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Review

# Making a membrane on the other side of the wall☆

Kerrie L. May, Thomas J. Silhavy *

*Department of Molecular Biology, Princeton University, Lewis Thomas Laboratory, Washington Road, Princeton, NJ 08544, USA*

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ABSTRACT

The outer membrane (OM) of Gram-negative bacteria is positioned at the frontline of the cell's interaction with its environment and provides a barrier against influx of external toxins while still allowing import of nutrients and excretion of wastes. It is a remarkable asymmetric bilayer with a glycolipid surface-exposed leaflet and a glycerophospholipid inner leaflet. Lipid asymmetry is key to OM barrier function and several different systems actively maintain this lipid asymmetry. All OM components are synthesized in the cytosol before being secreted and assembled into a contiguous membrane on the other side of the cell wall. Work in recent years has uncovered the pathways that transport and assemble most of the OM components. However, our understanding of how phospholipids are delivered to the OM remains notably limited. Here we will review seminal works in phospholipid transfer performed some 40 years ago and place more recent insights in their context. This article is part of a Special Issue entitled: Bacterial Lipids edited by Russell E. Bishop.



## 1. Architecture of the Gram negative envelope

Bacteria are typically classified into two groups based on their characteristic Gram-staining phenotype [1]. Gram-positive organisms have a single cytoplasmic membrane surrounded by a thick peptidoglycan layer that acts as an exoskeleton and is responsible for the positive Gram-staining status. In contrast, Gram-negative bacteria have a thin peptidoglycan layer and are instead fortified by an additional, outer membrane (OM). The two, inner and outer, membranes are separated by the periplasmic space, an aqueous and highly viscous compartment that accommodates the peptidoglycan cell wall [1] (Fig. 1).

The double membrane envelope structure enables distinct compartmentalization of discrete sets of proteins, lipids and lipoproteins that enable these organelles to perform highly specialized functions. The OM, which will be the focus of this review, is an essential organelle that functions as a formidable and selective permeability barrier [2]. In addition to protecting the cell against harsh environments and toxic compounds (e.g. bile salts and antibiotics), the OM enables efficient uptake of nutrients and efflux of waste products and toxic compounds. The OM is a biologically distinct asymmetric lipid bilayer, with lipopolysaccharide (LPS) in the outer leaflet and glycerophospholipids (commonly referred to as phospholipids, PLs) on the inner leaflet [3,4] (Fig. 1). This bilayer structure serves as a platform from which OM proteins perform their important cellular functions. Furthermore, it is the frontline from which these bacteria engage with and manipulate their environment or their host.

## 2. OM composition and biogenesis

### 2.1. OM proteins and lipoproteins

There are two major types of proteins in the OM: transmembrane β-barrel proteins (termed OMPs) and bilayer-anchored lipoproteins (Fig. 1). OMPs are synthesized in the cytoplasm and targeted to the Sec translocase via an N-terminal signal sequence and then translocated into the periplasm [5,6]. Because OMPs are hydrophobic transmembrane proteins, chaperones are required to guide them across the aqueous periplasm [7]. OMPs adopt a β-barrel structure by a cylindrical folding of *anti*-parallel β-sheets [5,6]. In recent years, it has become clear that the five member Bam complex at the OM is responsible for OMP β-barrel folding and membrane insertion [5,6,8,9] (Fig. 2).

Lipoproteins are similarly directed to the periplasm via an N-terminal signal sequence but possess an adjoining lipobox motif at the C-terminal end of the signal sequence that enables coordinated signal sequence cleavage and triacylation of a conserved cysteine residue within the lipobox [10]. As a result, all lipoproteins newly emerging from the Sec translocon are initially tethered to the IM: first via the transmembrane signal peptide, and then as triacylated mature lipoproteins [11,12]. Some lipoproteins remain in the IM but most are designated for transport to the OM via the Lol pathway [10] (Fig. 2). The identity of +2 and +3 residues adjacent to the acylated cysteine determine localization [13,14]. Recent reviews have described the current state of

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* Corresponding author.
*E-mail address:* tsilhavy@princeton.edu (T.J. Silhavy).

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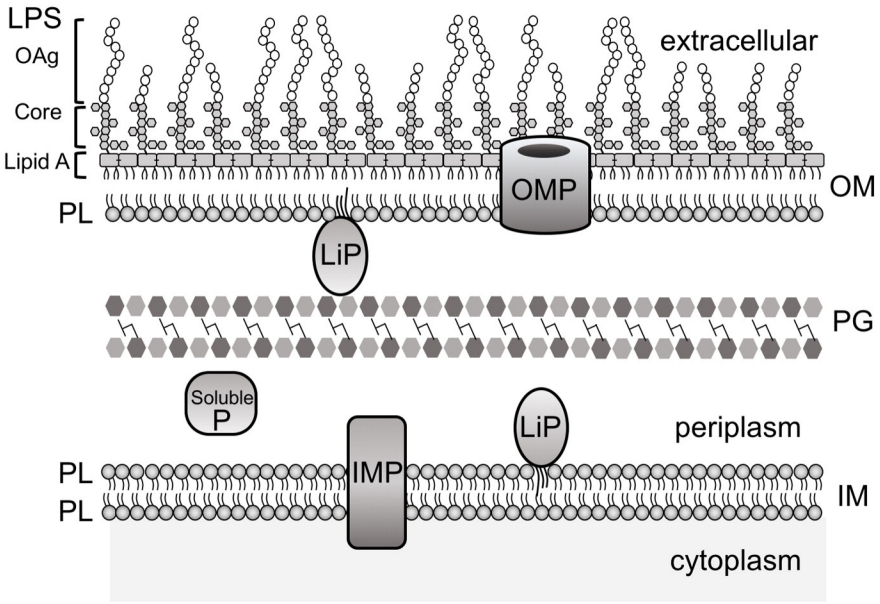


**Fig. 1.** – Architecture of the Gram-negative envelope. The outer membrane (OM) and inner membrane (IM) are separated by an aqueous periplasm that contains the peptidoglycan (PG) cell wall. The asymmetric distribution of lipids in the OM is shown with lipopolysaccharide (LPS) in the outer leaflet and glycerophospholipid (PL) in the inner-leaflet. LPS consists of a tripartite structure of lipid A, a core oligosaccharide component, and an O-antigen (OAg) polysaccharide chain that extends into the extracellular milieu. The three major membrane proteins are shown and include integral membrane proteins (IMP), lipoproteins (LiP) and outer membrane proteins (OMP). Soluble proteins (SolubleP) also exist both in the cytoplasm and periplasm.

knowledge about the Lol pathway and lipoprotein assembly into the OM, including lipoproteins that are surface exposed [15,16].

### 2.2. The OM bilayer

#### 2.2.1. Lipopolysaccharide (LPS)

In terms of the lipid bilayer, the outer leaflet of the OM consists almost exclusively of the glycolipid LPS [3]. The tripartite structure of LPS consists of lipid A component, a core oligosaccharide component, and an O-antigen (OAg) polysaccharide chain that extends into the extracellular milieu [17] (Fig. 1). The Oag component is not synthesized in the model organism *E. coli* K-12 [18], but it plays an important role in protecting commensal strains in their host environment and is also a crucial virulence determinant in pathogenic strains [19]. Lipid A is a diglucosamine phosphate-based lipid (Fig. 3A) that is a notoriously potent activator of the innate immune system and is commonly referred to as 'endotoxin' [20]. LPS is anchored to the OM bilayer via the lipid A moiety [17]. The core oligosaccharide can be divided into a conserved inner core that generally consists of two units of 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) and three units of L-glycero-D-manno-heptose (Hep), all linked together in discreet alpha-glycosidic linkages and subject to regulated covalent modifications with specific phosphoryl and glycosyl moieties, and a variable outer core to which the Oag polysaccharide is attached [17,20]. Phosphoryl substituents of the LPS core

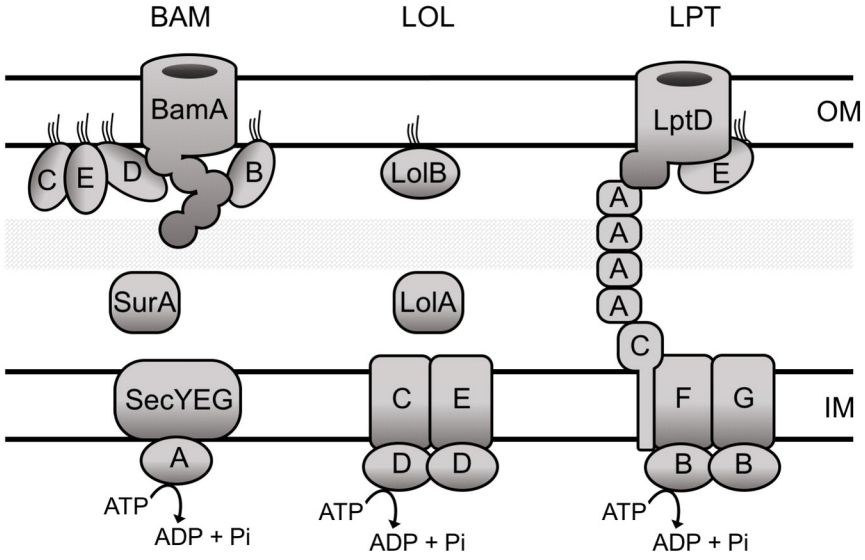


**Fig. 2.** – OM biogenesis machinery. Depicted are the components of three essential cellular machines required for outer membrane (OM) biogenesis. Chaperones, e.g. SurA and Skp deliver β-barrel outer membrane proteins to the β-barrel assembly machine (BAM) for assembly into the OM. While OM lipoproteins are delivered via the Lol machine. The LipoPolysaccharide Transport (LPT) Pathway transports LPS from the inner membrane (IM) to the cell surface via a hydrophobic conduit formed by the oligomerization of LptA. The molecular details of these machines are discussed in Section 2.

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