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The double life of a bacterial lipoprotein

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Abstract

It has been known for many years that the small lipoprotein Lpp, which is the most abundant protein in *E. coli*, exists in two forms. The "bound" form of the protein is tethered to the outer membrane (OM) by its N-terminal lipid moiety and covalently attached to the cell wall by its C-terminal lysine residue. The exact location of the "free" form, however, has never been determined. In this issue of *Molecular Microbiology*, Cowles *et al.* demonstrate that the free form of Lpp is an integral OM protein whose C-terminus is exposed on the cell surface. The new study provides the first example of a lipoprotein that has a dual localization and adds to a growing body of evidence that lipoproteins can span the OM despite the lack of an obvious transmembrane segment. Furthermore, the new results raise intriguing questions about the assembly of both lipoproteins and other types of OM proteins.

Bacterial lipoproteins are a subset of membrane proteins that are covalently modified with diacylglycerol at their N-terminal cysteine residue. Lipoproteins all contain a conserved motif called a lipobox [L(A/S)(G/A)|C] that surrounds the signal peptide cleavage site. After lipoproteins are translocated across the inner membrane (IM) through the Sec machinery, the lipobox is recognized by an acyl transferase on the periplasmic side of the membrane. Although a few lipoproteins remain in the IM, most of them are transported to the outer membrane (OM) by a dedicated sorting machinery known as the Lol system (Narita *et al.*, 2004). Following their transit through the periplasm, lipoproteins are anchored to the OM by their lipid moieties. Bioinformatic and expression studies have shown that *E. coli* produces more than 90 lipoproteins (Brokx *et al*., 2004). Lipoproteins have been shown to play a role in stabilization of the outer membrane, substrate transport, outer membrane protein assembly and cell signaling, but many lipoproteins have no known function. Unlike IM proteins (which are built around α-helical membrane spanning domains) and typical integral OM proteins (which contain a characteristic β barrel), lipoproteins are structurally diverse and share only the N-terminal lipid moiety in common.

The analysis of lipoprotein topology has generated major surprises. In general lipoproteins are relatively hydrophilic proteins that lack obvious membrane spanning segments. Furthermore, many lipoproteins function in the periplasm or interact with either periplasmic proteins or the peptidoglycan and often remain intact when cells are treated with exogenous proteases. For all of these reasons lipoproteins are usually depicted as globular, peripheral membrane proteins that are tethered to the OM by their lipid tail. A growing body of evidence, however, has challenged this simple view and has indicated that at least some *E. coli* lipoproteins adopt an unexpected transmembrane orientation in which the N-terminus presumably remains anchored to the inner leaflet of the OM while one or more distal segments are exposed on the cell surface. These proteins have been implicated in the

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secretion of large polymers including DNA, proteins and polysaccharides (Manning *et al*., 1980; Drummelsmith and Whitfield, 2000; Robinson *et al*., 2006). Although their topology is unknown, several other lipoproteins appear to be directly involved in other protein secretion phenomena in pathogenic *E. coli* and other Gram-negative pathogens (Ramer *et al*., 1996; Mundy *et al*., 2003; Bose and Taylor, 2005). The *Bordetalla pertussis* protein SphB1, a member of the autotransporter superfamily, is another lipoprotein that likely traverses the OM (Coutte *et al*., 2003). *Borrelia burgdorferi* produces a multitude of surface-exposed lipoproteins that may be unique. Available evidence suggests that these proteins are secreted in their entirety and that their N-terminal lipids are transferred from the inner leaflet of the OM to the outer leaflet (Brandt *et al*., 1990; Schulze *et al*., 2010).

A new study corroborates the conclusion that lipoproteins can span the OM and adds a new twist to the story of lipoprotein topology (Cowles *et al*., 2011). In this study, the authors addressed a long-standing mystery about a very small (58 residue) lipoprotein called Lpp, which is the most abundant protein in *E. coli*. Lpp exists in two distinct forms. About of a third of the protein is found in a "bound" form in which the C-terminal lysine residue is covalently attached to the peptidoglycan by transpeptidases (Magnet *et al*., 2007). The observation that deletion of the C-terminal residue severely impairs OM integrity (Zhang and Wu, 1992) suggests that the bound form plays an important role in maintaining the architecture of the cell envelope. Curiously, about two-thirds of the protein is found in a "free" form, whose function is unknown. Although Lpp has been studied for 40 years, the intracellular location of the free form has never been determined. Partly through the use of a broadly applicable biotinylation approach, the authors now show that the last few residues of Lpp are surface exposed and that the bulk of the protein is embedded in the OM. While IM proteins that have a dual topology have been identified (Rapp *et al*., 2006), bacterial pore-forming toxins (e.g., α-hemolysin) are known to exist in both membrane-embedded and soluble forms, and eukaryotic proteins have been shown to be localized to multiple intracellular compartments (Shaffer *et al*., 2005; Colombo *et al*., 2005), Lpp is the first example of a lipoprotein that has a dual localization.

Given that lipoproteins do not have conventional membrane spanning segments, how can they stably integrate into the OM and adopt both membrane-inserted and soluble conformations? Studies involving electron microscopy and x-ray crystallography have shown that CsgG, which is required for the secretion of the subunits of curli fibers, and Wza, which secretes capsular polysaccharides, form large oligomeric complexes with a central pore that presumably functions as a transport channel (Dong *et al*., 2006; Robinson *et al*, 2006). In the case of Wza, eight protomers each contribute a single C-terminal amphipathic α helix to a unique α-helical barrel structure that spans the OM. One might imagine that after the Wza monomers (and perhaps the monomers of other channel-forming lipoproteins) are targeted to the OM, multiple subunits interact and form anα-helical barrel in the periplasm. Because the external surface of the α-helical barrel is hydrophobic, it readily partitions into the OM. Interestingly, both the free form of Lpp and a soluble recombinant form that lacks the N-terminal cysteine and C-terminal lysine residues have been isolated as trimers (Choi *et al*., 1986; Shu *et al*., 2000; Cowles *et al*., 2011). Crystallographic analysis of the recombinant protein revealed a parallel three-stranded coiled-coil structure. This α-helical structure is much too hydrophilic to insert into a lipid bilayer and therefore might correspond to the bound form of Lpp. To account for the biogenesis of the free form of Lpp, one might imagine that multiple trimers interact and, like the α helices of Wza, form a lipophilic superhelical assembly. Indeed a model for such a structure that contains six subunits has been proposed (Inouye, 1974). Alternatively, a single Lpp trimer might undergo a conformational change that exposes internal hydrophobic residues. The finding that the secondary structure of cholesterol-dependent cytolysins such as perfringolysin O changes dramatically during the transition from the soluble to the membrane-bound state shows that

significant structural rearrangements are possible (Shatursky *et al*., 1999). In any case, it is possible that both forms of Lpp are derived from a common pool of precursors and that those trimers that are not retained in the periplasm by covalent attachment to the peptidoglycan are inserted into the OM by default (Fig. 1), but this model needs to be tested experimentally.

In light of the discovery that the free form of Lpp is an integral membrane protein, it will now be of great interest to determine whether other lipoproteins that have traditionally been depicted as periplasmic proteins also span the OM. Indeed by using the biotinylation method they have developed, Cowles and co-workers have obtained evidence that at least a fraction of a variety of different *E. coli* lipoproteins is surface exposed (Cowles and Silhavy, Abstract K-1306, 2010 American Society for Microbiology meeting). Cell surface exposure of the *Haemophilus influenzae* lipoprotein Pal might explain the otherwise puzzling observation that human antibodies raised against it are bacteriocidal (Murphy *et al*., 1986). In principle, membrane-embedded lipoproteins might serve several different functions. As the architecture of Wza illustrates, membrane integration of lipoproteins facilitates the creation of novel types of membrane channels. Like β barrel proteins, lipoproteins lack hydrophobic membrane spanning segments that might cause their retention in the IM, but unlike β barrel proteins, which form rather rigid channels, they oligomerize to form channels that have the potential to be highly dynamic. In addition, like mammalian proteins that have a dual localization, the membrane-embedded and periplasmic forms of lipoproteins might have independent functions (Shaffer *et al*., 2005). Finally, it is conceivable that the two forms of a lipoprotein are interchangeable, and that one form serves as a reservoir for the other. It is not known whether the free form of Lpp has any of these functions, but because the Lpp sequence is highly conserved it seems unlikely that the membrane localization of the free form is just an epiphenomenon.

Perhaps the biggest challenge will be to determine the mechanism by which lipoproteins are integrated into the OM. Because lipoproteins are targeted to the OM by their lipid tails and, at least in the case of *E. coli* Wza and Lpp, integrate into the OM without an accompanying translocation of large polypeptide segments, it is tempting to speculate that their membrane insertion is spontaneous. History has proven, however, that membrane protein insertion reactions that were once thought to occur spontaneously often turn out to be catalyzed by cellular factors. The insertion of β barrel proteins into the OM, for example, is now known to be catalyzed by a heterooligomer called the Bam complex (Voulhoux *et al*., 2003; Wu *et al*., 2005; Hagan *et al*., 2010). Recent work has shown that depletion of the *Borrelia* BamA ortholog leads to a decrease in the level of surface-exposed lipoproteins (Lenhart and Akins, 2010). *Borrelia* lipoproteins may be unusual, though, in that they are essentially secreted proteins rather than integral membrane proteins. Regardless of whether lipoprotein biogenesis is catalyzed by the Bam complex or some other factor, the finding that lipoproteins can be exposed on the cell surface adds to a growing suspicion that the OM is the site of a variety of unconventional polypeptide transport reactions that may not involve obvious channels. It seems very unlikely that either β barrel proteins or lipoproteins could be integrated into the OM by passing through the pore formed by BamA, which is itself a β barrel protein. Perhaps BamA and the four lipoprotein subunits of the Bam complex catalyze the membrane integration of β barrel proteins (and lipoproteins as well) by forming an oligomeric channel that is gated laterally. If such a channel could also open to the extracellular space, this hypothesis might explain the biogenesis of bacterial autotransporters, proteins whose large extracellular domain is secreted by an enigmatic mechanism that appears to involve the Bam complex (Ieva and Bernstein, 2009). Alternatively, the Bam complex might facilitate the membrane integration of β barrel proteins (and possibly surface-exposed lipoproteins) by an entirely novel mechanism that

does not involve the formation of a channel. In any case, the mysteries surrounding all of these phenomena suggest that there is still a lot to learn about the biogenesis of OM proteins.

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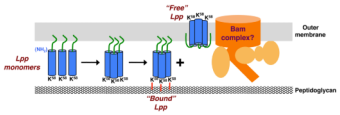

Fig. 1.

Model of the biogenesis of Lpp. In *E. coli*, Lpp is found in both “free” and “bound” forms. The free form is derived from Lpp trimers that exist as a hydrophilic coiled-coil in solution and that presumably assemble after the protein is targeted to the OM. The free form is integrated into the OM and its C terminus is exposed on the cell surface. Membrane integration may involve the activity of the Bam complex or another factor. Lpp trimers might also form higher order oligomers in the OM. The bound form of Lpp is covalently attached to the peptidoglycan by the ε-amino group of its C-terminal lysine residue (K^{58}), but its oligomeric state is unknown. One possibility is that the free and bound forms are derived from a common pool or trimers. In this model, trimers that are not linked to the peptidoglycan are integrated into the OM by default.