6%) and palmitoleic acid (9.4%) (Hantke & Braun, 1973), corresponding to the fatty acid moieties of phospholipids. Phospholipids that compose membranes of *Staphylococcus aureus* include phosphatidylglycerol, cardiolipin, lysyl-phosphatidylglycerol, and mono- and diglucosyl-diglyceride but not phosphatidylethanolamine (White & Frerman, 1968, Fischer, 1994). Mass spectrometry data has shown that lipoproteins in *S. aureus* are modified with C17:0 to C20:0 at the *sn*-1 position of the diacylglyceryl group and with C15:0 at the *sn*-2 position. The α-amino group of lipidated diacylglyceryl-cysteine have fatty acids of varying lengths, from C15:0 to C20:0 (Kurokawa *et al.*, 2012). The lipid moiety of lipoproteins thus reflects the fatty acid composition of phospholipids and varies among bacterial species.

Phosphatidylglycerol:prolipoprotein diacylglyceryl transferase (Lgt)

Modification of prolipoprotein by Lgt is the first step in lipoprotein modification (Tokunaga *et al.*, 1982). Upon membrane insertion of prolipoprotein by Sec or Tat, Lgt transfers the diacylglyceryl group from phosphatidylglycerol onto the sulfhydryl group of the invariant cysteine in the lipobox resulting in a thioether-linked diacylglyceryl-prolipoprotein and a *sn*-glycerol-1-phosphate by-product (Lai *et al.*, 1980, Sankaran & Wu, 1994). Signal sequences of lipoproteins share common features including one or several positively charged residues at the amino terminus followed by a hydrophobic stretch of about 20 amino acids that includes

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the lipobox. Based on studies with Lpp, the consensus sequence of the lipobox was determined as: $(L)_{-3}$-$(A/S)_{-2}$-$(G/A)_{-1}$-C_{+1}, in which the lipid-modified cysteine at position +1 becomes the first amino acid in the mature protein (Wu *et al.*, 1983, Wu, 1996, Madan Babu & Sankaran, 2002, Babu *et al.*, 2006). This consensus sequence has been documented in the PROSITE database under pattern entry PS00013/PDOC00013 (http://www.expasy.ch/prosite/). Lipobox motifs are similar in firmicutes but more variable in spirochetes (Haake, 2000, Sutcliffe & Harrington, 2002), the -3 position in particular is more loosely defined in the latter, where a leucine residue is often replaced by another hydrophobic amino acid. A high percentage of ORFs in spirochetes encode lipoproteins, some of which are highly abundant proteins (Haake, 2000). For Lpp it has also been demonstrated that residue G14 in the signal peptide sequence is important for lipidation by Lgt, and that a signal sequence shortened by several residues still leads to modified protein (Wu *et al.*, 1977, Yem & Wu, 1977, Lin *et al.*, 1978, Lin *et al.*, 1980, Inouye *et al.*, 1984, Pollitt *et al.*, 1985). The first biochemical characterization of Lgt was reported in the 1980-s using crude membrane extracts. The optimal pH and reaction temperature for Lgt are pH 7.8 and 37°C, respectively (Tokunaga *et al.*, 1984). Lgt is stable in membrane vesicles up to 65°C, the enzyme is active in N-octyl-β-D-glucoside but many other detergents are not compatible with enzyme activity (Tokunaga *et al.*, 1984, Sankaran & Wu, 1994). PG is specific as acyl donor since PE and CL could not be used as substrate in the *in vitro* assay using a synthetic peptide, suggesting that the polar headgroup of the phospholipid plays an important role in the Lgt reaction (Sankaran & Wu, 1994). The gene encoding Lgt was identified by screening a collection of temperature sensitive mutants of *S. enterica* for the accumulation of unmodified Lpp and was shown to be located at the 5' end of *thyA* (Williams *et al.*, 1989, Bell-Pedersen *et al.*, 1991, Gan *et al.*, 1993). The enzyme is localized in the cytoplasmic membrane and spans the membrane by seven transmembrane segments (Fig. 2.) (Pailler *et al.*, 2012). The majority of essential

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residues are located in the membrane, however, it is currently unknown how Lgt interacts with its substrates PG and prolipoprotein (Qi *et al.*, 1995, Sankaran *et al.*, 1997, Pailler *et al.*, 2012). Residues H103 and Y235 were identified as conserved by comparing four Lgt sequences and were shown to be essential for Lgt activity (Sankaran *et al.*, 1997). These residues are among twelve residues highly conserved in proteobacteria, firmicutes and actinobacteria (Pailler *et al.*, 2012). A more recent analysis on conserved residues highlighted the existence of a so-called Lgt signature motif; two amino acids in this sequence (N146 and G154) were shown to be absolutely required for function (Pailler *et al.*, 2012). Together, these data show that regions H103-G108 and G142-G154 and residues Y26 and Y235 are critical for Lgt function. Initial kinetics studies were performed with crude membrane extracts but further biochemical characterization of the *E. coli* enzyme turned out difficult due to overproduction defects and instability of the purified enzyme (Sankaran *et al.*, 1997, Selvan & Sankaran, 2008, Pailler *et al.*, 2012). Lgt has been purified recently from *Lactococcus lactis* with which kinetic studies were performed (Banerjee & Sankaran, 2012), unfortunately, assay conditions were not comparable with those described previously (Sankaran *et al.*, 1997, Sundaram *et al.*, 2012), making it difficult to compare enzyme activities from different bacterial species.

Lgt of *S. aureus* can complement a temperature sensitive mutant of *E. coli*, suggesting that Lgt is functionally conserved among bacterial species (Qi *et al.*, 1995). Deletion mutants of *lgt* in firmicutes often lead to shedding of lipoproteins into the culture supernatant in non-lipidated precursor form with uncleaved signal peptide (Leskelä *et al.*, 1999) or processed by an unidentified signal peptidase (Stoll *et al.*, 2005, Denham *et al.*, 2009) or Lsp (Baumgärtner *et al.*, 2007, Henneke *et al.*, 2008). Two Lgt homologues were identified in *Streptomyces coelicolor*, Lgt1 and Lgt2, which can both complement the *lgt* mutant of *Streptomyces scabies*, suggesting that both enzymes are functional enzymes (Widdick *et al.*, 2011). A

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double mutant could not be obtained suggesting that Lgt activity is essential in *S. coelicolor* (Thompson *et al.*, 2010). Lipoproteins were purified from the complementing strains and the composition of their N-terminal fatty acid was analyzed by mass spectrometry (Widdick *et al.*, 2011). Several fatty acid acylated peptides were missing in these strains compared to wild type *S. scabies*, suggesting that subtle differences in diacylglyceryl transferase activity might exist. The gene encoding Lgt in *Mycobacterium tuberculosis* and two genes in *Mycobacterium smegmatis* have recently been identified (Tschumi *et al.*, 2012). The MSMEG_3222 gene was shown to be responsible for the transfer of diacylglyceryl onto lipoproteins, and although less conserved than MSMEG_3222 the role of the second *lgt* gene (MSMEG_5408) in lipoprotein modification has not yet been addressed (Tschumi *et al.*, 2012). An *lgt* deletion mutant could not be obtained in *M. tuberculosis* suggesting that this gene is essential (Sassetti *et al.*, 2003, Tschumi *et al.*, 2012). Strikingly, MSMEG_3222 is not essential in *M. smegmatis* and deletion did not completely abolish incorporation of radioactive palmitate suggesting that either MSMEG_5408 or another, as yet unknown, enzyme possesses Lgt activity but is perhaps less efficient than MSMEG_3222 (Tschumi *et al.*, 2012). Absence of Lgt resulted in massive release of lipoproteins in the culture media but none of these proteins contained a signal peptide, suggesting that they are processed by Lsp or other signal peptidases (Tschumi *et al.*, 2012). No clear phenotype on cell wall biogenesis was observed.

Detailed biochemical analyses and comparison of kinetic parameters of various Lgt enzymes will contribute to a better understanding of the mechanism of the reaction and are predicted to highlight differences and similarities in enzyme activity. The thiol group of prolipoprotein probably performs a nucleophilic attack on the carbonyl group of PG where Lgt provides the catalytic residues to support transfer of diacylglyceryl onto prolipoprotein, and positions the two substrates close together. PG is the sole acyl donor in the Lgt reaction, suggesting that the glycerol-phosphate headgroup plays an important role in substrate recognition by Lgt.

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Lipoprotein signal peptidase (Lsp)

Lsp (SPase II) is localized in the cytoplasmic membrane (Tokunaga *et al.*, 1984) and spans this membrane by four transmembrane segments with both the amino and carboxyl termini facing the cytoplasm (Munoa *et al.*, 1991) (Fig. 2). Five conserved regions were identified, i.e. region I (amino acids residues 23-27 in *E. coli* sequence), region II (residues 53-58), region III (residues 104-115) and region IV (residues 121-123) and region V (residues 137-141) (Munoa *et al.*, 1991, Tjalsma *et al.*, 1999). The role of these conserved regions in the peptidase reaction has not yet been addressed but probably plays a role in substrate recognition and/or binding. The active site of Lsp is composed of at least two aspartate residues (D114 and D141 in *E. coli*) (Tjalsma *et al.*, 1999). Modification of lipoproteins by addition of diacylglyceryl catalyzed by Lgt is a prerequisite for processing by Lsp in proteobacteria (Tokunaga *et al.*, 1982). The Lsp enzymes of actinomycetes have an N-terminal extension, truncations of which led to loss of Lsp function, either due to inactivation or instability of the enzyme (Thompson *et al.*, 2010). Biochemical studies with Lsp were performed in the early 1980-s and showed that Lsp is temperature resistant in isolated membranes, but unlike Lnt still active at 80°C (Hussain *et al.*, 1982). In detergent-solubilized membranes Lsp is active at a reaction temperature between 37°C-45°C with a pH optimum of 7.9 (Tokunaga *et al.*, 1984). The enzyme does not require phospholipids and is active in non-ionic detergents.

Initially two enzymatic activities were shown to be involved in the proteolytic cleavage of lipoproteins, Lsp that cleaves off the signal peptide of diacylglyceryl-proLpp and a signal peptide peptidase responsible for the degradation of signal peptide (SppA or Protease IV) (Suzuki *et al.*, 1978, Novak & Dev, 1988). SppA is an intramembrane protease (Pacaud, 1982, Pacaud, 1982) utilizing a Ser/Lys dyad for catalysis (Wang *et al.*, 2008). It adopts a

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tetrameric structure with a hydrophobic interior providing access to the catalytic site (Ichihara *et al.*, 1986, Kim *et al.*, 2008) and cleaves its substrate in the hydrophobic region (Novak & Dev, 1988). Recently, Saito *et al.* (Saito *et al.*, 2011) provided evidence that peptidase RseP (YaeL in *E. coli*), which belongs to the zinc-metalloprotease group (S2P) of the intramembrane-cleaving proteases (I-CLiPs) family of proteases (Urban, 2009), is responsible for cleavage of signal peptides including those obtained after cleavage by Lsp. RseP is not the exclusive signal peptide peptidase since some signal peptides were cleaved in the absence of RseP and SppA. The sole natural substrate of RseP is the anti-σ^E factor RseA that upon cell envelope stress is cleaved by DegS and further processed by RseP resulting in activation of σ^E and the expression of stress-induced genes (Alba *et al.*, 2002). RseP has four transmembrane segments with its N- and C-termini facing the periplasm (Kanehara *et al.*, 2001). The periplasmic PDZ domain is specific for regulated processing of RseA after truncation by DegS. Removal of the PDZ domain led to intramembrane cleavage of RseA (Hizukuri *et al.*, 2014), suggesting that this domain is not required for processing of lipoprotein signal peptides.

Lsp proteins from firmicutes can complement an *lsp* temperature sensitive mutant of *E. coli*, suggesting that the enzymes are functionally conserved as seen for Lgt (Pragai *et al.*, 1997), however kinetic analyses have not yet been performed to determine specificity and mechanism of the reaction. Lsp does not process prolipoprotein without S-diacylglyceryl modification in proteobacteria, indicating that the enzyme specifically recognizes the fatty acid acylated cysteine. Processing of Lpp occurs both in *E. coli* and *Bacillus subtilis* but at different rates (Hayashi *et al.*, 1985). Apo-Lpp accumulates in *B. subtilis* probably due to inefficient N-acylation or N-acetylation of apoLpp (Kurokawa *et al.*, 2012). Furthermore, the two organisms differ in their susceptibility to globomycin as shown by the processing of penicillinase (Hayashi & Wu, 1983). *Myxococcus xanthus* possesses four *lsp* genes, two of

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which are part of the antibiotic TA biosynthetic cluster (Xiao & Wall, 2014). The Lsp enzymes can complement an *lsp* depletion strain of *E. coli* only in the absence of Lpp suggesting that Lsp-s of *M. xanthus* are less efficient than Lsp of *E. coli*. Lipoproteins of *Streptococcus uberis* are processed by Eep, a homologue of enhanced expression of pheromone from *Enterococcus faecalis* (An *et al.*, 1999), in the absence of Lsp in late-stationary-phase cells (Denham *et al.*, 2009).

Structural information is not available for any of the lipoprotein modification enzymes, including Lsp. Other bacterial signal peptidases, such as signal peptidase I (leader peptidase) and type IV pilin signal peptidase (prepilin peptidase), share their membrane-embedded characteristics and the hydrophobic nature of their substrates. The catalytic mechanism (Paetzel, 2014) and the Ser/Lys active site (Wang *et al.*, 2008) of leader peptidase have been reported. A soluble version of the enzyme, lacking its two N-terminal transmembrane segments, is active *in vitro* (Kuo *et al.*, 1993), although slightly less compared to full-length protein and it requires detergents or phospholipids for its activity (Tschantz *et al.*, 1995). Several crystal structures of the soluble form have been solved (Kim *et al.*, 2008) and a recent NMR structure suggests that the signal peptide remains in the bilayer in an unstructured conformation and that the active site of leader peptidase functions at the membrane surface (De Bona *et al.*, 2012) or well within the lipid bilayer (Paetzel, 2014). The membrane is probably important in stabilizing the signal peptide while under reconstituted conditions the detergents or phospholipids would serve an analogous role. Several inhibitors have been identified including penem-type inhibitors (Allsop *et al.*, 1996) and lipopeptides (Paetzel *et al.*, 2002). Differences in specificity between leader peptidases from different species may be explained by structural variations in the active site (Auclair *et al.*, 2012). Prepilin peptidase is, like Lsp, an aspartic protease that belongs to the class of aspartyl protease I-CLiPs (Erez *et al.*, 2009) and is involved in processing of pre-pilin proteins (LaPointe & Taylor, 2000). I-

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CLiPs are involved in intramembrane proteolysis liberating membrane-tethered domains of membrane proteins that can function as regulatory molecules, and are found in many different organisms (Erez *et al.*, 2009). Prepilin peptidase is an integral membrane protein and processing of prepilin has been postulated to occur at the cytoplasmic side of the membrane, allowing the subsequent addition of methyl groups onto phenylalanine (Strom & Lory, 1987). A topology model was proposed based on data obtained with reporter fusion proteins and topology prediction programs; prepilin peptidases have eight transmembrane segments with their N- and C-termini are located in the periplasm, and the catalytic Asp dyad is facing the cytoplasm (LaPointe & Taylor, 2000). The crystal structure of aspartyl protease FlaK, has recently been solved (Hu *et al.*, 2011). The two active site Asp residues are located on top of two α-helices, housed in a hydrophilic cavity in the membrane and facing the cytoplasm, similar to the structure of presenilin (Hu *et al.*, 2011, Lu *et al.*, 2014). Lsp probably shares structural features with both leader peptidase and aspartyl proteases. It interacts with diacylglyceryl-prolipoprotein as leader peptidase with signal peptides of secreted proteins in such a way that the diacylglyceryl-cysteine is accessible to the Asp dyad active site as in aspartyl proteases, with the only difference that the active site is facing the periplasm.

Globomycin – a specific inhibitor of Lsp

Globomycin was first identified in 1978, as an antibiotic produced by various Streptomyces strains with spheroplast-forming (global-shape) activity on *E. coli* (Inukai *et al.*, 1978, Inukai *et al.*, 1978). It specifically inhibits proteobacteria such as *E. coli* (MIC 0.4 μg/ml for *E. coli* B) but not *Pseudomonas aeruginosa*. It is also less active against firmicutes (for example, MIC >100 μg/ml for *S. aureus* and *B. subtilis*) (Inukai *et al.*, 1978). Globomycin is a lipophilic cyclic peptide with molecular formula $C_{32}H_{57}N_5O_2$ and a molecular weight of 655 (Nakajima *et al.*, 1978) (Fig. 3). Indications that the target of globomycin is Lsp were

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reported in the last paper in a series of articles on globomycin discovery and its characterization by Mamoru Arai's group (Inukai *et al.*, 1978). It was later shown that diacylglyceryl-proLpp that accumulated in the cytoplasmic membrane in cells grown in the presence of globomycin (Hussain *et al.*, 1980) could be converted into mature protein *in vitro* when cell envelope fractions were incubated under optimal conditions. The gene encoding Lsp was identified in *E. coli* by selecting clones from the Carbon-Clarke collection for globomycin resistance, or by screening for complementation of a strain that is temperature sensitive in the processing of diacylglyceryl-proLpp (Tokunaga *et al.*, 1983, Yamagata *et al.*, 1983). Kinetics studies showed that globomycin inhibits Lsp in a non-competitive manner with a K_i of 36 nM, and a K_m value of 6 μM was determined for diacylglyceryl-prolipoprotein substrate (Dev *et al.*, 1985). Globomycin is mycobactericidal at elevated concentrations but killing is independent of Lsp in *M. tuberculosis*, suggesting an alternative pathway of inhibition in this bacterium (Banaiee *et al.*, 2007). Globomycin analogues have been described that are more potent against proteobacteria and exhibit antibacterial activity against firmicutes (Kiho *et al.*, 2003, Kiho *et al.*, 2004). A long alkyl side chain, the lipophilic part of the molecule, increases activity and also shows moderate activity against *S. aureus*. The hydroxyl group of L-Ser is essential but not that of L-allo-Thr (Kiho *et al.*, 2004). The lipid moiety of globomycin is probably required to anchor the antibiotic into the membrane to allow correct positioning of the peptidic part for interaction with the enzyme, but also seems to play a direct role in globomycin-enzyme interaction. Differences in membrane composition and/or membrane topology of Lsp might explain differences in antibacterial activities of globomycin and its analogues on Lsp of different bacterial species. Another antibiotic, TA or myxovirescin isolated from *Myxococcus xantus*, has been shown to target Lsp (Xiao *et al.*, 2012). Antibiotic TA is a cyclic macrolactam lactone that is active against proteobacteria and some firmicutes (Rosenberg *et al.*, 1973).

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Apolipoprotein N-acyltransferase (Lnt)

The membrane localization of Lnt of *E. coli* was demonstrated by Gupta and Wu in 1991 (Gupta & Wu, 1991), in the same study it was demonstrated that Lnt has a broad pH optimum (pH 6.5-7.5) and that it requires the presence of detergents for its activity *in vitro*. In 2005 the membrane topology of Lnt and the phenotype of an *lnt* depletion mutant was reported (Robichon *et al.*, 2005). Several essential residues in Lnt were identified that are mainly located in the catalytic periplasmic domain and include the catalytic triad E-K-C (Vidal-Ingigliardi *et al.*, 2007). It was shown that growth of the *lnt* depletion mutant could be restored by Lnt homologues of proteobacteria, but not actinobacteria (Vidal-Ingigliardi *et al.*, 2007). The reaction catalyzed by Lnt involves two steps. In the first step, the *sn*-1 of PE reacts with the active site cysteine of Lnt resulting in the formation of a thioester-acyl enzyme intermediate and a lysophospholipid by-product (Jackowski & Rock, 1986, Buddelmeijer & Young, 2010, Hillmann *et al.*, 2011). In the second step, the acyl group is transferred to apolipoprotein resulting in triacylated, mature lipoprotein (Buddelmeijer & Young, 2010). Kinetics studies showed that Lnt follows a ping-pong mechanism whereby lyso-phospholipid produced in the first reaction is released before the second substrate, apolipoprotein, is bound and modified. With PE and a synthetic diacylated peptide (FSL-1) a K_m value of 8.0 μM and a V_{max} value of 350 $\mu mol_{FSL\text{-}1}\ min^{-1}\ \mu mol^{-1}_{Lnt}$ were determined, corresponding to a specific activity of 5.6 $\mu mol_{FSL\text{-}1}\ min^{-1}\ mg^{-1}_{Lnt}$. Interestingly, similar kinetics were obtained with PagP that catalyzes the transfer of an acyl group from PE to lipid A (Bishop *et al.*, 2000). Although acyltransferases Lnt and PagP are involved in completely different pathways, their substrates are highly abundant; 5 x 10^5 molecules of Lpp and 2 x 10^6 molecules of lipid A per cell (Braun & Rehn, 1969, Raetz, 1986). Furthermore, similar K_m values have been reported for

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Lsp and Lnt of *E. coli* for diacylglyceryl-proLpp and apo-lipopeptide, respectively (Dev *et al.*, 1985, Hillmann *et al.*, 2011).

Lnt is highly efficient in the second step of the reaction but less in the first step: k_{cat}/K_m of 4.8 x 10^3 for PE versus 7.3 x 10^5 for FSL-1. Based on the kinetic data, the number of Lnt molecules per cell was estimated between 100 and 200, indicating that the *N*-acyltransferase reaction is fast and efficient since lipoproteins are very abundant. PE is the preferred substrate with an optimal fatty acid chain length of C16-18, more specifically palmitate or oleate with a saturated acyl chain in position *sn*-1 and a non-saturated acyl chain at *sn*-2 (Jackowski & Rock, 1986, Hillmann *et al.*, 2011). Phospholipids with small polar headgroups can also serve as acyl donor (Gupta *et al.*, 1991, Hillmann *et al.*, 2011). PagP and LpxA, which is involved in the first step of lipopolysaccharide biosynthesis, possess a carbon-ruler that makes these enzymes highly specific for the chain length of the acyl donor substrate (Wyckoff *et al.*, 1998, Ahn *et al.*, 2004, Williams & Raetz, 2007). It is unlikely that Lnt has carbon ruler capacity since the acyl chain length and composition of the phospholipid substrate are variable. On the other hand, the *S*-diacylglyceryl moiety of the apolipoprotein substrate probably determines substrate specificity due to variations in lipid composition depending on the bacterial species.

The gene encoding Lnt was first identified in *S. enterica* by screening the same library as described for *lgt* for the accumulation of apol-Lpp (Gupta *et al.*, 1993). The mutation causing the temperature sensitive defect in Lnt was a substitution of glutamate 435 to lysine; a similar mutation in *E. coli* did not result in a temperature sensitive phenotype (Robichon *et al.*, 2005). Lnt activity in Actinomycetes was first reported in *M. smegmatis* (Tschumi *et al.*, 2009). A strain of *M. smegmatis* deleted for *lnt* was viable and resulted in the accumulation of the apolipoprotein form of two model lipoproteins LppX and LprF, directly demonstrating its *N*-acyltransferase activity (Tschumi *et al.*, 2009, Brülle *et al.*, 2010). Lnt of mycobacteria transfers either palmitate or tuberculostearic acid (in *M. bovis*) from phospholipids onto

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apolipoprotein (Brülle *et al.*, 2013). Phospholipids of mycobacteria possess mainly octadecanoic acid and tuberculostearic acid at the *sn*-1 position and palmitate at the *sn*-2 position (Okuyama *et al.*, 1967), suggesting that Lnt has different substrate specificity in these species. Lnt of *M. smegmatis* was unable to complement a conditional *lnt* null mutant of *E. coli*, suggesting that indeed these enzymes have different substrate specificity. Furthermore, the enzyme was correctly inserted into the cytoplasmic membrane and shown to have a similar membrane topology as Lnt of *E. coli* (Baulard *et al.*, 2003). Among the essential residues in Lnt of *E. coli*, W237 and Y388 are not conserved in Lnt of *M. smegmatis* (Vidal-Ingigliardi *et al.*, 2007, Tschumi *et al.*, 2009). These residues were shown to be involved in the second step of the reaction, *N*-acylation of apolipoprotein, suggesting they might play a role in substrate specificity (Buddelmeijer & Young, 2010). The *lnt* gene in *M. smegmatis* (MSMEG_3860 or ppm2) is located adjacent to *ppm1* encoding polyprenol monophosphomannose (Ppm) synthase that is involved in the transfer of mannose from GDP-mannose to polyprenol phosphate, an important step in the biosynthesis of mannose-containing lipoglycans (Gurcha *et al.*, 2002). Ppm synthase is essential for viability in *M. smegmatis* (Rana *et al.*, 2012).

In *M. tuberculosis ppm1* (Rv2051c) encodes a two-domain protein, in which the N-terminal domain is Lnt and the C-terminal domain Ppm synthase. The Lnt domain of Ppm1 enhances Ppm synthase activity (Gurcha *et al.*, 2002). In mycobacteria where Lnt and Ppm synthase are encoded by two genes, the proteins were shown to interact (Baulard *et al.*, 2003). In both cases Lnt is thought to stabilize the Ppm synthase (Gurcha *et al.*, 2002, Baulard *et al.*, 2003). A second *lnt*-like gene could be identified in *M. tuberculosis* (Rv2261c and Rv2262c) and *M. bovis* (BCG_2279c), but the active site cysteine residue is replaced by serine (Brülle *et al.*, 2013). The role of these enzymes in lipoprotein modification is currently unknown.

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Lipoproteins in Mycobacteria and Streptomyces are often, in addition to being acylated, glycosylated (Espitia & Mancilla, 1989, Herrmann *et al.*, 1996, Gonzalez-Zamorano *et al.*, 2009, Sartain & Belisle, 2009, Wehmeier *et al.*, 2009, Brülle *et al.*, 2010). Recent findings demonstrated a direct correlation between *N*-acylation and glycosylation of lipoproteins in *Corynebacterium glutamicum* (Mohiman *et al.*, 2012). Lnt (Cg-Ppm2) of *C. glutamicum* catalyzes *N*-acylation of apolipoproteins by addition of a palmitoyl moiety similar to *M. smegmatis* (Tschumi *et al.*, 2009, Mohiman *et al.*, 2012). Lipid modifications in *C. glutamicum* included a diacylglyceryl moiety (C16:0, C18:1) and a *N*-palmitoyl group (C16:0) similar to lipoproteins of *E. coli* (Mohiman *et al.*, 2012). *N*-acylation of lipoproteins was not affected in a Cg-*ppm1* mutant but proteins were no longer *O*-glycosylated with hexose units, indicating that Ppm synthase is required for glycosylation of lipoproteins in *C. glutamicum*. In a Cg-*ppm2* mutant lipoproteins are only diacylated and not glycosylated (Mohiman *et al.*, 2012). A Cg-*ppm1* deletion mutant does not synthesize lipoglycans, furthermore, their biosynthesis is also affected in the absence of Cg-*ppm2* (Lnt), probably because Ppm synthase is less stable under these conditions (Gibson *et al.*, 2003). Interestingly, Ppm1 of *M. tuberculosis* is able to complement both *N*-acylation and glycosylation defects in *C. glutamicum* (Mohiman *et al.*, 2012) and to restore *N*-acylation in *M. smegmatis* (Tschumi *et al.*, 2009). The Lnt domain of Ppm1 alone does not restore *N*-acylation but does restore glycosylation in *C. glutamicum*. This strengthens the interpretation that the activities of Lnt and Ppm synthase are tightly linked and illustrate that these enzymes operate in a common pathway implicated in envelope biosynthesis.

All Streptomyces species have two *lnt* genes (Widdick *et al.*, 2011). In *S. scabies*, Lnt1 is responsible for *N*-acylation of lipoproteins since lipoproteins are diacylated in *lnt1* and *lnt1lnt2* mutants. In the absence of Lnt2, however, both di- and triacylated proteins were

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detected, suggesting a role for Lnt2 in efficient *N*-acylation of lipoproteins (Widdick *et al.*, 2011). Since these bacteria undergo different developmental stages, gene expression and activity of lipoprotein modifying enzymes might be tightly regulated depending on environmental conditions. In this respect it is interesting to note that the degree of fatty acid acylation varies greatly in firmicutes depending on growth conditions. For example, diacylated lipoproteins accumulated in *S. aureus* during late-exponential growth at low pH (Kurokawa *et al.*, 2012). New protein synthesis is required for both accumulation of diacylated proteins and N-acylation of these proteins during pH upshift. These data suggest that regulation of *lnt* expression or pH-mediated enzyme activity control *N*-acylation of lipoproteins. Assuming that phospholipids are also substrate for *N*-acylation in firmicutes, Lnt-like enzymes of these species probably have different specificities for the acyl-donor substrate than those of proteobacteria.

After completion of the Lnt reaction, the lysophosphatidylethanolamine, or 2-acyl-glycerophosphoethanolamine (2-acyl-GPE) by-product can either be degraded by lysophospholipase PldB (Hsu *et al.*, 1991) or recycled in the synthesis of phospholipid via the acyl-acyl carrier protein synthetase/2-acylglycerophosphoethanolamine acyltransferase pathway (Cooper *et al.*, 1989, Jackowski *et al.*, 1994). 2-acyl-GPE first needs to be translocated to the inner leaflet of the cytoplasmic membrane, a process catalyzed by LplT, before it can be used as a substrate for PL synthesis (Harvat *et al.*, 2005). The Aas enzyme is a cytoplasmic membrane protein (Cooper *et al.*, 1989) that contains both enzymatic activities with acyltransferase located at the N-terminal end and acyl ACP synthetase located at the C-terminal end (Jackowski *et al.*, 1994). In the first step of the reaction acyl-ACP synthetase adds saturated fatty acids onto ACP and in the second step fatty acid is transferred to the *sn*-1 position of 2-acyl-GPE by the acyltransferase resulting in the formation of new PE.

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Alternatives for removal of 2-acyl-GPE are the production of PE and GPE from two molecules of GPE (Homma & Nojima, 1982), and another phospholipase that degrades 2-acyl-GPE (Doi & Nojima, 1975).

Sorting of lipoproteins to the outer membrane

The majority of lipoproteins reside in the inner leaflet of the outer membrane (Tokuda *et al.*, 2007), although recent examples of surface-exposed lipoproteins have been reported (see below). They reach this cellular location by a specific *l*ipoprotein *o*uter membrane *l*ocalization pathway (Lol) that is composed of five proteins: a cytoplasmic ATP-ase LolD, two cytoplasmic membrane proteins LolC and LolE, a periplasmic chaperone LolA and an outer membrane receptor LolB (Fig. 4). Sorting and translocation of lipoproteins by Lol is dependent on the lipoprotein-sorting signal determined by specific residues located C-terminally to the diacylglyceryl-cysteine. The Lol pathway has been described in great detail in several excellent reviews (Tokuda *et al.*, 2014, Zuckert, 2014) and will only be briefly discussed in light of functionality with enzymes involved in lipoprotein modification. *N*-acylation of lipoproteins by Lnt is essential for the Lol-dependent release of lipoproteins from the cytoplasmic membrane (Fukuda *et al.*, 2002), however, overexpression of *lolCDE* can compensate for lack of *N*-acylation, in the absence of Lpp, and results in the release of diacylated lipoproteins from the membrane and correct outer membrane localization (Narita & Tokuda, 2011). However, the efficiency of outer membrane targeting is dependent on the nature of the lipoprotein (Narita & Tokuda, 2011). Model lipoproteins containing an outer membrane-sorting signal are not localized in the outer membrane, neither released from the cytoplasmic membrane in absence of Lnt. This suggests that the *N*-acyl moiety of lipoproteins plays an important role in lipoprotein translocation through the periplasm. Mature lipoprotein first interacts with LolE, is then transferred to LolC, and upon ATP hydrolysis by LolD, the

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interaction between LolC and LolA leads to charging of LolA with lipoprotein (Okuda & Tokuda, 2009). The interaction between LolA and lipoprotein is weaker than the interaction between LolB and lipoprotein, driving transfer from LolA to LolB and preventing back-transfer to LolCE (Taniguchi *et al.*, 2005). It is unknown how lipoproteins interact with the ABC transporter LolCDE and by which mechanism lipoproteins are released from the cytoplasmic membrane. Crystal structures of LolA and soluble LolB, lacking its lipid-anchor, have been solved (Takeda *et al.*, 2003). The calculated size of the cavity is predicted to accommodate one but is not large enough for three fatty acid chains. Structure-function analyses of LolA of *P. aeruginosa* showed that one large hydrophobic surface patch is likely to be involved in binding of acyl chains (Remans *et al.*, 2010). Decreasing its hydrophobic nature abolished LolA-dependent release of lipoproteins from the cytoplasmic membrane (Remans *et al.*, 2010). The fatty acid acyl moiety of lipoprotein, together with its sorting signal, clearly plays a role in substrate recognition by the Lol machinery. LolC and LolE share a similar membrane topology and their periplasmic domains share sequence identity with each other as well as with LolB (Okuda & Tokuda, 2009). LolA interacts specifically with LolC and not with LolE. Differences in binding affinity between LolE and LolC for lipoprotein, similar as described for LolA and LolB, may explain the molecular mechanism of transfer. Characterization of the lipid-protein interaction by structural- and biophysical studies is likely to provide more insight into the mechanism of Lol-dependent targeting of lipoproteins to the outer membrane.

Inhibitors of the Lol machinery were identified in a chemical genomic approach (Pathania *et al.*, 2009) which utilized a combination of three *in vitro* assays (Ito *et al.*, 2007). Treatment of cells with MAC13243 led to the accumulation of Lpp in the cytoplasmic membrane. It directly interacts with LolA (Pathania *et al.*, 2009). Further characterization of this compound led to the finding that a degradation product is the active species (Barker *et al.*, 2013). This

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thiourea compound is an analog of A22, an inhibitor of bacterial actin MreB (Barker *et al.*, 2013) and seems to inhibit bacterial growth in a non-specific fashion.

The Lol machinery is not fully conserved in proteobacteria, LolB is only found in β- and γ-proteobacteria and also LolCE are often missing (Okuda & Tokuda, 2011). Proteomics analyses showed that lipoproteins reside in the outer membrane of diderm actinomycetes but the sorting mechanism is completely unknown.

Lipoproteins involved in key processes of envelope biosynthesis

The most abundant lipoprotein in γ-proteobacteria is Lpp that is covalently linked to murein (peptidoglycan) and the first protein shown to be lipidated (Braun & Rehn, 1969). Lpp is also the most abundant protein in the cell and plays an important role in homeostasis of the cell envelope. A recent study identified a σ^E-regulated small RNA, MicL, which specifically targets *lpp* (Guo *et al.*, 2014). The mechanism of regulation allows decrease of Lpp translation and acceleration of degradation of the stable *lpp* mRNA. σ^E up-regulates proteins that facilitate assembly of outer membrane proteins and transport of LPS under stress conditions. At the same time it down-regulates OMP synthesis by up-regulation of sRNAs such as MicL (Guo *et al.*, 2014). A strain deleted for *lpp* is very sensitive to EDTA and cationic dyes and somewhat sensitive to detergents and it also leaks periplasmic proteins into the extracellular space (Hirota *et al.*, 1977, Yem & Wu, 1978). One-third of Lpp is covalently linked to peptidoglycan, while two-third is in the cell envelope but not anchored to the cell wall, the so-called free form (Inouye *et al.*, 1972). There is a dynamic equilibrium between the two forms and the protein is homogeneously distributed over the cell (Hiemstra *et al.*, 1986, Hiemstra *et al.*, 1987). The enzymes responsible for cross-linking Lpp to peptidoglycan were identified in 2008 (Magnet *et al.*, 2008). YbiS was shown to catalyze the covalent anchoring of Lpp to peptidoglycan, but two other enzymes ErfK and YcfS could also perform

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this reaction. These enzymes belong to the family of L,D-transpeptidases found in firmicutes (Mainardi *et al.*, 2005, Magnet *et al.*, 2007). YbiS is expected to cleave the peptide bond between meso-DAP and D-Ala and to link meso-DAP to the L-Lys residue in Lpp (Magnet *et al.*, 2008). Mis-localization of Lpp in the cytoplasmic membrane is lethal when cross-linked to the peptidoglycan (Yakushi *et al.*, 1997). The highly abundant Lpp homologue in *P. aeruginosa*, OprI exist in a free form and is not covalently cross-linked to peptidoglycan (Mizuno & Kageyama, 1979, Cornelis *et al.*, 1989). When produced in *E. coli* it is exposed on the surface of cells (Cornelis *et al.*, 1996). Interestingly, the free form of *E. coli* Lpp is also exposed on the cell surface (Cowles *et al.*, 2011), although detection of Lpp by immunogold labeling was only successful upon permeabilization of the outer membrane with TRIS-EDTA (Hiemstra *et al.*, 1986). Lpp is an elongated trimer composed of long α-helical domains that adopt a coiled coil, which reflects its repetitive characteristics (Braun & Bosch, 1972, Choi *et al.*, 1986). The last amino acids of the C-terminus form a hydrophobic capping motif that is important for binding to PG and possibly involved in the recognition of lipoprotein by ligase YbiS (Zhang & Wu, 1992, Shu *et al.*, 2000). It is thus likely that Lpp has a dual orientation, similar as described for peptidoglycan-associated Lipoprotein P6 of *Haemophilus influenza* (Michel *et al.*, 2013), although the correlation between surface-exposed orientation and biological function is not yet known.

Surface exposed lipoproteins are abundant in *Borrelia*, which lacks LPS in the outer membrane (for a recent review see (Zuckert, 2014)). The N-terminal membrane-tethering peptide keeps lipoproteins of *Borrelia burgdorferi* in a translocation compatible state that allow them to be secreted to the cell surface probably via a flippase complex. Several other examples of surface-exposed lipoproteins have been reported in proteobacteria, including proteins that are translocated via type II secretion machinery (PulA) (Pugsley *et al.*, 1986) or via an autotransporter mechanism (NalP and SphB1) (Coutte *et al.*, 2003, van Ulsen *et al.*,

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2003). In case of NalP of *Neisseria meningitidis*, it was demonstrated that lipidation is required for efficient release of surface-exposed proteins by delaying its own release from the cell surface through autocatalytic processing (Roussel-Jazede *et al.*, 2013). Similarly, non-lipidated SphB1 is efficiently secreted but is unable to cleave its substrate filamentous haemagglutinin FHA (Coutte *et al.*, 2003), which is important for virulence of *Bordetella pertussis* (Coutte *et al.*, 2003).

The Lpt pathway transports LPS from the cytoplasmic membrane to the cell surface (Ruiz *et al.*, 2009). LptE is an essential OM lipoprotein that stabilizes the LptD OM channel responsible for correct translocation of LPS (Bos *et al.*, 2004, Chng *et al.*, 2010). Recent studies showed that LptE forms a plug inside the β-barrel structure of LptD (Freinkman *et al.*, 2011) and that it changes the biophysical state of LPS to allow transport and OM insertion (Malojcic *et al.*, 2014). The N-terminal lipid anchor is not required for function since a variant of LptE lacking the signal sequence including the cysteine complements a *lptE* deletion strain and is able to form a complex with LptD (Chng *et al.*, 2010). LptD is inserted into the OM by the β-barrel assembly machine (Bam complex) (Chimalakonda *et al.*, 2011). Four lipoproteins (BamB-E) are part of the Bam complex, of which BamD is essential for the assembly and/or insertion of proteins in the OM (Malinverni *et al.*, 2006). BamC is surface exposed (Webb *et al.*, 2012). How the lipid moiety is orientated in the outer membrane is still an open question.

Biosynthesis of peptidoglycan is catalyzed by several membrane-bound enzymes, among which the bifunctional penicillin-binding proteins PBP1A and PBP1B. These enzymes each require an outer membrane lipoprotein, LpoA and LpoB, respectively, for their function (Paradis-Bleau *et al.*, 2010, Typas *et al.*, 2010). The Lpo proteins bind specifically to their cognate PBP and stimulate transpeptidase activity. LpoB with an inner membrane retention signal is functional as long as the flexible N-terminal domain is present (Typas *et al.*, 2010). It is currently unkown whether non-lipidated LpoA is functional.

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The role of lipoprotein modification in virulence

The role of individual lipoproteins on virulence of pathogenic bacteria has been studied (for a review see (Kovacs-Simon *et al.*, 2010)). Signaling of the host immune system by lipoproteins and lipopeptides occurs via Toll-like receptor 2, either via TLR2-1 in case of diacylated proteins or via TLR2-6 heterodimers in case of triacylated proteins. The lipid moiety plays an important role in the interaction with these receptors (Jin *et al.*, 2007, Kang *et al.*, 2009). In recent years the effect of lipoprotein modification on virulence has been addressed using *lgt* and *lsp* deletion mutants of pathogenic bacteria and depending on the pathogen and the infection model used, various effects on virulence were obtained.

For example, impaired colonization of the nasopharynx and a decrease in virulence in pneumonia and septicaemia mouse models was reported for *Streptococcus pneumoniae* deleted for *lgt* (Petit *et al.*, 2001, Chimalapati *et al.*, 2012). TLR-2 signaling is dependent on pneumococcal lipoproteins and NF-κB and cytokine release were reduced in a Δ*lgt* stain (Tomlinson *et al.*, 2014). Intracellular survival of *Listeria monocytogenes* was affected (Baumgärtner *et al.*, 2007) and the Δ*lgt* strain was attenuated in a mouse model (Machata *et al.*, 2008). Lipoproteins are released into the culture supernatant of this stain (Baumgärtner *et al.*, 2007). Inactivation of *lsp* in *L. monocytogenes* led to a severe growth defect in macrophages and a reduced capacity of phagosomal escape leading to attenuated virulence (Reglier-Poupet *et al.*, 2003). Expression of the *lsp* gene was strongly induced inside phagosomes probably caused by environmental conditions similar to pH-regulation of *N*-acylation in *S. aureus* (Reglier-Poupet *et al.*, 2003, Kurokawa *et al.*, 2012). Inefficient germination of spores of a *lgt* deletion strain of *Bacillus anthracis* led to attenuation of virulence (Okugawa *et al.*, 2012). Lipoproteins of an *lsp* deletion mutant of *M. tuberculosis* are not processed and the strain was attenuated in an animal model of tuberculosis (Sander *et*

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al., 2004, Rampini *et al.*, 2008). Reduced innate immune activation was observed with a Δ*lgt* strain of *Streptococcus suis* (Wichgers Schreur *et al.*, 2011), but no difference in virulence was observed between a wild type strain and a *lsp* deletion mutant in an infection model in piglets (De Greeff *et al.*, 2003). Deletion of either *lgt* or *lsp* in *Streptococcus sanguinis* has only a slight effect on endocarditis virulence (Das *et al.*, 2009). No significant effect on virulence was observed in *Streptococcus equi* (Hamilton *et al.*, 2006, Das *et al.*, 2009). Escape of immune recognition caused lethal infections by a Δ*lgt* strain of *S. aureus* but a Δ*lsp* strain was attenuated (Bubeck-Wardenburg *et al.*, 2006). Enhanced lethality was shown in a mouse model for neonatal sepsis by Δ*lgt* of *Streptococcus agalactiae* (Henneke *et al.*, 2008). Stimulation of the immune system via TLR2 during early stages of infection is important for elimination of pathogens. The examples described here clearly show that both the lipid moiety and the functional characteristics of lipoproteins determine virulence capacity.

Protein lipidation in other microbes

Lipoproteins are predicted in Archaea, but orthologous genes for lipoprotein modifying enzymes could not been identified in their genomes. Recent findings in *Haloferax volcanii* show that the Tat-dependent secretion of predicted lipoproteins depends on the conserved cysteine in the putative lipobox (Gimenez *et al.*, 2007, Storf *et al.*, 2010). Future studies will provide direct evidence for lipidation of these proteins and will demonstrate whether phospho-, glyco- or phospho-glyco-lipids that constitute archaeal membranes, and are substantially different from their bacterial counterparts, are the source of modification (Villanueva *et al.*, 2014).

In eukaryotes, including yeast, various types of protein lipidation exist including *S*-palmitoylation, *N*-myristoylation and *S*-prenylation (Aicart-Ramos *et al.*, 2011). In the process of *S*-palmitoylation, palmitate is transferred onto a cysteine residue catalyzed by

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protein palmitate transferases (PAT) containing DHHC (Asp-His-His-Cys) cysteine rich domains that results in the formation of a thioester-linked palmitoylated protein (Dietrich & Ungermann, 2004). The PAT enzymes are polytopic integral membrane proteins located in the ER or Golgi. The active site is facing the cytoplasm where acyl-CoA functions as the acyl donor. Multiple DHHC enzymes have been identified in *Saccharomyces cerevisiae* but their substrate specificity is still poorly understood. Recent work on the Erf2-Erf4 PAT complex in *S. cerevisiae* showed that it catalyzes a two-step reaction in which Erf2 undergoes auto-acylation (Mitchell *et al.*, 2010). Erf2 transfers the palmitate group onto the substrate and Erf4 is required for stabilization of Erf2 and for the formation of the Erf2-palmitate intermediate (Mitchell *et al.*, 2012). *S*-palmitoylation is a reversible process due to the action of acyl-protein thioesterases. Together these two processes allow for regulated control of membrane-associated proteins, such as small GTPase Ras, the function of which depends on their lipid anchor.

Several examples have been described of bacterial effector proteins that contain eukaryotic lipidation motifs and that are modified by lipids upon entry into host cells. A farnesyl or geranylgeranyl isopreny lipid moiety is added to cysteine residue of the conserved sequence leading to enhanced membrane affinity and localization of effector proteins to membranes of host cells (Ivanov *et al.*, 2010).

Concluding remarks

Lipoproteins are highly abundant in bacteria, and require therefore efficient maturation. The acyltransferases and signal peptidase involved in this pathway are not abundant, estimated to only a few hundred molecules per cell. It is very likely that these enzymes exist in a complex to guarantee complete and efficient lipoprotein modification. The existence of a lipoprotein modification complex was already suggested in 1982 (Tokunaga *et al.*, 1982). This presumed

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complex might co-localize and function together with other essential protein complexes including the Sec and Tat secretion machineries. In this respect it is intriguing to understand how the lipoprotein modification machinery recognizes non-folded- and fully folded substrates that are translocated via Sec and Tat, respectively. The signal peptide of both types of lipoprotein substrates including the early mature region of the precursor protein is thought to exist in a flexible conformation inside the translocon. Lateral opening of the Sec channel can be envisaged for insertion of lipoproteins into the lipid bilayer (Chatzi *et al.*, 2014), whether lateral gating of the Tat complex occurs is currently unknown (Patel *et al.*, 2014). Once inserted into the membrane, Lgt binds the signal peptide and the first few amino acids of the mature protein and catalyzes the first step in the lipidation pathway.

Several examples clearly demonstrate that some essential lipoproteins are perfectly functional without their lipid anchor what raises the following questions: what is the role of the lipid moiety, and how does the degree of lipidation influence protein function? Unlike membrane proteins, lipoproteins are expected to be more mobile within lipid bilayers giving them more flexibility, although this has not been experimentally tested. For lipoproteins in monoderm bacteria it has been postulated that the N-terminal lipid anchor keeps the protein close to the membrane while the extended functional domain participates in various biological processes. Many lipoproteins in these organisms fulfill equivalent functions as periplasmic proteins in diderm bacteria. Accessory proteins such as LptE and LpoA/B interact strongly with their partner proteins, for which the lipid part is not essential, although the process in which they are involved might be more efficient when these proteins are present in lipidated form. In the case of lipoproteins that themselves fulfill specific functions and not in complex with other proteins, such as surface-exposed proteases or targeting of proteins to the OM, the lipid anchor becomes essential. The lipoprotein modification pathway seems to be tightly regulated since a lipoprotein can exist in both di- or triacylated form in one bacterial species, depending

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on growth conditions. The fact that multiple copies of genes encoding modification enzymes exist in bacteria that undergo various developmental stages also suggest that regulation of their expression is environmentally controlled.

The lipoprotein modification pathway represents an attractive target for antibiotic development. It is essential for the viability of all proteobacteria examined to date, which include many important human pathogens. Furthermore, Lsp and Lnt are membrane-bound with catalytic domains facing the bacterial periplasm. This topology makes at least two steps of lipoprotein biogenesis accessible to small molecules that will be capable of inhibiting enzymes without a need to traverse the relatively impermeable cytoplasmic membrane or risk elimination by the action of efflux pumps. For future studies on lipoprotein modification and function it is important to include proteomic analysis of membrane-bound and secreted lipoproteins and to determine the lipidation state and lipid composition by mass spectrometry (Cain *et al.*, 2014). These approaches not only allow the identification and characterization of specific lipoproteins that act as virulence factors but also demonstrate a correlation between lipidation and the stage of infection. Structural and mechanistic properties of enzymes involved in lipidation of proteins in various organisms will highlight similarities and differences and will provide ways of finding new species specific antibacterial agents.

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Figure legends

Figure 1. Lipoprotein modification pathway in bacteria

Lipoprotein modification occurs in the cytoplasmic membrane (CM) by the sequential action of three membrane-bound enzymes Lgt, Lsp and Lnt. Prolipoprotein is inserted into the membrane via the Sec or Tat secretion machinery through recognition of its signal peptide (SP). This sequence also contains the lipobox (LB) specific for bacterial lipoproteins. Lgt catalyzes the transfer of a diacylglyceryl group from phosphatidylglycerol (PG) onto prolipoprotein resulting in diacylglyceryl-prolipoprotein. Cleavage of the signal peptide by Lsp liberates the α-amino group of diacylglyceryl-cysteine that then becomes acylated by Lnt resulting in mature triacylated lipoprotein. Lnt preferentially uses phosphatidylethanolamine (PE) as acyl donor. Alternative N-acyl modifications have been observed in lipoproteins from firmicutes and mollicutes.

Figure 2. Membrane topology of modification enzymes

Schematic representation of membrane topology and essential residues (in red) in Lgt, Lsp and Lnt. Numbering of amino acids correspond to sequences of *E. coli* proteins. C=cytoplasm, CM=cytoplasmic membrane, P=periplasm. Catalytic site residues are shown in yellow. Conserved domains are indicated as white boxes. See text for details.

Figure 3. Structure of globomycin

Globomycin is a lipophilic cyclic peptide. Derivatives have been synthesized and analyzed for their activity against a variety of bacteria, in particular the length of the lipophilic part (A), and the role of the hydroxyl groups of threonine (B) and serine (C) have been addressed.

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Figure 4. Lol machinery in proteobacteria

The Lol machinery is involved in translocation of mature (triacylated) lipoproteins from the cytoplasmic membrane (CM) to the outer membrane (OM). The ABC transporter component of the machinery is composed of membrane proteins LolC and LolE and cytoplasmic ATPase LolD. The periplasmic chaperone LolA binds the lipoprotein, upon interaction with LolC and hydrolysis of ATP by LolD, and transfers it to receptor protein LolB. The lipoprotein is then inserted into the outer membrane. Several lipoproteins are located on the cell surface; however, the mechanism for their translocation and the orientation of the fatty acids in the membrane is not yet understood.

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References:

Ahn VE, Lo EI, Engel CK, Chen L, Hwang PM, Kay LE, Bishop RE & Prive GG (2004) A hydrocarbon ruler measures palmitate in the enzymatic acylation of endotoxin. *The EMBO journal* **23**: 2931-2941.

Aicart-Ramos C, Valero RA & Rodriguez-Crespo I (2011) Protein palmitoylation and subcellular trafficking. *Biochimica et biophysica acta* **1808**: 2981-2994.

Alba BM, Leeds JA, Onufryk C, Lu CZ & Gross CA (2002) DegS and YaeL participate sequentially in the cleavage of RseA to activate the sigma(E)-dependent extracytoplasmic stress response. *Genes & development* **16**: 2156-2168.

Allsop A, Brooks G, Edwards PD, Kaura AC & Southgate R (1996) Inhibitors of bacterial signal peptidase: a series of 6-(substituted oxyethyl)penems. *The Journal of antibiotics* **49**: 921-928.

Ames GF (1968) Lipids of Salmonella typhimurium and Escherichia coli: structure and metabolism. *Journal of bacteriology* **95**: 833-843.

An FY, Sulavik MC & Clewell DB (1999) Identification and characterization of a determinant (eep) on the Enterococcus faecalis chromosome that is involved in production of the peptide sex pheromone cAD1. *Journal of bacteriology* **181**: 5915-5921.

Asanuma M, Kurokawa K, Ichikawa R, Ryu K, Chae J, Dohmae N, Lee BL & Nakayama H (2010) Structural evidence of α-aminoacylated lipoproteins of *Staphylococcus aureus*. *FEBS J* **278**: 716-728.

Auclair SM, Bhanu MK & Kendall DA (2012) Signal peptidase I: cleaving the way to mature proteins. *Protein science : a publication of the Protein Society* **21**: 13-25.

Babu MM, Priya ML, Selvan AT, Madera M, Gough J, Aravind L & Sankaran K (2006) A database of bacterial lipoproteins (DOLOP) with functional assignments to predicted lipoproteins. *J Bacteriol* **188**: 2761-2773.

Banaiee N, Jacobs WR & Ernst JD (2007) LspA-independent action of globomycin on Mycobacterium tuberculosis. *The Journal of antimicrobial chemotherapy* **60**: 414-416.

Banerjee S & Sankaran K (2012) First ever isolation of bacterial prolipoprotein diacylglyceryl transferase in single step from Lactococcus lactis. *Protein Expr Purif* **87**: 120-128.

Barker CA, Allison SE, Zlitni S, Nguyen ND, Das R, Melacini G, Capretta FA & Brown ED (2013) Degradation of MAC13243 and studies of the interaction of resulting thiourea compounds with the lipoprotein targeting chaperone LolA. *Bioorganic and Medicinal Chemistry Letters* **23**: 2426-2431.

Baulard AR, Gurcha SS, Engohang-Ndong J, Gouffi K, Locht C & Besra GS (2003) *In vivo* interaction between the polyprenol phosphate mannose synthase Ppm1 and the integral

For Peer Review

ScholarOne Support 1-434/964-4100

membrane protein Ppm2 from *Mycobacterium smegmatis* revealed by a bacterial two-hybrid system. *J Biol Chem* **278**: 2242-2248.

Baumgärtner M, Karst U, Gerstel B, Loessner M, Wehland J & Jansch L (2007) Inactivation of *lgt* allows systematic characterization of lipoproteins from *Listeria monocytogenes*. *J Bacteriol* **189**: 313-324.

Bell-Pedersen D, Galloway Salvo JL & Belfort M (1991) A transcription terminator in the thymidylate synthase (thyA) structural gene of Escherichia coli and construction of a viable thyA::Kmr deletion. *Journal of bacteriology* **173**: 1193-1200.

Bishop RE, Gibbons HS, Guina T, Trent MS, Miller SI & Raetz CR (2000) Transfer of palmitate from phospholipids to lipid A in outer membranes of gram-negative bacteria. *The EMBO journal* **19**: 5071-5080.

Bos MP, Tefsen B, Geurtsen J & Tommassen J (2004) Identification of an outer membrane protein required for the transport of lipopolysaccharide to the bacterial cell surface. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 9417-9422.

Braun V & Rehn K (1969) Chemical characterization, spatial distribution and function of a lipoprotein (murein-lipoprotein) of the *E. coli* cell wall. *European journal of biochemistry / FEBS* **10**: 426-438.

Braun V & Bosch V (1972) Sequence of the murein-lipoprotein and the attachment site of the lipid. *European journal of biochemistry / FEBS* **28**: 51-69.

Brülle JK, Tschumi A & Sander P (2013) Lipoproteins of slow-growing Mycobacteria carry three fatty acids and are N-acylated by apolipoprotein N-acyltransferase BCG_2070c. *BMC Microbiol* **13**: 223.

Brülle JK, Grau T, Tschumi A, Auchli Y, Burri R, Polsfuss S, Keller PM, Hunziker P & Sander P (2010) Cloning, expression and charaterization of *Mycobacterium tuberculosis* lipoprotein LprF. *Biochem Biophys Res Comm* **391**: 679-684.

Bubeck-Wardenburg J, Williams WA & Missiakas D (2006) Host defenses against *Staphylococcus aureus* infection require recognition of bacterial lipoproteins. *Proc Natl Acad Sci U S A* **103**: 13831-13836.

Buddelmeijer N & Young R (2010) The essential *Escherichia coli* Apolipoprotein N-acyltransferase (Lnt) exists as an extracytoplasmic thioester acyl-enzyme intermediate. *Biochemistry* **49**: 341-346.

Cain JA, Solis N & Cordwell SJ (2014) Beyond gene expression: the impact of protein post-translational modifications in bacteria. *Journal of proteomics* **97**: 265-286.

Chatzi KE, Sardis MF, Economou A & Karamanou S (2014) SecA-mediated targeting and translocation of secretory proteins. *Biochimica et biophysica acta* **1843**: 1466-1474.

For Peer Review

ScholarOne Support 1-434/964-4100

Chimalakonda G, Ruiz N, Chng SS, Garner RA, Kahne D & Silhavy TJ (2011) Lipoprotein LptE is required for the assembly of LptD by the beta-barrel assembly machine in the outer membrane of Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 2492-2497.

Chimalapati S, Cohen JM, Camberlein E, MacDonald N, Durmort C, Vernet T, Hermans PW, Mitchell T & Brown JS (2012) Effects of deletion of the Streptococcus pneumoniae lipoprotein diacylglyceryl transferase gene lgt on ABC transporter function and on growth in vivo. *PLoS One* **7**: e41393.

Chng SS, Ruiz N, Chimalakonda G, Silhavy TJ & Kahne D (2010) Characterization of the two-protein complex in Escherichia coli responsible for lipopolysaccharide assembly at the outer membrane. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 5363-5368.

Choi D-S, Yamada H, Mizuno T & Mizushima S (1986) Trimeric structure and localization of the major lipoprotein in the cell surface of *Escherichia coli*. *J Biol Chem* **261**: 8953-8957.

Cooper CL, Hsu L, Jackowski S & Rock CO (1989) 2-Acylglycerolphosphoethanolamine acyltransferase/acyl-acyl carrier protein synthetase is a membrane-associated acyl carrier protein binding protein. *The Journal of biological chemistry* **264**: 7384-7389.

Cordova-Davalos LE, Espitia C, Gonzalez-Ceron G, Arreguin-Espinosa R, Soberon-Chavez G & Servin-Gonzalez L (2014) Lipoprotein N-acyl transferase (Lnt1) is dispensable for protein O-mannosylation by Streptomyces coelicolor. *FEMS microbiology letters* **350**: 72-82.

Cornelis P, Bouia A, Belarbi A, Guyonvarch A, Kammerer B, Hannaert V & Hubert JC (1989) Cloning and analysis of the gene for the major outer membrane lipoprotein from Pseudomonas aeruginosa. *Molecular microbiology* **3**: 421-428.

Cornelis P, Cote-Sierra J, Lim Jr A*, et al.* (1996) Development of new cloning vectors for the production of immunogenic outer membrane fusion proteins in *Escherichia coli*. *Bio/Technol* **14**: 203-208.

Coutte L, Willery E, Antoine R, Drobecq H, Locht C & Jacob-Dubuisson F (2003) Surface anchoring of bacterial subtilisin important for maturation function. *Mol Microbiol* **49**: 529-539.

Coutte L, Alonso S, Reveneau N, Willery E, Quatannens B, Locht C & Jacob-Dubuisson F (2003) Role of adhesin release for mucosal colonization by a bacterial pathogen. *The Journal of experimental medicine* **197**: 735-742.

Cowles CE, Li Y, Semmelhack MF, Cristea IM & Silhavy TJ (2011) The free and bound forms of Lpp occupy distinct subcellular locations in Escherichia coli. *Molecular microbiology* **79**: 1168-1181.

For Peer Review

ScholarOne Support 1-434/964-4100

Cronan JE, Jr. & Rock CO (1996) Biosynthesis of membrane lipids. *Escherichia coli and Salmonella: molecular and cellular biology,*(Neidhardt FC, ed.) p.^pp. ASM, Washington D.C.

Das S, Kanamoto T, Ge X, Xu P, Unoki T, Munro CL & Kitten T (2009) Contribution of lipoproteins and lipoprotein processing to endocarditis virulence in Streptococcus sanguinis. *J Bacteriol* **191**: 4166-4179.

De Bona P, Deshmukh L, Gorbatyuk V, Vinogradova O & Kendall DA (2012) Structural studies of a signal peptide in complex with signal peptidase I cytoplasmic domain: the stabilizing effect of membrane-mimetics on the acquired fold. *Proteins* **80**: 807-817.

De Greeff A, Hamilton A, Sutcliffe IC, Buys H, Van Alphen L & Smith HE (2003) Lipoprotein signal peptidase of Streptococcus suis serotype 2. *Microbiology (Reading, England)* **149**: 1399-1407.

Denham EL, Ward PN & Leigh JA (2009) In the absence of Lgt, lipoproteins are shed from Streptococcus uberis independently of Lsp. *Microbiology (Reading, England)* **155**: 134-141.

Dev IK, Harvey RJ & Ray PH (1985) Inhibition of prolipoprotein signal peptidase by globomycin. *J Biol Chem* **260**: 5891-5894.

Dietrich LE & Ungermann C (2004) On the mechanism of protein palmitoylation. *EMBO reports* **5**: 1053-1057.

Doerrler WT & Raetz CR (2002) ATPase activity of the MsbA lipid flippase of Escherichia coli. *The Journal of biological chemistry* **277**: 36697-36705.

Doerrler WT, Reedy MC & Raetz CRH (2001) An *Escherichia coli* mutant defective in lipid export. *The Journal of biological chemistry* **276**: 11461-11464.

Doerrler WT, Gibbons HS & Raetz CR (2004) MsbA-dependent translocation of lipids across the inner membrane of Escherichia coli. *The Journal of biological chemistry* **279**: 45102-45109.

Doi O & Nojima S (1975) Lysophospholipase of Escherichia coli. *The Journal of biological chemistry* **250**: 5208-5214.

Doshi R & van Veen HW (2013) Substrate binding stabilizes a pre-translocation intermediate in the ATP-binding cassette transport protein MsbA. *The Journal of biological chemistry* **288**: 21638-21647.

Erez E, Fass D & Bibi E (2009) How intramembrane proteases bury hydrolytic reactions in the membrane. *Nature* **459**: 371-378.

Espitia C & Mancilla R (1989) Identification, isolation and partial characterization of Mycobacterium tuberculosis glycoprotein antigens. *Clinical and experimental immunology* **77**: 378-383.

For Peer Review

ScholarOne Support 1-434/964-4100

Fischer W (1994) Lipoteichoic acid and lipids in the membrane of Staphylococcus aureus. *Medical microbiology and immunology* **183**: 61-76.

Freinkman E, Chng SS & Kahne D (2011) The complex that inserts lipopolysaccharide into the bacterial outer membrane forms a two-protein plug-and-barrel. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 2486-2491.

Fukuda A, Matsuyama S-I, Hara T, Nakayama J, Nagasawa H & Tokuda H (2002) Aminoacylation of the N-terminal cysteine is essential for Lol-dependent release of lipoproteins from membranes but does not depend on lipoprotein sorting signals. *J Biol Chem* **277**: 43512-43518.

Gan K, Gupta SD, Sankaran K, Schmid MB & Wu HC (1993) Isolation and characterization of a temperature-sensitive mutant of *Salmonella typhimurium* defective in prolipoprotein modification. *J Biol Chem* **268**: 16544-16550.

Gan K, Sankaran K, Williams MG, Aldea M, Rudd KE, Kushner SR & Wu HC (1995) The *umpA* gene of *Escherichia coli* encodes phosphatidylglycerol: prolipoprotein diacylglyceryl transferase (*lgt*) and regulates thymidylate synthase levels through translational coupling. *J Bacteriol* **177**: 1879-1882.

Gibbons HS, Wolschendorf F, Abshire M, Niederweis M & Braunstein M (2007) Identification of two Mycobacterium smegmatis lipoproteins exported by a SecA2-dependent pathway. *Journal of bacteriology* **189**: 5090-5100.

Gibson KJ, Eggeling L, Maughan WN, Krumbach K, Gurcha SS, Nigou J, Puzo G, Sahm H & Besra GS (2003) Disruption of Cg-Ppm1, a polyprenyl monophosphomannose synthase, and the generation of lipoglycan-less mutants in *Corynebacterium glutamicum*. *J Biol Chem* **278**: 40842-40850.

Gimenez MI, Dilks K & Pohlschroder M (2007) Haloferax volcanii twin-arginine translocation substates include secreted soluble, C-terminally anchored and lipoproteins. *Molecular microbiology* **66**: 1597-1606.

Gonzalez-Zamorano M, Mendoza-Hernandez G, Xolalpa W, Parada C, Vallecillo AJ, Bigi F & Espitia C (2009) Mycobacterium tuberculosis glycoproteomics based on ConA-lectin affinity capture of mannosylated proteins. *Journal of proteome research* **8**: 721-733.

Gullon S, Arranz EI & Mellado RP (2013) Transcriptional characterisation of the negative effect exerted by a deficiency in type II signal peptidase on extracellular protein secretion in Streptomyces lividans. *Applied microbiology and biotechnology* **97**: 10069-10080.

Guo MS, Updegrove TB, Gogol EB, Shabalina SA, Gross CA & Storz G (2014) MicL, a new sigmaE-dependent sRNA, combats envelope stress by repressing synthesis of Lpp, the major outer membrane lipoprotein. *Genes & development* **28**: 1620-1634.

For Peer Review

ScholarOne Support 1-434/964-4100

Gupta SD & Wu HC (1991) Identification and subcellular localization of apolipoprotein N-acyltransferase in *Escherichia coli*. *FEMS Microbiol Lett* **78**: 37-42.

Gupta SD, Dowham W & Wu HC (1991) Phosphatidylethanolamine is not essential for the N-acylation of apolipoprotein in *Escherichia coli*. *J Biol Chem* **266**: 9983-9986.

Gupta SD, Gan K, Schmid MB & Wu HC (1993) Characterization of a temperature-sensitive mutant of *Salmonella typhimurium* defective in apolipoprotein N-acyltransferase. *J Biol Chem* **268**: 16551-16556.

Gurcha SS, Baulard AR, Kremer L, Locht C, Moody DB, Muhlecker W, Costello CE, Crick DC, Brennan PJ & Besra GS (2002) Ppm1, a novel polyprenol monophosphomannose synthase from *Mycobacterium tuberculosis*. *Biochem J* **365**: 441-450.

Haake DA (2000) Spirochaetal lipoproteins and pathogenesis. *Microbiology (Reading, England)* **146 (Pt 7)**: 1491-1504.

Hamilton A, Robinson C, Sutcliffe IC, Slater J, Maskell DJ, Davis-Poynter N, Smith K, Waller A & Harrington DJ (2006) Mutation of the maturase lipoprotein attenuates the virulence of *Streptococcus equi* to a greater extent than does loss of general lipoprotein lipidation. *Infect Immun* **74**: 6907-6919.

Hantke K & Braun V (1973) Covalent binding of lipid to protein. Diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the *Escherichia coli* outer membrane. *Eur J Biochem* **34**: 284-296.

Harvat E, Zhang Y-M, Tran C, Zhang Z, Frank M, Rock C & Saier MJ (2005) Lysophospholipid flipping across the *Escherichia coli* inner membrane catalyzed by a transporter (LplT) belonging to the major facilitator superfamily. *J Biol Chem* **280**: 12028-12034.

Hayashi S & Wu HC (1983) Biosynthesis of Bacillus licheniformis penicillinase in Escherichia coli and in Bacillus subtilis. *Journal of bacteriology* **156**: 773-777.

Hayashi S & Wu HC (1985) Accumulation of prolipoprotein in Escherichia coli mutants defective in protein secretion. *Journal of bacteriology* **164**: 949-954.

Hayashi S, Chang SY, Chang S, Giam CZ & Wu HC (1985) Modification and processing of internalized signal sequences of prolipoprotein in *Escherichia coli* and in *Bacillus subtilis*. *J Biol Chem* **260**: 5753-5759.

Henneke P, Dramsi S, Mancuso G*, et al.* (2008) Lipoproteins are critical TLR2 activating toxins in group B streptococcal sepsis. *J Immunol* **180**: 6149-6158.

Herrmann JL, O'Gaora P, Gallagher A, Thole JER & Young DB (1996) Bacterial glycoproteins: a link between glycosylation and proteolytic cleavage of a 19 kDa antigen from *Mycobacterium tuberculosis*. *EMBO J* **15**: 3547-3554.

For Peer Review

ScholarOne Support 1-434/964-4100

Hiemstra H, de Hoop MJ, Inouye M & Witholt B (1986) Induction kinetics and cell surface distribution of *Escherichia coli* lipoprotein under *lac* promoter control. *Journal of bacteriology* **168**: 140-151.

Hiemstra H, Nanninga N, Woldringh CL, Inouye M & Witholt B (1987) Distribution of newly synthesized lipoprotein over the outer membrane and the peptidoglycan sacculus of an *Escherichia coli* lac-lpp strain. *Journal of bacteriology* **169**: 5434-5444.

Hillmann F, Argentini M & Buddelmeijer N (2011) Kinetics and phospholipid specificity of apolipoprotein N-acyltransferase. *J Biol Chem* **286**: 27936-27946.

Hirota Y, Suzuki H, Nishimura Y & Yasuda S (1977) On the process of cellular division in *Escherichia coli*: A mutant of *E. coli* lacking a murein-lipoprotein. *Proc Natl Acad Sci USA* **74**: 1417-1420.

Hizukuri Y, Oda T, Tabata S, Tamura-Kawakami K, Oi R, Sato M, Takagi J, Akiyama Y & Nogi T (2014) A structure-based model of substrate discrimination by a noncanonical PDZ tandem in the intramembrane-cleaving protease RseP. *Structure* **22**: 326-336.

Homma H & Nojima S (1982) Synthesis of various phospholipids from 2-acyl lysophospholipids by Escherichia coli extract. *Journal of biochemistry* **91**: 1103-1110.

Hsu L, Jackowski S & Rock C (1991) Isolation and characterization of *Escherichia coli* K-12 mutants lacking both 2-acylglycerophosphoethanolamine acyltransferase and acyl-acyl carrier protein synthetase activity. *J Biol Chem* **266**: 13783-13788.

Hu J, Xue Y, Lee S & Ha Y (2011) The crystal structure of GXGD membrane protease FlaK. *Nature* **475**: 528-531.

Hussain M, Ichihara S & Mizushima S (1980) Accumulation of glyceride-containing precursor of the outer membrane lipoprotein in the cytoplasmic membrane of *Escherichia coli* treated with globomycin. *J Biol Chem* **255**: 3707-3712.

Hussain M, Ichihara S & Mizushima S (1982) Mechanism of signal peptide cleavage in the biosynthesis of the major lipoprotein of the *Escherichia coli* outer membrane. *J Biol Chem* **257**: 5177-5182.

Ichihara S, Suzuki T, Suzuki M & Mizushima S (1986) Molecular cloning and sequencing of the sppA gene and characterization of the encoded protease IV, a signal peptide peptidase, of Escherichia coli. *The Journal of biological chemistry* **261**: 9405-9411.

Inouye M, Shaw J & Shen C (1972) The assembly of a structural lipoprotein in the envelope of Escherichia coli. *The Journal of biological chemistry* **247**: 8154-8159.

Inouye S, Vlasuk GP, Hsiung H & Inouye M (1984) Effects of mutations at glycine residues in the hydrophobic region of the Escherichia coli prolipoprotein signal peptide on the secretion across the membrane. *The Journal of biological chemistry* **259**: 3729-3733.

For Peer Review

ScholarOne Support 1-434/964-4100

Inouye S, Wang S, Sekizawa J, Halegoua S & Inouye M (1977) Amino acid sequence for the peptide extension on the prolipoprotein of the Escherichia coli outer membrane. *Proceedings of the National Academy of Sciences of the United States of America* **74**: 1004-1008.

Inukai M, Takeuchi K, Shimizu K & Arai M (1978) Mechanism of action of globomycin. *J Antibiot* **31**: 1203-1205.

Inukai M, Enokita R, Torikata A & Nakahara M (1978) Globomycin, a new peptide antibiotic with spheroplast-forming activity. I. Taxonomy of producing organisms and fermentation. *J Antibiot* **31**: 410-420.

Inukai M, Nakajima M, Osawa M, Haneishi T & Arai M (1978) Globomycin, a new peptide antibiotic with speroplast-forming activity. II Isolation and physico-chemical and biological characterization. *J Antibiot* **31**: 421-425.

Ito H, Ura A, Oyamada Y, Yoshida H, Yamagishi J, Narita S, Matsuyama S & Tokuda H (2007) A new screening method to identify inhibitors of the Lol (localization of lipoproteins) system, a novel antibacterial target. *Microbiology and immunology* **51**: 263-270.

Ivanov SS, Charron G, Hang HC & Roy CR (2010) Lipidation by the host prenyltransferase machinery facilitates membrane localization of Legionella pneumophila effector proteins. *The Journal of biological chemistry* **285**: 34686-34698.

Jackowski S & Rock CO (1986) Transfer of fatty acids from the 1-position of phosphatidylethanolamine to the major outer membrane lipoprotein of *Escherichia coli*. *J Biol Chem* **261**: 11328-11333.

Jackowski S, Jackson PD & Rock CO (1994) Sequence and function of the aas gene in Escherichia coli. *The Journal of biological chemistry* **269**: 2921-2928.

Jan G, Fontenelle C, Le Henaff M & Wroblewski H (1995) Acylation and immunological properties of Mycoplasma gallisepticum membrane proteins. *Research in microbiology* **146**: 739-750.

Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H & Lee JO (2007) Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. *Cell* **130**: 1071-1082.

Kanehara K, Akiyama Y & Ito K (2001) Characterization of the yaeL gene product and its S2P-protease motifs in Escherichia coli. *Gene* **281**: 71-79.

Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG & Lee JO (2009) Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. *Immunity* **31**: 873-884.

For Peer Review

ScholarOne Support 1-434/964-4100

Kiho T, Nakayama M, Yasuda K, Miyakoshi S, Inukai M & Kogen H (2003) Synthesis and antimicrobial activity of novel globomycin analogues. *Bioorg Med Chem Lett* **13**: 2315-2318.

Kiho T, Nakayama M, Yasuda K, Miyakoshi S, Inukai M & Kogen H (2004) Structure-activity relationships of globomycin analogues as antibiotics. *Bioorganic & medicinal chemistry* **12**: 337-361.

Kim AC, Oliver DC & Paetzel M (2008) Crystal structure of a bacterial signal Peptide peptidase. *Journal of molecular biology* **376**: 352-366.

Kovacs-Simon A, Titball RW & Michell SL (2010) Lipoproteins of bacterial pathogens. *Infect Immun* **79**: 548-561.

Kuo DW, Chan HK, Wilson CJ, Griffin PR, Williams H & Knight WB (1993) Escherichia coli leader peptidase: production of an active form lacking a requirement for detergent and development of peptide substrates. *Archives of biochemistry and biophysics* **303**: 274-280.

Kurokawa K, Kim MS, Ichikawa R, Ryu KH, Dohmae N, Nakayama H & Lee BL (2012) Environment-mediated accumulation of diacyl lipoproteins over their triacyl counterpart in *Staphylococcus aureus*. *J Bacteriol* **194**: 3299-3306.

Kurokawa K, Ryu KH, Ichikawa R, *et al.* (2012) Novel bacterial lipoprotein structures conserved in low-GC content Gram-positive bacteria are recognized by Toll-like receptor 2. *J Biol Chem* **287**: 13170-13181.

Kurokawa K, Lee H., Roh K.-B., Asanuma M., Kim Y S, Nakayama H, Shiratsuchi A., choi Y, Takeuchi O, Kang H J, Dohmae N, Nakanishi Y, Akira S, Sekimizu K, Lee B L (2009) The triacylated ATP binding cluster transporter substrate-binding lipoprotein of *Staphylococcus aureus* functions as a native ligand for Toll-like receptor 2. *J Biol Chem* **284**: 8406-8411.

Lai J-S, Philbrick WM & Wu HC (1980) Acyl moieties in phospholipids are tha precursors for the fatty acids in murein lipoprotein in *Escherichia coli*. *J Biol Chem* **255**: 5384-5387.

LaPointe CF & Taylor RK (2000) The type 4 prepilin peptidases comprise a novel family of aspartic acid proteases. *The Journal of biological chemistry* **275**: 1502-1510.

Leskelä S, Wahlström E, V.P. K & Sarvas M (1999) Lipid modification of prelipoproteins is dispensable for growth but essential for efficient protein secretion in *Bacillus subtilis:* characterization of the *lgt* gene. *Mol Microbiol* **31**: 1075-1085.

Lin JJ, Kanazawa H, Ozols J & Wu HC (1978) An *Escherichia coli* mutant with an amino acid alteration within the signal sequence of outer membrane prolipoprotein. *Proc Natl Acad Sci USA* **75**: 489-495.

Lin JJC, Giam CZ & Wu HC (1980) Assembly of the outer membrane lipoprotein in *Escherichia coli*. *The Journal of biological chemistry* **255**: 807-811.

For Peer Review

ScholarOne Support 1-434/964-4100

Lu P, Bai XC, Ma D, *et al.* (2014) Three-dimensional structure of human gamma-secretase. *Nature* **512**: 166-170.

Machata S, Tchatalbachev S, Mohamed W, Jansch L, Hain T & Chakraborty T (2008) Lipoproteins of *Listeria monocytogenes* are critical for virulence and TLR2-mediated immune activation. *J Immunol* **181**: 2028-2035.

Madan Babu M & Sankaran K (2002) DOLOP--database of bacterial lipoproteins. *Bioinformatics (Oxford, England)* **18**: 641-643.

Magnet S, Dubost L, Marie A, Arthur M & Gutmann L (2008) Identification of the L,D-transpeptidases for peptidoglycan cross-linking in Escherichia coli. *Journal of bacteriology* **190**: 4782-4785.

Magnet S, Arbeloa A, Mainardi JL, *et al.* (2007) Specificity of L,D-transpeptidases from gram-positive bacteria producing different peptidoglycan chemotypes. *The Journal of biological chemistry* **282**: 13151-13159.

Mainardi JL, Fourgeaud M, Hugonnet JE, Dubost L, Brouard JP, Ouazzani J, Rice LB, Gutmann L & Arthur M (2005) A novel peptidoglycan cross-linking enzyme for a beta-lactam-resistant transpeptidation pathway. *The Journal of biological chemistry* **280**: 38146-38152.

Malinverni JC, Werner J, Kim S, Sklar JG, Kahne D, Misra R & Silhavy TJ (2006) YfiO stabilizes the YaeT complex and is essential for outer membrane protein assembly in Escherichia coli. *Molecular microbiology* **61**: 151-164.

Malojcic G, Andres D, Grabowicz M, George AH, Ruiz N, Silhavy TJ & Kahne D (2014) LptE binds to and alters the physical state of LPS to catalyze its assembly at the cell surface. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 9467-9472.

Michel LV, Snyder J, Schmidt R, Milillo J, Grimaldi K, Kalmeta B, Khan MN, Sharma S, Wright LK & Pichichero ME (2013) Dual orientation of the outer membrane lipoprotein P6 of nontypeable haemophilus influenzae. *Journal of bacteriology* **195**: 3252-3259.

Mitchell DA, Mitchell G, Ling Y, Budde C & Deschenes RJ (2010) Mutational analysis of Saccharomyces cerevisiae Erf2 reveals a two-step reaction mechanism for protein palmitoylation by DHHC enzymes. *The Journal of biological chemistry* **285**: 38104-38114.

Mitchell DA, Hamel LD, Ishizuka K, Mitchell G, Schaefer LM & Deschenes RJ (2012) The Erf4 subunit of the yeast Ras palmitoyl acyltransferase is required for stability of the Acyl-Erf2 intermediate and palmitoyl transfer to a Ras2 substrate. *The Journal of biological chemistry* **287**: 34337-34348.

For Peer Review

ScholarOne Support 1-434/964-4100

Mizuno T & Kageyama M (1979) Isolation and characterization of major outer membrane proteins of Pseudomonas aeruginosa strain PAO with special reference to peptidoglycan-associated protein. *Journal of biochemistry* **86**: 979-989.

Mohiman N, Argentini M, Batt SM, Cornu D, Masi M, Eggeling L, Besra G & Bayan N (2012) The ppm operon is essential for acylation and glycosylation of lipoproteins in *Corynebacterium glutamicum*. *PloS One* **7**: e46225.

Munoa FJ, Miller KW, Beers R, Graham M & Wu HC (1991) Membrane topology of *Escherichia coli* prolipoprotein signal peptidase (signal peptidase II). *J Biol Chem* **266**: 17667-17672.

Nakajima M, Inukai M, Haneishi T, Terahara A & Arai M (1978) Globomycin, a new peptide antibiotic with spheroplast-forming activity. III. Structural determination of globomycin. *J Antibiot* **31**: 426-432.

Nakayama H, Kurokawa K & Lee BL (2012) Lipoproteins in bacteria: structures and biosynthetic pathways. *FEBS J* **279**: 4247-4268.

Narita S & Tokuda H (2011) Overexpression of LolCDE allows deletion of the Escherichia coli gene encoding apolipoprotein N-acyltransferase. *J Bacteriol* **193**: 4832-4840.

Novak P & Dev IK (1988) Degradation of a signal peptide by protease IV and oligopeptidase A. *Journal of bacteriology* **170**: 5067-5075.

Okuda S & Tokuda H (2009) Model of mouth-to-mouth transfer of bacterial lipoproteins through inner membrane LolC, periplasmic LolA, and outer membrane LolB. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 5877-5882.

Okuda S & Tokuda H (2011) Lipoprotein sorting in bacteria. *Annual review of microbiology* **65**: 239-259.

Okugawa S, Moayeri M, Pomerantsev AP, Sastalla I, Crown D, Gupta PK & Leppla SH (2012) Lipoprotein biosynthesis by prolipoprotein diacylglyceryl transferase is required for efficient spore germination and full virulence of *Bacillus anthracis*. *Mol Microbiol* **83**: 96-109.

Okuyama H, Kankura T & Nojima S (1967) Positional distribution of fatty acids in phospholipids from Mycobacteria. *Journal of biochemistry* **61**: 732-737.

Pacaud M (1982) Purification and characterization of two novel proteolytic enzymes in membranes of Escherichia coli. Protease IV and protease V. *The Journal of biological chemistry* **257**: 4333-4339.

Pacaud M (1982) Identification and localization of two membrane-bound esterases from Escherichia coli. *Journal of bacteriology* **149**: 6-14.

Paetzel M (2014) Structure and mechanism of Escherichia coli type I signal peptidase. *Biochimica et biophysica acta* **1843**: 1497-1508.

For Peer Review

ScholarOne Support 1-434/964-4100

Paetzel M, Karla A, Strynadka NC & Dalbey RE (2002) Signal peptidases. *Chem Rev* **102**: 4549-4580.

Pailler J, Aucher W, Pires M & Buddelmeijer N (2012) Phosphatidylglycerol::prolipoprotein diacylglyceryltransferase (Lgt) of *E. coli* has seven transmembrane segments and its essential residues are embedded in the membrane. *J Bacteriol* **194**: 2142-2151.

Paradis-Bleau C, Markovski M, Uehara T, Lupoli TJ, Walker S, Kahne DE & Bernhardt TG (2010) Lipoprotein cofactors located in the outer membrane activate bacterial cell wall polymerases. *Cell* **143**: 1110-1120.

Parsons JB & Rock CO (2013) Bacterial lipids: metabolism and membrane homeostasis. *Progress in lipid research* **52**: 249-276.

Patel R, Smith SM & Robinson C (2014) Protein transport by the bacterial Tat pathway. *Biochimica et biophysica acta* **1843**: 1620-1628.

Pathania R, Zlitni S, Barker C, Das R, Gerritsma DA, Lebert J, Awuah E, Melacini G, Capretta FA & Brown ED (2009) Chemical genomics in *Escherichia coli* identifies an inhibitor of bacterial lipoprotein targeting. *Nature chemical biology* **5**: 849-856.

Petit CM, Brown JR, Ingraham K, Bryant AP & Holmes DJ (2001) Lipid modification of prelipoproteins is dispensable for growth *in vitro* but essential for virulence in *Streptococcus pneumoniae*. *FEMS Microbiol Lett* **200**: 229-233.

Pollitt S, Inouye S & Inouye M (1985) A functional prolipoprotein signal peptide with a deletion of four amino acid residues from the hydrophobic region. *The Journal of biological chemistry* **260**: 7965-7969.

Pragai Z, Tjalsma H, Bolhuis A, van Dijl JM, Venema G & Bron S (1997) The signal peptidase II (*Isp*) gene of *Bacillus subtilis*. *Microbiol* **143**: 1327-1333.

Pugsley AP, Chapon C & Schwartz M (1986) Extracellular pullulanase of *Klebsiella pneumoniae* is a lipoprotein. *Journal of bacteriology* **166**: 1083-1088.

Qi HY, Sankaran K, Gan K & Wu HC (1995) Structure-function relationship of bacterial prolipoprotein diacylglyceryl transferase: functionally significant conserved regions. *J Bacteriol* **177**: 6820-6824.

Raetz CR (1986) Molecular genetics of membrane phospholipid synthesis. *Annual review of genetics* **20**: 253-295.

Rampini SK, Selchow P, Keller C, Ehlers S, Bottger EC & Sander P (2008) LspA inactivation in Mycobacterium tuberculosis results in attenuation without affecting phagosome maturation arrest. *Microbiology (Reading, England)* **154**: 2991-3001.

For Peer Review

ScholarOne Support 1-434/964-4100

Rana AK, Singh A, Gurcha SS, Cox LR, Bhatt A & Besra GS (2012) Ppm1-encoded polyprenyl monophosphomannose synthase activity is essential for lipoglycan synthesis and survival in mycobacteria. *PLoS One* **7**: e48211.

Reglier-Poupet H, Frehel C, Dubail I, Beretti JL, Berche P, Charbit A & Raynaud C (2003) Maturation of lipoproteins by type II signal peptidase is required for phagosomal escape of Listeria monocytogenes. *J Biol Chem* **278**: 49469-49477.

Remans K, Pauwels K, van Ulsen P, Buts L, Cornelis P, Tommassen J, Savvides SN, Decanniere K & Van Gelder P (2010) Hydrophobic surface patches on LolA of Pseudomonas aeruginosa are essential for lipoprotein binding. *Journal of molecular biology* **401**: 921-930.

Robichon C, Vidal-Ingigliardi D & Pugsley AP (2005) Depletion of apolipoprotein N-acyltransferase causes mislocalization of outer membrane lipoproteins in *Escherichia coli*. *J Biol Chem* **280**: 974-983.

Rogers SD, Bhave MR, Mercer JFB, Camakaris J & Lee BTO (1991) Cloning and characterization of *cutE* a gene involved in copper transport in *Escherichia coli*. *J Bacteriol* **173**: 6742-6748.

Rosenberg E, Vaks B & Zuckerberg A (1973) Bactericidal action of an antibiotic produced by Myxococcus xanthus. *Antimicrobial agents and chemotherapy* **4**: 507-513.

Roussel-Jazede V, Grijpstra J, van Dam V, Tommassen J & van Ulsen P (2013) Lipidation of the autotransporter NalP of Neisseria meningitidis is required for its function in the release of cell-surface-exposed proteins. *Microbiology (Reading, England)* **159**: 286-295.

Ruiz N, Kahne D & Silhavy TJ (2009) Transport of lipopolysaccharide across the cell envelope: the long road of discovery. *Nature reviews* **7**: 677-683.

Saito A, Hizukuri Y, Matsuo E, Chiba S, Mori H, Nishimura O, Ito K & Akiyama Y (2011) Post-liberation cleavage of signal peptides is catalyzed by the site-2 protease (S2P) in bacteria. *Proc Natl Acad Sci U S A* **108**.

Sander P, Rezwan M, Walker B, *et al.* (2004) Lipoprotein processing is required for virulence of *Mycobacterium tuberculosis*. *Mol Microbiol* **52**: 1543-1552.

Sankaran K & Wu HC (1994) Lipid modification of bacterial prolipoprotein. Transfer of diacylglyceryl moiety from phosphatidylglycerol. *J Biol Chem* **269**: 19701-19706.

Sankaran K, Gan K, Rash B, Qi H-Y, Wu HC & Rick PD (1997) Roles of histidine-103 and tyrosine-235 in the function of the prelipoprotein diacylglyceryl transferase of *Escherichia coli*. *J Bacteriol* **179**: 2944-2948.

Sartain MJ & Belisle JT (2009) N-Terminal clustering of the O-glycosylation sites in the *Mycobacterium tuberculosis* lipoprotein SodC. *Glycobiology* **19**: 38-51.

For Peer Review

ScholarOne Support 1-434/964-4100

Sassetti CM, Boyd DH & Rubin EJ (2003) Genes required for mycobacterial growth defined by high density mutagenesis. *Molecular microbiology* **48**: 77-84.

Selvan AT & Sankaran K (2008) Localization and characterization of prolipoprotein diacylglyceryl transferase (Lgt) critical in bacterial lipoprotein biosynthesis. *Biochimie* **90**: 1647-1655.

Serebryakova MV, Demina IA, Galyamina MA, Kondratov IG, Ladygina VG & Govorun VM (2011) The acylation state of surface lipoproteins of mollicute *Acholeplasma laidlawii*. *J Biol Chem* **286**: 22769-22776.

Shruthi H, Anand P, Murugan V & Sankaran K (2010) Twin arginine translocase pathway and fast-folding lipoprotein biosynthesis in E. coli: interesting implications and applications. *Molecular bioSystems* **6**: 999-1007.

Shu W, Liu J, Ji H & Lu M (2000) Core structure of the outer membrane lipoprotein from *Escherichia coli* at 1.9 A resolution. *J Mol Biol* **299**: 1101-1112.

Siboo IR, Chaffin DO, Rubens CE & Sullam PM (2008) Characterization of the accessory Sec system of Staphylococcus aureus. *Journal of bacteriology* **190**: 6188-6196.

Stoll H, Dengjel J, Nerz C & Gotz F (2005) *Staphylococcus aureus* deficient in lipidation of prelipoproteins is attenuated in growth and immune activation. *Infect Immun* **73**: 2411-2423.

Storf S, Pfeiffer F, Dilks K, Chen ZQ, Imam S & Pohlschroder M (2010) Mutational and bioinformatic analysis of haloarchaeal lipobox-containing proteins. *Archaea* **2010**.

Strom MS & Lory S (1987) Mapping of export signals of Pseudomonas aeruginosa pilin with alkaline phosphatase fusions. *Journal of bacteriology* **169**: 3181-3188.

Sundaram S, Banerjee S & Sankaran K (2012) The first nonradioactive fluorescence assay for phosphatidylglycerol:prolipoprotein diacylglyceryl transferase that initiates bacterial lipoprotein biosynthesis. *Analytical biochemistry* **423**: 163-170.

Sutcliffe IC & Harrington DJ (2002) Pattern searches for the identification of putative lipoprotein genes in Gram-positive bacterial genomes. *Microbiology (Reading, England)* **148**: 2065-2077.

Suzuki H, Nishimura Y, Yasuda S, Nishimura A, Yamada M & Hirota Y (1978) Murein-lipoprotein of *Escherichia coli*: a protein involved in the stabilization of bacterial cell envelope. . *Mol Gen Genet* **167**: 1-9.

Takeda K, Miyatake H, Yokota N, Matsuyama S, Tokuda H & Miki K (2003) Crystal structures of bacterial lipoprotein localization factors, LolA and LolB. *EMBO J* **22**: 3199-3209.

For Peer Review

ScholarOne Support 1-434/964-4100

Taniguchi N, Matsuyama S & Tokuda H (2005) Mechanisms underlying energy-independent transfer of lipoproteins from LolA to LolB, which have similar unclosed {beta}-barrel structures. *The Journal of biological chemistry* **280**: 34481-34488.

Thompson BJ, Widdick DA, Hicks MG, Chandra G, Sutcliffe IC, Palmer T & Hutchings MI (2010) Investigating lipoprotein biogenesis and function in the model Gram-positive bacterium *Streptomyces coelicolor*. *Mol Microbiol* **77**: 943-957.

Tjalsma H, Zanen G, Venema G, Bron S & van Dijl JM (1999) The potential active site of the lipoprotein-specific (type II) signal peptidase of *Bacillus subtilis. J Biol Chem* **274**: 28191-28197.

Tokuda H, Matsuyama S & Tanaka-Masuda K (2007) Structure, function and transport of lipoproteins in *Escherichia coli*. *The Periplasm,*(Ehrmann M, ed.) p.^pp. 67-79. ASM Press, Washington, D.C.

Tokuda H, Sander P, Lee BL, Okuda S, Grau T, Tschumi A, Brülle JK, Kurokawa K & Nakayama H (2014) Bacterial lipoproteins: biogenesis, virulence/pathogenicity and trafficking. *Bacterial membranes: structural and molecular biology,*(Remaut H & Fronzes R, eds.), p.^pp. Caister Academic Press.

Tokunaga M, Tokunaga H & Wu HC (1982) Post-translational modification and processing of *Escherichia coli* prolipoprotein *in vitro*. *Proc Natl Acad Sci U S A* **79**: 2255-2259.

Tokunaga M, Loranger JM & WU HC (1983) Isolation and characterization of an *Escherichia coli* overproducing prolipoprotein signal peptidase. *The Journal of biological chemistry* **258**: 12102-12105.

Tokunaga M, Loranger JM & Wu HC (1984) Prolipoprotein modification and processing enzymes in *Escherichia coli*. *J Biol Chem* **259**: 3825-3830.

Tomlinson G, Chimalapati S, Pollard T*, et al.* (2014) TLR-Mediated Inflammatory Responses to Streptococcus pneumoniae Are Highly Dependent on Surface Expression of Bacterial Lipoproteins. *J Immunol*.

Tschantz WR, Paetzel M, Cao G, Suciu D, Inouye M & Dalbey RE (1995) Characterization of a soluble, catalytically active form of Escherichia coli leader peptidase: requirement of detergent or phospholipid for optimal activity. *Biochemistry* **34**: 3935-3941.

Tschumi A, Grau T, Albrecht D, Rezwan M, Antelmann H & Sander P (2012) Functional analyses of mycobacterial lipoprotein diacylglyceryl transferase and comparative secretome analysis of a mycobacterial lgt mutant. *Journal of bacteriology* **194**: 3938-3949.

Tschumi A, Nai C, Auchli Y, Hunziker P, Gehrig P, Keller P, Grau T & Sander P (2009) Identification of apolipoprotein N-acyltransferase (Lnt) in Mycobacteria. *J Biol Chem* **284**: 27146-27156.

For Peer Review

ScholarOne Support 1-434/964-4100

Typas A, Banzhaf M, van den Berg van Saparoea B, *et al.* (2010) Regulation of peptidoglycan synthesis by outer-membrane proteins. *Cell* **143**: 1097-1109.

Urban S (2009) Making the cut: central roles of intramembrane proteolysis in pathogenic microorganisms. *Nature reviews* **7**: 411-423.

van Ulsen P, van Alphen L, ten Hove J, Fransen F, van der Ley P & Tommassen J (2003) A Neisserial autotransporter NalP modulating the processing of other autotransporters. *Molecular microbiology* **50**: 1017-1030.

Vidal-Ingigliardi D, Lewenza S & Buddelmeijer N (2007) Identification of essential residues in apolipoprotein N-acyltransferase, a member of the CN hydrolase family. *J Bacteriol* **189**: 4456-4464.

Villanueva L, Damste JS & Schouten S (2014) A re-evaluation of the archaeal membrane lipid biosynthetic pathway. *Nature reviews* **12**: 438-448.

Wang P, Shim E, Cravatt B, Jacobsen R, Schoeniger J, Kim AC, Paetzel M & Dalbey RE (2008) Escherichia coli signal peptide peptidase A is a serine-lysine protease with a lysine recruited to the nonconserved amino-terminal domain in the S49 protease family. *Biochemistry* **47**: 6361-6369.

Ward A, Reyes CL, Yu J, Roth CB & Chang G (2007) Flexibility in the ABC transporter MsbA: Alternating access with a twist. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 19005-19010.

Webb CT, Selkrig J, Perry AJ, Noinaj N, Buchanan SK & Lithgow T (2012) Dynamic association of BAM complex modules includes surface exposure of the lipoprotein BamC. *Journal of molecular biology* **422**: 545-555.

Wehmeier S, Varghese AS, Gurcha SS, Tissot B, Panico M, Hitchen P, Morris HR, Besra GS, Dell A & Smith MC (2009) Glycosylation of the phosphate binding protein, PstS, in Streptomyces coelicolor by a pathway that resembles protein O-mannosylation in eukaryotes. *Molecular microbiology* **71**: 421-433.

White DC & Frerman FE (1968) Fatty acid composition of the complex lipids of Staphylococcus aureus during the formation of the membrane-bound electron transport system. *Journal of bacteriology* **95**: 2198-2209.

Wichgers Schreur PJ, Rebel JM, Smits MA, van Putten JP & Smith HE (2011) Lgt processing is an essential step in Streptococcus suis lipoprotein mediated innate immune activation. *PLoS One* **6**: e22299.

Widdick DA, Hicks MG, Thompson BJ, Tschumi A, Chandra G, Sutcliffe IC, Brulle JK, Sander P, Palmer T & Hutchings MI (2011) Dissecting the complete lipoprotein biogenesis pathway in *Streptomyces scabies*. *Mol Microbiol* **80**: 1395-1412.

For Peer Review

ScholarOne Support 1-434/964-4100

Williams AH & Raetz CR (2007) Structural basis for the acyl chain selectivity and mechanism of UDP-N-acetylglucosamine acyltransferase. *Proc Natl Acad Sci USA* **104**: 13543-13550.

Williams MG, Fortson M, Dykstra CC, Jensen P & Kushner SR (1989) Identification and genetic mapping of the structural gene for an essential *Escherichia coli* membrane protein. *J Bacteriol* **171**: 565-568.

Wu HC (1996) Biosynthesis of lipoproteins. *Escherichia coli and Salmonella Cellular and Molecular Biology,* Vol. 1 (Neidhardt FC, Curtiss III R, Ingraham JL, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M & Umbarger HE, eds.), p.^pp. 1005-1014. ASM Press, Washington D.C. USA.

Wu HC, Hou C, Lin JJ & Yem DW (1977) Biochemical characterization of a mutant lipoprotein of Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America* **74**: 1388-1392.

Wu HC, Lai JS, Hayashi S & Giam CZ (1982) Biogenesis of membrane lipoproteins in Escherichia coli. *Biophysical journal* **37**: 307-315.

Wu HC, Tokunaga M, Tokunaga H, Hayashi S & Giam CZ (1983) Posttranslational modification and processing of membrane lipoproteins in bacteria. *Journal of cellular biochemistry* **22**: 161-171.

Wyckoff TJ, Lin S, Cotter RJ, Dotson GD & Raetz CR (1998) Hydrocarbon rulers in UDP-N-acetylglucosamine acyltransferases. *J Biol Chem* **273**: 32369-32372.

Xiao Y & Wall D (2014) Genetic redundancy, proximity, and functionality of lspA, the target of antibiotic TA, in the Myxococcus xanthus producer strain. *Journal of bacteriology* **196**: 1174-1183.

Xiao Y, Gerth K, Müller R & Wall D (2012) Myxobacterium-produced antiobiotic TA (Myxovirescin) inhibits type II signal peptidase. *Antimicrob Agents and Chemother* **56**: 2014-2021.

Yakushi T, Tajima T, Matsuyama S-I & Tokuda H (1997) Lethality of the covalent linkage between mislocalized major outer membrane lipoprotein and the peptidoglycan of *Escherichia coli*. *J Bacteriol* **179**: 2857-2862.

Yamagata H, Daishima K & Mizushima S (1983) Cloning and expression of a gene coding for the prolipoprotein signal peptidase of Escherichia coli. *FEBS letters* **158**: 301-304.

Yem DW & Wu HC (1977) Genetic characterization of an Escherichia coli mutant altered in the structure of murein lipoprotein. *Journal of bacteriology* **131**: 759-764.

Yem DW & Wu HC (1978) Physiological characterization of an Escherichia coli mutant altered in the structure of murein lipoprotein. *Journal of bacteriology* **133**: 1419-1426.

For Peer Review

ScholarOne Support 1-434/964-4100

Zhang WY & Wu HC (1992) Alterations of the carboxyl-terminal amino acid residues of *Escherichia coli* lipoprotein affect the formation of murein-bound lipoprotein. *The Journal of biological chemistry* **257**: 19560-19564.

Zhou Z, White KA, Polissi A, Georgopoulos C & Raetz CR (1998) Function of Escherichia coli MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis. *The Journal of biological chemistry* **273**: 12466-12475.

Zuckert WR (2014) Secretion of Bacterial Lipoproteins: Through the Cytoplasmic Membrane, the Periplasm and Beyond. *Biochim Biophys Acta*.

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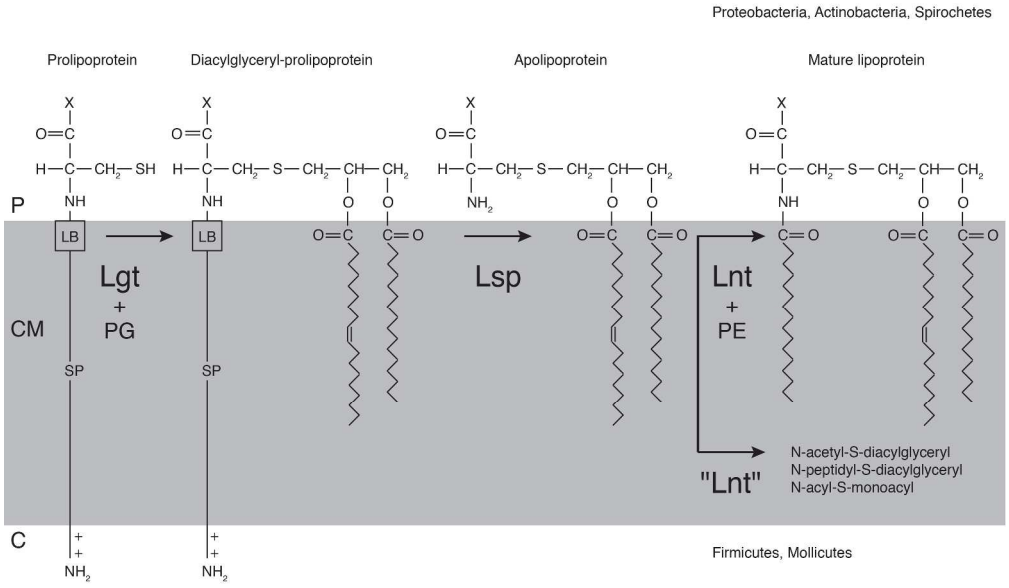
Table 1. Essentiality of genes encoding lipoprotein modifying enzymes in diderm bacteria.

	lgt	*lsp*	*lnt*
E. coli	*lgt* (*ump*A) (Gan *et al.*, 1995)	*lsp* (Tokunaga *et al.*, 1983, Yamagata *et al.*, 1983)	*lnt* (*cut*E) (Gupta & Wu, 1991, Rogers *et al.*, 1991, Gupta *et al.*, 1993, Robichon *et al.*, 2005)
S. enterica	*lgt* (Gan *et al.*, 1993)		*lnt* (Gupta *et al.*, 1993)
M. xantus		*lsp*1, *lsp*2, *lsp*3, *lsp*4 (Xiao & Wall, 2014)	
M. smegmatis	*lgt* (MSMEG_3222) (Tschumi *et al.*, 2012)		*lnt* (MSMEG_3860) (*ppm*2) (Tschumi *et al.*, 2009)
M. tuberculosis	*lgt* (Rv1614) (Sassetti *et al.*, 2003, Tschumi *et al.*, 2012)	*lsp* (Rv1539) (Sander *et al.*, 2004, Rampini *et al.*, 2008)	*ppm*1 (Rv2051c) (Tschumi *et al.*, 2009)
M. bovis			*lnt* (BCG_2070c) (Brülle *et al.*, 2013)
C. glutamicum			*lnt* (NCgl1424) (Mohiman *et al.*, 2012)
S. coelicolor	*lgt*1 (SCO2034), *lgt*2 (SCO7822) (Thompson *et al.*, 2010)	*lsp* (SCO2074) (Thompson *et al.*, 2010)	*lnt*1 (SCO1014), *lnt*2 (SCO1336) (Widdick *et al.*, 2011, Cordova-Davalos *et al.*, 2014)
S. scabies	*lgt* (SCAB68531) (Widdick *et al.*, 2011)	*lsp* (SCAB68121) (Widdick *et al.*, 2011)	*lnt*1 (SCAB83111), *lnt*2 (SCAB76621) (Widdick *et al.*, 2011)
S. lividans		*lsp* (Gullon *et al.*, 2013)	

Essential genes are shown in red, non-essential genes are shown in blue. Table includes only published findings. See text for further details.

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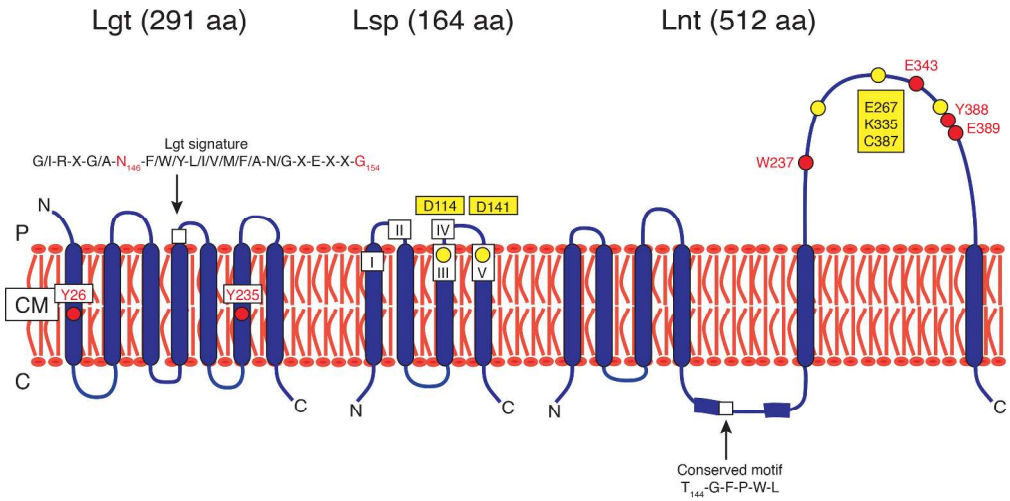
FIGURE 1



290x191mm (300 x 300 DPI)

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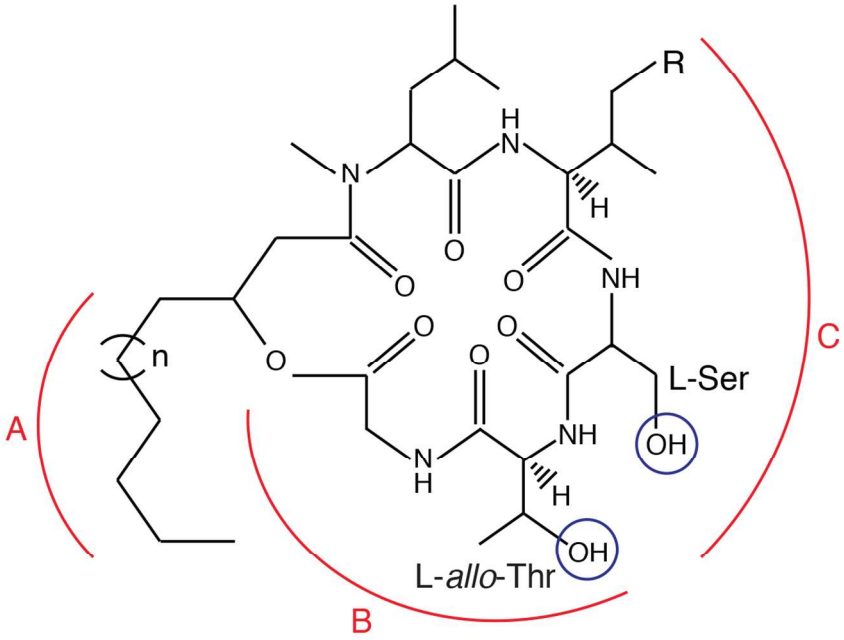
FIGURE 2



293x167mm (300 x 300 DPI)

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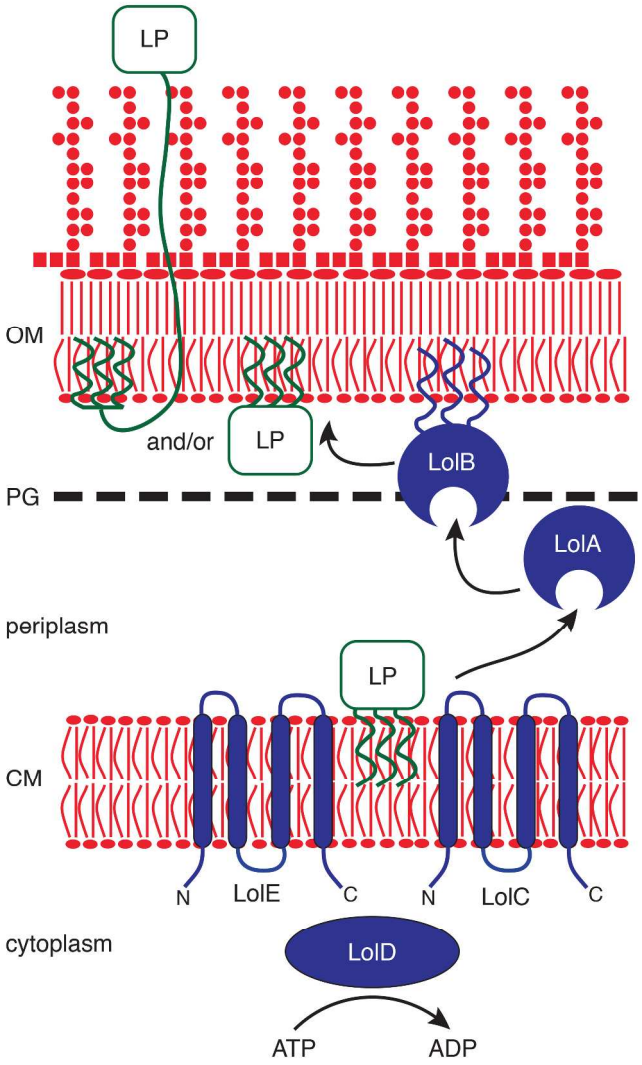
FIGURE 3



128x98mm (300 x 300 DPI)

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FIGURE 4



180x320mm (300 x 300 DPI)