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Translocation of lipoproteins to the surface of gram negative bacteria

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The surface of many Gram-negative bacteria contains lipidated protein molecules referred to as surface lipoproteins or SLPs. SLPs play critical roles in host immune evasion, nutrient acquisition and regulation of bacterial stress response, and have been extensively studied as vaccine antigens. The aim of this review is to summarize the recent studies that have investigated the biosynthetic and translocation pathways used by different bacterial species to deliver SLPs to the surface. We will specifically focus on Slam, a novel outer membrane protein first discovered in pathogenic *Neisseria* sp., that is involved in translocation of SLPs across the outer membrane.

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Introduction

Bacteria often utilize surface exposed protein molecules to interact with their environment [1]. A subset of these surface molecules are peripherally attached soluble proteins referred to as lipoproteins [2]. Lipoproteins contain an N-terminal cysteine residue that is post-translationally modified with three lipid groups, which serve as membrane anchors. The lipoproteins present on the surface are referred to as surface lipoproteins or SLPs and have been found to be involved in key cellular pathways for nutrient acquisition, cellular adhesion and stress response. Several SLPs have been identified to date in Gram-negative bacteria and have been reviewed previously [3,4]. Gram-negative SLPs, such as fHbp and NHBA in *Neisseria meningitidis* [5,6], TbpB in *Haemophilus parasuis* [7], OspA in *Borrelia burdorferi* and Tp0751 in *Treponema pallidum* to list a few, have been investigated as vaccine antigens. The cellular pathway for the translocation of lipoproteins across the inner membrane and through the periplasm to the inner leaflet of the outer membrane is well characterized, whereas the translocation systems used by SLPs to move across the outer membrane has only been identified for a handful of SLPs [4]. In this review, we aim to summarize the recent work conducted on the biogenesis of SLPs. We will specifically focus on strategies used by SLPs to cross the outer membrane, with an emphasis on Slams, a recently identified family of outer membrane proteins [8••].

Role of surface lipoproteins (SLPs) in Gram-negative bacteria

Surface lipoproteins or SLPs are anchored to the outer membrane via three fatty acyl chains that are post-translationally attached to their N-termini [4]. The first reported SLP was TraT, a protein of the F sex factor in *Escherichia coli* [9]. Several other SLPs were identified soon after in other Gram-negative bacteria such as *Klebsiella* [10], *Neisseria* [11] and *Spirochetes* [12,13]. There has been an increase in the reports of SLPs with distinct structures and surface topologies from different bacterial species in recent years [14]. Most of the recent SLPs were first identified by functional proteomics screens searching for vaccine antigens. Upon identification of a putative SLP, a number of experimental assays can be performed to confirm their lipidation and surface localization [15]. SLPs can be further characterized by biochemical and genetic studies to elucidate their biological function [3]. As SLPs contain soluble protein domains, they are also amenable to biophysical and structural characterization.

From information available to date, it appears that different bacteria contain a different number of SLPs. On one extreme is *E. coli* that contains three SLPs (RscF, Lpp and BamC) that are only partially surface displayed. At the other extreme are *Sphirochetes* such as *Borrelia burgdorferi* (causative agent of Lyme disease) where 86 of the 125 predicted lipoproteins are surface displayed [12,16]. Most bacterial species are probably in the middle of the two extremes, such as *N. meningitidis*, a host-restricted human pathogen that causes septicaemia and meningitis. *N. meningitidis* contains 8 SLPs that include fully exposed mammalian transferrin binding SLP TbpB, autotransporter protease NalP that also contains a transmembrane domain, and AniA, a partially surface exposed nitrite reductase [17]. Another interesting family of SLPs are found in *Bacteroides sp.*, a family of mutualistic bacteria found in mammalian guts that breakdown complex carbohydrates [4]. Most of the Bacteroides SLPs recognize specific carbohydrates and/or cleave polysaccharide

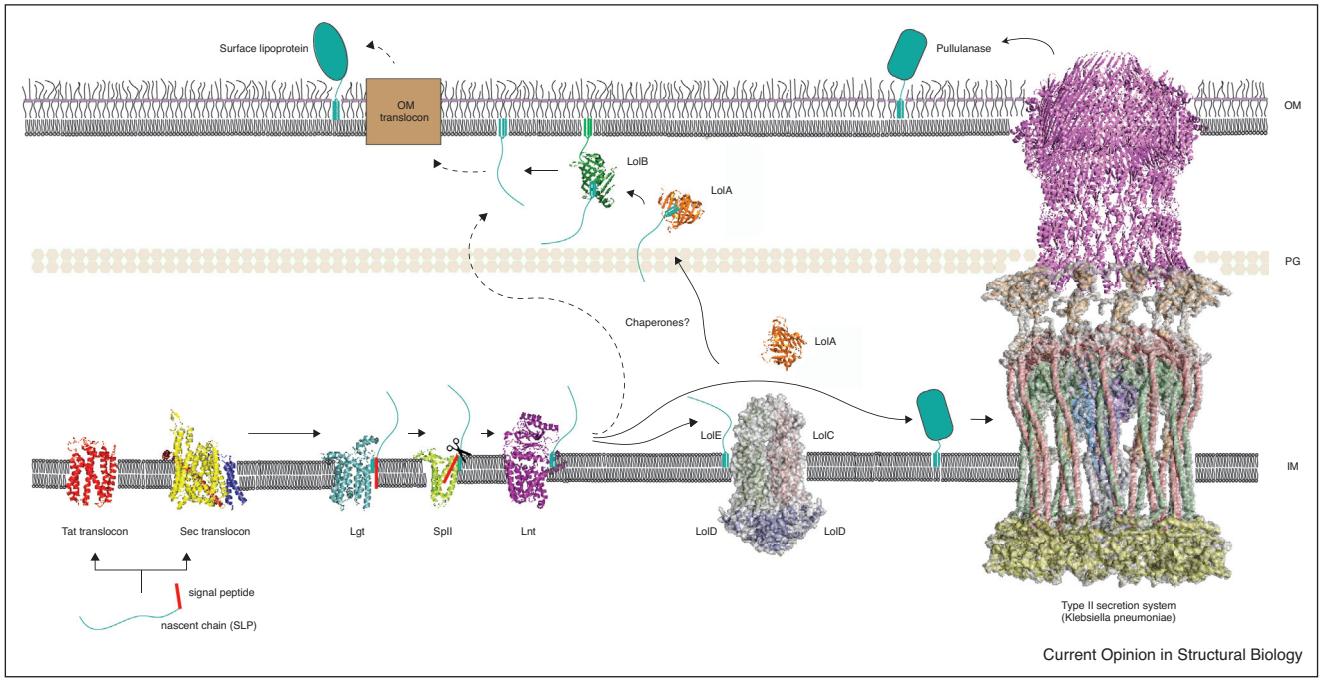
bonds. Recent study by Glenwright *et al.* provided the first structural snapshot of large complexes formed by *Bacteroides* SLPs with a membrane-spanning TonB-dependent receptor [18•].

Taken together, these studies have established that SLPs are present in most, if not all Gram-negative bacteria. But this brings forward a more central question as to why Gram-negative bacteria have surface lipoproteins? We can think of several reasons. First, given that SLPs often contain large and flexible anchor peptides on their N-terminus, they can extend much further from the cell surface than OM proteins and capture their interaction partners or substrates. Second, because of their peripheral attachment, SLPs often have greater mobility [19] than OM proteins, which cluster in supramolecular complexes within the outer membrane [20]. Third, unlike OM proteins that are not specifically targeted for degradation, SLPs can be removed by a single proteolytic cut like seen in the case with the SLP LbpB and protease NalP [21]. Fourth, SLPs often contain catalytic subunits (such as proteases, hydrolases) that can detoxify substrates on the surface before their subsequent uptake. Finally, unlike secreted hemophores and siderophores, surface-bound SLPs involved in iron acquisition cannot be hijacked by surrounding bacteria (social cheats). As more SLPs are discovered and studied, we will be able to discern the myriad roles they play in bacterial cell biology.

Transport of SLPs to the outer membrane

The translocation pathway used by lipoproteins is shown in Figure 1. Lipoprotein precursors are synthesized in the cytoplasm with an N-terminal signal peptide. The last four residues at the C terminus of the signal peptide comprise the conserved region referred to as the lipobox motif ([LVI] [ASTVI] [GAS] C). The cysteine residue at the last position of the lipobox eventually becomes the first residue of the mature lipoprotein [22]. Most prepro-lipoproteins contain the Sec-specific secretion signal peptide, but preprolipoproteins with a twin-arginine translocation (Tat) specific secretion system have also been reported [23]. Upon translocation across the inner membrane, preprolipoproteins undergo a series of post-translational modifications by enzymes, diacylglyceryl

Figure 1



Transport of lipoproteins to the outer membrane. The key proteins and protein complexes implicated in transport of lipoproteins to the outer membrane are shown. Lipoproteins (green) contain an N-terminal signal peptide (red) that is recognized by the Sec (SecY — yellow, SecE — dark red and secG — blue, PDB ID: 5AWW) or the Tat translocon (TatC — TV red, PDB ID: 4B4A). Once inside the periplasm, the lipoproteins are post-translationally modified by three enzymes: Lgt (cyan, PDB ID: 5AZB), SpII (light green, PDB ID: 5DIR) and Lnt (purple, PDB ID: 5AZB). Most lipoproteins are then transported across the periplasm by the five member Lol system composed of LolA (orange, PDB ID: 1IWL), LolB (green, PDB ID: 1IWM) and LolCDE (LolC — salmon, LolD dimer — dark blue and LolE — olive green). The model of LolCDE was obtained from the structure of LptBFG (PDB ID: 5UDF)and is shown in surface representation. Lipoprotein pullulanase in *Klebsiella pneumoniae* bypasses the Lol system by using the Type II secretion system, shown as a trans-envelope complex. PulD secretin model (purple) was obtained from structure of GspD from *Vibrio cholerae* (PDB ID: 5WQ8). The inner membrane Pul complex model (PulC — gold, PulE –dark yellow, PulF — grey, Pul GHIJK — light blue, PulL — magenta and PulM — green) is shown in surface representation. The structure of type IVa pilus machinery from *Myxococcus xanthus* (PDB ID: 3JC9) was used as a model for the inner membrane Pul complex.