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Review

Secretion of Bacterial Lipoproteins: Through the Cytoplasmic Membrane, the Periplasm and Beyond ☆

Wolfram R. Zückert *

University of Kansas School of Medicine, Department of Microbiology, Molecular Genetics and Immunology, Kansas City, KS, United States

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ABSTRACT

Bacterial lipoproteins are peripherally anchored membrane proteins that play a variety of roles in bacterial physiology and virulence in monoderm (single membrane-enveloped, e.g., gram-positive) and diderm (double membrane-enveloped, e.g., gram-negative) bacteria. After export of prolipoproteins through the cytoplasmic membrane, which occurs predominantly but not exclusively via the general secretory or Sec pathway, the proteins are lipid-modified at the cytoplasmic membrane in a multistep process that involves sequential modification of a cysteine residue and cleavage of the signal peptide by the signal II peptidase Lsp. In both monoderms and diderms, signal peptide processing is preceded by acylation with a diacylglycerol through preprolipoprotein diacylglycerol transferase (Lgt). In diderms but also some monoderms, lipoproteins are further modified with a third acyl chain through lipoprotein *N*-acyl transferase (Lnt). Fully modified lipoproteins that are destined to be anchored in the inner leaflet of the outer membrane (OM) are selected, transported and inserted by the Lol (lipoprotein outer membrane localization) pathway machinery, which consists of the inner-membrane (IM) ABC transporter-like LolCDE complex, the periplasmic LolA chaperone and the OM LolB lipoprotein receptor. Retention of lipoproteins in the cytoplasmic membrane results from Lol avoidance signals that were originally described as the "+2 rule". Surface localization of lipoproteins in diderms is rare in most bacteria, with the exception of several spirochetal species. Type 2 (T2SS) and type 5 (T5SS) secretion systems are involved in secretion of specific surface lipoproteins of γ-proteobacteria. In the model spirochete *Borrelia burgdorferi*, surface lipoprotein secretion does not follow established sorting rules, but remains dependent on N-terminal peptide sequences. Secretion through the outer membrane requires maintenance of lipoproteins in a translocation-competent unfolded conformation, likely through interaction with a periplasmic holding chaperone, which delivers the proteins to an outer membrane lipoprotein flippase. This article is part of a Special Issue entitled: Protein Trafficking & Secretion.

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1. Introduction

Since the description of the first prokaryotic lipoprotein in the cell envelope of *Escherichia coli* by Braun and colleagues over four decades ago [1,2], this class of peripherally anchored membrane proteins has been increasingly recognized to play important roles in basic bacterial physiology such envelope stability, cell division, sporulation, conjugation, nutrient acquisition, signal transduction, transport and protein folding, but also in bacterial pathogenic mechanisms such as adhesion, colonization, invasion and persistence through immune evasion. Proper localization of these lipoproteins is of utmost importance for their function and hinges on an efficient lipoprotein modification and transport pathway and accurate lipoprotein sorting machinery. This review will focus on *cis* and *trans* factors that help compartmentalize the bacterial lipoproteome according to individual lipoprotein function within the bacterial envelope. Mechanistically, localization is relatively simple in monoderm (or single membrane-enveloped) bacteria such as the firmicutes, where only export through the cytoplasmic membrane and acylation is required for proper and stable localization on the bacterial surface. In diderm (or double membrane-enveloped) bacteria such as the γ-proteobacteria, acylated and therefore partially hydrophobic proteins destined for the outer membrane face a formidable hurdle in the aqueous periplasmic space, which is overcome with the help of a lipoprotein-specific chaperoned pathway. Surface localization of lipoproteins utilizes specific outer membrane porins and is rare in most eubacterial species, with the exception of some spirochetes, where it appears to be the norm.

2. Lipoprotein domain structure-function

All lipoproteins are translated in the cytoplasm as preprolipoprotein precursors with several structural and functional domains that can be recognized at the primary, secondary and tertiary structural level

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* Mail Stop 3029, 3025 Wahl Hall West, 3901 Rainbow Boulevard, Kansas City, KS 66160. Tel.: +1 913 588 7061; fax: +1 913 588 7295.
E-mail address: wzueckert@kumc.edu.

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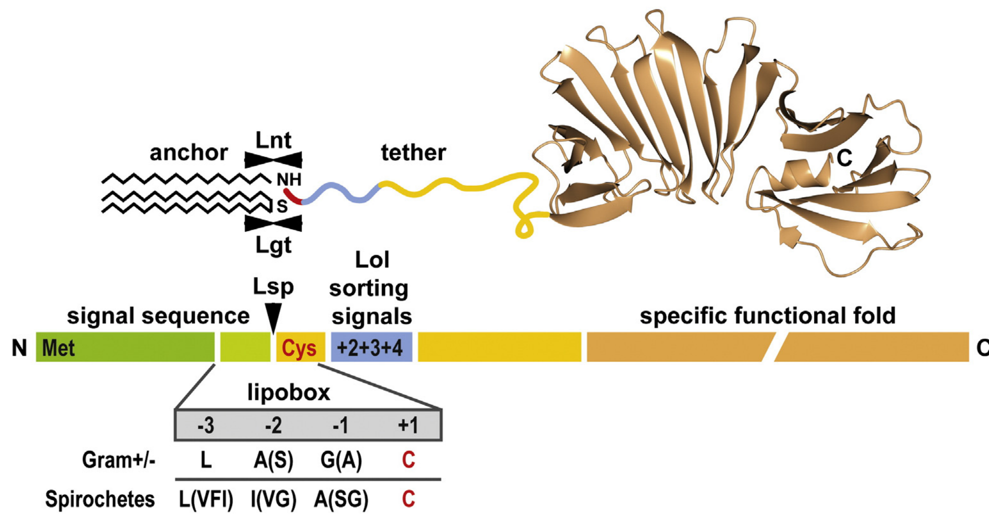

Fig. 1. Lipoprotein domain structure. Lipoproteins are translated in the bacterial cytoplasm as preprolipoprotein precursors. An N-terminal signal peptide (in green) targets the protein for export of the protein through the cytoplasmic membrane. Diacylation at a conserved Cys residue (in red) is mediated by Lgt via the Cys sulfhydryl group. Lsp recognizes the lipobox residues and cleaves the signal peptide. This makes the N-terminal amine group available for Lnt-mediated modification with a third acyl chain, which completes the membrane anchor. The N-terminus of the mature lipoprotein contains sorting signals (in blue) recognized by the Lol pathway in diderm bacteria. Generally, the N-terminus is also intrinsically disordered, providing a flexible "tether" (in yellow) for proper positioning and function in the bacterial envelope. The C-terminal portion of the polypeptide (in orange) assumes a fold specific for the protein's function. The structure of *B. burgdorferi* OspA (PDB Accession # 1osp) is used for illustrative purposes. See text for details.

(Fig. 1). The most N-terminal domain is on average 20 amino acids in length and forms the signal (or leader) peptide [3]. In contrast to the signal peptides of secreted soluble proteins, the C-termini of lipoprotein signal peptides contain a four-amino-acid motif called the "lipobox" [4], which forms the molecular basis for several *in silico* algorithms that are used to predict lipoprotein genes in bacterial genomes [5,6]. Maybe not surprisingly, the originally canonical lipobox sequence has degenerated as more and more lipoproteins were identified, not in a small part powered by the exponential increase in sequenced bacterial genomes and the associated proteomic analyses. So are lipobox sequences a bit more degenerate in spirochetes than in gram-positive and -negative bacteria [6] (Fig. 1). Today, the only conserved residue within the motif remains a cysteine that will become the target of acylation and the new N-terminal amino acid of the mature lipoprotein, i.e., the residue at position +1. *In silico* predictions as well as structural information on a number of lipoproteins indicate that the residues following the +1 cysteine lack any predicted or observed secondary structure. This indicates that this second domain is intrinsically disordered and forms a "tether" that links the lipid anchor to the third domain, which folds into a tertiary and sometimes quaternary structure and executes the protein-specific functions (Fig. 1). Tether lengths can vary quite dramatically from lipoprotein to lipoprotein [7]. As extreme examples, the crystal structure of Braun's lipoprotein Lpp does not reveal any significant N-terminal disorder [8], while the *Borrelia burgdorferi* surface lipoprotein BBA66 has stretch of about 170 disordered N-terminal amino acids [9]. As discussed in more detail below, the tether peptides contain lipoprotein sorting information, but they are also thought – by means of their extension – to properly position lipoproteins for optimal function within the at times complex bacterial envelope architecture [10].

3. Lipoprotein modification

Preprolipoproteins generally cross the cytoplasmic membrane as unfolded proteins via the general secretory (Sec) pathway with the help of YidC [11], but can also cross in an already folded conformation via the twin-arginine translocation (TAT) pathway [12–16] or with the help of a SecA variant [17] (see Fig. 2 for an overall model of lipoprotein secretion; *also see other chapters in this issue*). Upon this translocation event, preprolipoproteins are targeted for a two- or three-step posttranslational modification by three essential enzymes that are associated with the cytoplasmic membrane. Multiple paralogs can be found in some bacteria [18], but their functions remain to be determined.

3.1. Lipoprotein diacylglyceryl transferase Lgt

Preprolipoprotein diacylglyceryl transferase (Lgt) serves as the committing enzyme and was shown *in vitro* to catalyze the attachment of a negatively charged diacylglycerol moiety, particularly phosphatidylglycerol, to the thiol group of the conserved +1 position cysteine via a thioester bond [19]. The same experiments also showed that recognition of the signal peptide alone is sufficient for the reaction to occur. Structure-function information on Lgt is limited. Based on *in silico* predictions and C-terminal tagging with localization-sensitive reporters PhoA (periplasmic activity only) and GFP (cytoplasmic fluorescence only), *Escherichia coli* Lgt is a multipass integral protein with five transmembrane (TM) helices and a C terminus that is exposed to the cytoplasm [20]. Mutation of a conserved histidine residue (His^{103}) within the predicted TM helix 3 inactivated the enzyme. Mutations of two additional residues, Tyr^{235} in the predicted TM helix 4 and His^{196} in a predicted large cytoplasmic loop, also affected activity [21,22]. Pending the complete confirmation of Lgt topology, this suggests that the first step of lipoprotein modification may occur within the cytoplasmic membrane or at its interface with the cytoplasm. Somewhat puzzling has been the finding that recombinant Lgt retained full specific activity in an aqueous environment [23].

3.2. Signal peptidase II Lsp

In the next posttranslational modification step, the (partially) acylated prolipoproteins are cleaved by lipoprotein signal peptidase (Lsp), also known as signal peptidase II (SPase II) to distinguish it from signal peptidase I that processes non-lipidated exported proteins. Cleavage of the prolipoprotein occurs N terminally of the +1 position cysteine residue, i.e., within the lipobox [24]. Early topology experiments using fusions of Lsp fragments to PhoA and LacZ (cytoplasmic activity only) reporters produced a model with 4 TM helices that has remained unchallenged in later experiments and predictions [20,25]. In the *Bacillus subtilis* Lsp, five conserved residues within conserved domains (Asn^{99}, Asp^{102}, Asn^{126}, Ala^{128} and Asp^{129}) were shown to be functionally important, with residues Asp^{102} and Asp^{129} most likely involved in catalysis [26]. The positioning of the latter two residues at both ends of an extracytoplasmic loop

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