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Lipid Transport Across Bacterial Membranes

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*These authors contributed equally to this article, and their order was determined by a game of roulette

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Keywords

transport, lipids, bacteria, membranes, lipopolysaccharide, cell envelope

Abstract

The movement of lipids within and between membranes in bacteria is essential for building and maintaining the bacterial cell envelope. Moving lipids to their final destination is often energetically unfavorable and does not readily occur spontaneously. Bacteria have evolved several protein-mediated transport systems that bind specific lipid substrates and catalyze the transport of lipids across membranes and from one membrane to another. Specific protein flippases act in translocating lipids across the plasma membrane, overcoming the obstacle of moving relatively large and chemically diverse lipids between leaflets of the bilayer. Active transporters found in double-membraned bacteria have evolved sophisticated mechanisms to traffic lipids between the two membranes, including assembling to form large, multiprotein complexes that resemble bridges, shuttles, and tunnels, shielding lipids from the hydrophilic environment of the periplasm during transport. In this review, we explore our current understanding of the mechanisms thought to drive bacterial lipid transport.

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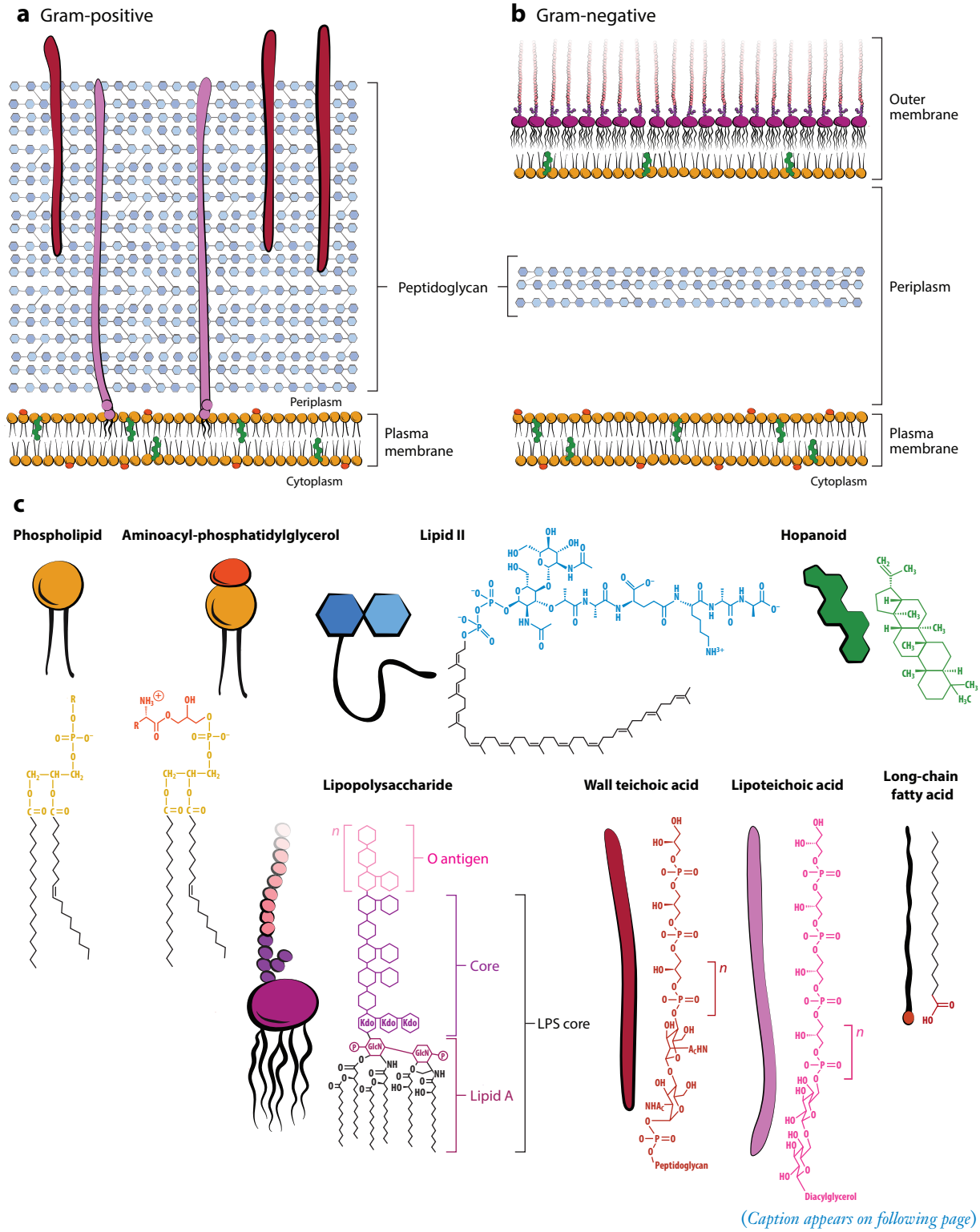
1. INTRODUCTION

Transporting lipids from one location to another is a fundamental challenge faced by all cells, whether between membrane-bound organelles in eukaryotes or to generate and maintain the plasma membrane and outer membrane (OM) in prokaryotes. In bacteria, the transport of lipids and other hydrophobic molecules is important for several cellular processes including importing nutrients, exporting toxic compounds such as detergents or antibiotics, and building their cell envelopes, which form the first line of defense for the cell against the environment. While eukaryotic cells depend on vesicular trafficking as well as protein-mediated mechanisms for lipid transport, bacteria lack vesicular transport pathways, and consequently, most bacterial lipids are individually trafficked to their destination via protein-based systems, several of which are discussed in this review.

In single-membraned bacteria (gram-positive), cell wall components and associated outer leaflet lipids need to be flipped across the membrane (**Figure 1*a***). Double-membraned bacteria (mostly gram-negative) face the additional challenge of moving lipids across the periplasmic space between the plasma membrane [also called the inner membrane] and the OM (**Figure 1*b***).



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

Architectures of the bacterial cell envelope. (*a*) Gram-positive and (*b*) gram-negative cell envelopes are depicted, highlighting the lipid composition of each. (*c*) Cartoon representation of lipids, corresponding to panels *a* and *b*, with examples of chemical structures of each lipid shown. Abbreviations: GlcN, glucosamine; Kdo, 3-deoxy-d-manno-octulosonic acid; LPS, lipopolysaccharide.

Mycobacteria are also double-membraned, but their cell envelope is quite distinct and evolutionarily divergent from that of gram-negative bacteria. Several recent reviews cover various aspects of the mycomembrane and mycobacterial lipid transport (Dulberger et al. 2020, Ma et al. 2020, Touchette & Seeliger 2017), which are not discussed in this review.

ATP in the cytoplasm and the proton gradient across the plasma membrane can provide the driving force for energy-dependent lipid transport across the lipid bilayer. However, the periplasm is devoid of ATP and other energy sources. Consequently, the transport of lipids between the plasma membrane and OM, as well as the remodeling of the OM, must somehow be coupled to energy in the plasma membrane, or the transport must be energetically neutral. This review focuses on the transport of individual lipid substrates across gram-positive and gram-negative bacterial cell envelopes.

2. KEY CONCEPTS

2.1. Architectures of Gram-Positive and Gram-Negative Bacterial Cell Envelopes

The cell envelope of gram-positive bacteria consists of a single lipid bilayer, the plasma membrane, which is surrounded by a peptidoglycan cell wall (**Figure 1*a***). In gram-negative bacteria, an additional lipid bilayer called the OM surrounds the cell (Salton & Kim 1996, Vollmer & Seligman 2010) (**Figure 1*b***). Much of our understanding of the cell envelope comes from studies in *Bacillus subtilis* and *Staphylococcus aureus* for gram-positive bacteria and in the model bacterium *Escherichia coli* for gram-negative bacteria.

The plasma membrane of *B. subtilis* is composed of ∼30% neutral glucolipids and ∼70% phospholipids (Willdigg & Helmann 2021), of which the majority are phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), with a minor component of cardiolipin (Clejan et al. 1986, den Kamp et al. 1969, Nickels et al. 2017, Uttlová et al. 2016) (**Figure 1*c***). The *E. coli* plasma membrane is primarily composed of PE (∼75%) and PG (∼20%) and, to a lesser extent, cardiolipin (∼5%) (Silhavy et al. 2010, Sohlenkamp & Geiger 2016). However, *E. coli* is not representative of the potential diversity in bacterial membrane lipids; for example, *E. coli* lacks phosphatidylcholine, which is often found in other Proteobacteria, such as *Pseudomonas aeruginosa* (Mahato & Sen 1997, Ourisson et al. 1987, Sohlenkamp & Geiger 2016). Both gram-positive and gram-negative bacterial plasma membranes often contain aminoacylated PG (**Figure 1*c***), such as lysyl-PG in *S. aureus* (Rajagopal & Walker 2017) or alanyl-PG in *P. aeruginosa* (Klein et al. 2009), which help to protect against antimicrobial peptides. Aside from phospholipids, bacterial membranes can also house a range of other lipid classes, such as glycolipids and hopanoids (Belin et al. 2018) (**Figure 1*c***). Present in both gram-negative and gram-positive bacteria, hopanoids are structurally diverse pentacyclic triterpenoid lipids thought to play a role in enhancing bacterial membrane integrity and impermeability by condensing the membrane (Belin et al. 2018, Mahato & Sen 1997, Malott et al. 2014, Ourisson et al. 1987, Sáenz et al. 2015, Sohlenkamp & Geiger 2016, Welander et al. 2009). The plasma membrane is asymmetric in both gram-positive and gram-negative bacteria (Barsukov et al. 1976, Bogdanov et al. 2020, Rothman & Kennedy 1977). For example, in *S. aureus*, aminoacylated phospholipids are preferentially localized in the outer leaflet in patient isolates, where they are important for resistance to antimicrobial peptides (Jones et al. 2008). In contrast, in

E. coli, ~75% of the PE in the plasma membrane is found in the inner leaflet (Bogdanov et al. 2020). The mechanisms by which much of this asymmetry is established and maintained remain unclear.

In gram-positive bacteria, a thick peptidoglycan cell wall, built with the essential precursor Lipid II (**Figure 1*c***), surrounds the plasma membrane (Breukink & de Kruijff 2006) (**Figure 1*a***). The peptidoglycan cell wall contains repeating units of the disaccharide N-acetyl glucosamine-N-acetyl muramic acid, with cross-linked peptide linkages. The peptidoglycan is arranged in multiple layers, creating a 30–100-nm-thick mesh-like network (Silhavy et al. 2010, Vollmer et al. 2008). The cell wall stabilizes the plasma membrane against turgor pressure, and its rigidity determines cell shape (Silhavy et al. 2010). Long anionic polymers called teichoic acids are distributed throughout the peptidoglycan and play a role in maintaining the gram-positive cell envelope structure, as well as aiding in antibiotic resistance and pathogenesis. Wall teichoic acids (WTAs) are covalently attached to the peptidoglycan, while lipoteichoic acids (LTAs) are anchored to the headgroups of membrane lipids. Together, WTAs and LTAs constitute 60% of the mass making up the gram-positive cell wall (Brown et al. 2013, Neuhaus & Baddiley 2003) (**Figure 1*c***). The overall peptidoglycan structure of the cell wall is similar in gram-negative bacteria. However, the cell wall of gram-negative bacteria is much thinner (2–7 nm) and lacks WTA and LTA polymers (Matias et al. 2003, Vollmer et al. 2008).

The OM of gram-negative bacteria is asymmetric: its inner leaflet is composed of phospholipids, while the outer leaflet is chiefly made up of the complex glycolipid lipopolysaccharide (LPS) (Kamio & Nikaido 1976) (**Figure 1*c***). LPS is the key determinant of the OM's low permeability to hydrophobic molecules such as detergents and antibiotics (Nikaido 1994, 2003; Whitfield & Trent 2014), which otherwise readily diffuse across conventional phospholipid bilayers. The asymmetry of the OM is a critical feature, with the bulky LPS facing the environment and forming a barrier that protects the cell from harmful compounds such as host innate immune components (Caboni et al. 2015, Clay et al. 2008, Goebel et al. 2008, Murray et al. 2006).

2.2. Active Transport: Energy-Dependent Movement Across and Between Membranes

Several energetic barriers complicate the transport of lipids, both within and between membranes. To move lipids between the leaflets of a bilayer, the polar headgroup must be moved through the hydrophobic, lipidic phase of the membrane, which is energetically unfavorable. Due to this barrier, the flipping of lipids between leaflets generally does not occur spontaneously. Moving lipids between membranes is also energetically unfavorable, as lipids must be extracted from the hydrophobic environment of the membrane and moved through the aqueous space that separates the two membranes. Thus, for the movement of lipids between leaflets, as well as between membranes, proteins are generally required as catalysts to reduce these energetic barriers. Finally, many lipids must be moved against a concentration gradient, and in order to do this efficiently, proteins coupled to a source of energy are often required.

Numerous families of membrane transporters have been described, employing different mechanisms to move lipids and other molecules across membranes. One of the most common mechanisms for the movement of substrate through a membrane transporter is called alternating access. In this mechanism, the transporter alternates between an inward-facing state, in which the substrate-binding pocket faces the cytoplasm, and an outward-facing state, in which the substrate-binding pocket faces the extracellular or periplasmic space (Jardetzky 1966) (**Figure 2*a*,*b***). By alternating between these two states but never forming a completely open channel, the transporter can facilitate the directional movement of molecules across a membrane while minimizing the leakage of other molecules through the substrate translocation pathway. Beyond alternating

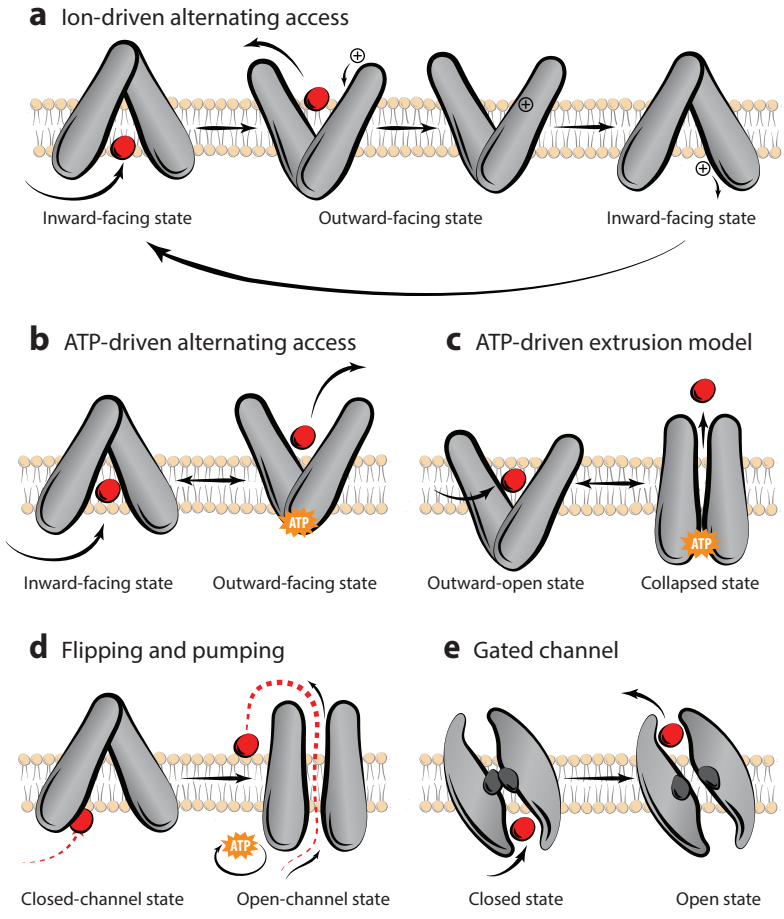


Figure 2

Common mechanisms of active transport. Transporters are depicted in gray, and red balls indicate a generic substrate in all parts of the figure. (*a*) Ion-driven alternating access mechanism. After substrate binding to the inward-facing state of the protein, conformational changes result in a transition to an outward-facing state. In the outward-facing state, the substrate is released and a counterion, indicated here by a cation, binds to the transporter and results in a conformational change back to an inward-facing state, resetting the transporter. (*b*) ATP-driven alternating access mechanism. After substrate binding to the inward-facing state of the protein, ATP binds and is hydrolyzed. ATP binding drives conformational change to the outward-facing state, transferring the substrate to the outer leaflet of the membrane or to the extracellular or periplasmic space (Holland & Blight 1999). (*c*) ATP-driven extrusion model. After substrate binding to the outward-open state of the transporter, ATP binding triggers a conformational change in the system that results in a collapsed state, in which the substrate-binding pocket closes and the substrate is extruded out of the transporter. (*d*) Putative flipping and pumping mechanism. After substrate binding to the closed-channel state of the protein, ATP binding and hydrolysis trigger conformational changes that allow the processive movement of a longer substrate (*red dashed line*) through an open channel in the transporter. Multiple cycles of ATP hydrolysis are thought to be necessary for the complete translocation of the substrate. (*e*) Gated-channel mechanism. In the resting state, the protein contains cytoplasm-facing and extracellular-facing cavities, separated by a gate. After substrate binding to the cytoplasm-facing cavity, the gate opens, allowing passage of the substrate to the extracellular-facing cavity and translocation into the outer leaflet.

access, another mechanism used by some lipid transporters is the extrusion mechanism (**Figure 2*c***), in which the transmembrane domains of the transporter collapse while the substrate is bound in a central pocket, expelling it out of the protein (discussed in more detail for individual transporters in Sections 4.3 and 5.1).

Active transporters use cellular energy to transport substrates against their concentration gradient (Forrest et al. 2011) and are classified into two main types: primary active transporters and secondary active transporters. Primary active transporters use chemical energy, such as that from ATP, to energize transport. ABC (ATP-binding cassette) transporters are common primary active transporters that use ATP as an energy source (Locher 2016). ABC transporters are composed of two core domains: a transmembrane domain, which is embedded in the plasma membrane, forming a path for the substrate transport, and cytoplasmic nucleotide-binding domains, which drive conformational changes in the transmembrane domains upon ATP binding and hydrolysis (Holland & Blight 1999) (**Figure 2*b–d***). Secondary active transporters, or coupled active transporters, rely on proton or ion gradients as their energy source (Forrest et al. 2011) (**Figure 2*a***). Secondary active lipid transporters usually consist of two transmembrane domains. The movement of protons or ions down their electrochemical gradient and through the transporter provides energy to drive conformational changes in the transmembrane domains, which in turn facilitates substrate transport.

3. FLIPPING LIPIDS ACROSS THE PLASMA MEMBRANE

Dedicated transporters mediate the flipping of many lipids across the plasma membrane, some of which are then utilized in cell wall and surface polysaccharide biosynthesis. This section discusses some of the best-studied examples of systems that are involved in the transport of lipid-linked precursors for peptidoglycan (Lipid II by MurJ), WTAs and LTAs (TarGH and LtaA, respectively), and the membrane lipid aminoacyl-PG (MprF).

3.1. MurJ

MurJ flips Lipid II across the plasma membrane and is essential for cell wall biosynthesis.

3.1.1. Function. Lipid II is an essential precursor used in the synthesis of the peptidoglycan cell wall. The headgroup contains the disaccharide-pentapeptide unit that is the basic building block of peptidoglycan, which is attached to a membrane anchor called undecaprenyl pyrophosphate (Und-PP, also called undecaprenyl diphosphate). The headgroup must be flipped across the plasma membrane from its site of biosynthesis inside the cell to the outer leaflet, where glycosyltransferases couple the disaccharide-pentapeptide unit to a growing polysaccharide chain (Chugunov et al. 2013). MurJ is the flippase that translocates Lipid II across the cell membrane in both gram-positive and gram-negative bacteria. Much of our understanding of the function of MurJ comes from studies in *E. coli*. Studies depleting or inactivating MurJ (Inoue et al. 2008, Ruiz 2008, Sham et al. 2014) revealed the protein to be essential for peptidoglycan biogenesis.

3.1.2. Components.

This system consists of a single component:

- MurJ: flippase, plasma membrane

3.1.3. Proposed transport mechanism. MurJ has been proposed to flip Lipid II across the plasma membrane, potentially using an alternating access mechanism driven by ion gradients (Kuk et al. 2017, 2019; Kumar et al. 2019; Rubino et al. 2020) (**Figure 2*a***). A single copy of MurJ contains two homologous domains that form a pseudodimer, with a cationic cavity at the pseudodimer

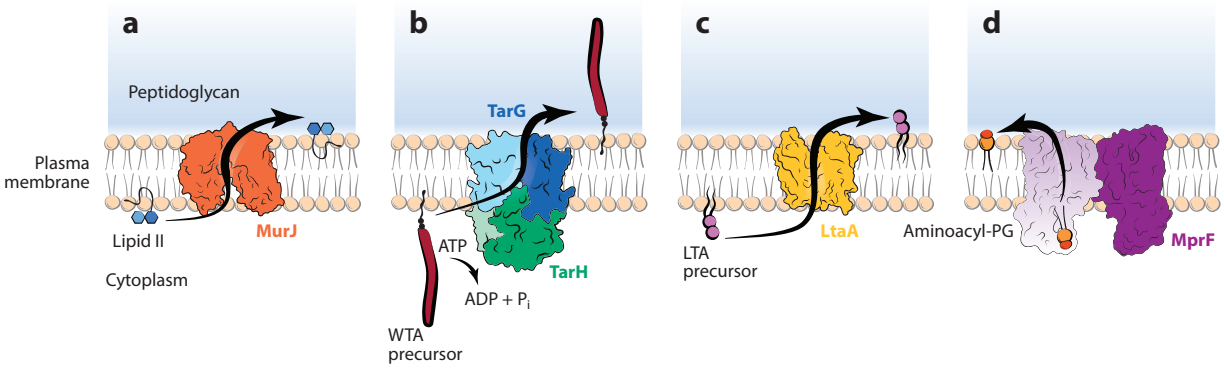


Figure 3

Transporters responsible for the translocation of substrates across the plasma membrane. (*a*) Flippase MurJ in an inward-facing state [PDB (Protein Data Bank) 6NC7], responsible for flipping Lipid II from the inner to the outer leaflet of the plasma membrane. (*b*) Flippase TarGH in an inward-facing state (PDB 6JBH), responsible for flipping the nascent Und-PP-WTA moiety from the inner to the outer leaflet of the plasma membrane. (*c*) Flippase LtaA in an outward-facing state (PDB 6S7V), responsible for flipping the LTA precursor, gentiobiosyl-diacylglycerol, from the inner to the outer leaflet of the plasma membrane. (*d*) Flippase MprF (PDB 7DUW), responsible for flipping aminoacyl-PG from the inner to the outer leaflet of the plasma membrane. Abbreviations: aminoacyl-PG, aminoacyl-phosphatidylglycerol; LTA, lipoteichoic acid; Und-PP, undecaprenyl pyrophosphate; WTA, wall teichoic acid.

interface that is complementary to the negatively charged Lipid II (Kuk et al. 2017, 2019; Ruiz 2008; Zheng et al. 2018) (**Figure 3*a***). Starting with MurJ in an inward-facing state (Kuk et al. 2017, Zheng et al. 2018), Lipid II can enter the central cavity from the inner leaflet. After substrate binding, conformational changes in MurJ lead to a transient closed state, followed by an outward-facing state (Kuk et al. 2019). In the outward-facing state, Lipid II is released for incorporation into the cell wall. Membrane potential has been shown to be important for returning MurJ to the inward-facing state, though a counterion has not been identified (Rubino et al. 2018). As many homologous transporters couple either H^+ or Na^+, one of these ions could play a role in transport (Kuk et al. 2019).

3.2. TarGH

TarGH flips a WTA precursor across the plasma membrane for incorporation into the cell wall.

3.2.1. Function. WTA is a polymer of ribitol-phosphate, or glycerol-phosphate in some species, modified with *N*-acetylglucosamine (Brown et al. 2013, Endl et al. 1983) (**Figure 1*c***). WTA is synthesized at the inner leaflet of the plasma membrane of gram-positive bacteria (Ellwood 1970, Xia et al. 2010) and is linked to the lipid carrier Und-PP (Brown et al. 2013, Caffalette et al. 2020). The ABC transporter TarGH is responsible for flipping nascent Und-PP-WTA from the inner leaflet to the outer leaflet of the plasma membrane (Brown et al. 2008, Caffalette et al. 2020, Chen et al. 2020, Schirner et al. 2011).

3.2.2. Components.

The components of this system are as follows:

- TarG: transmembrane component of the ABC transporter, plasma membrane
- TarH: ATPase component, cytoplasm

3.2.3. Proposed transport mechanism. TarGH is a type V ABC transporter (Thomas et al. 2020) consisting of two copies each of TarG and TarH (**Figure 3*b***). In its resting state, a substrate

tunnel at the interface between the TarG subunits is open at the cytosolic entrance, poised for substrate recognition and binding (Chen et al. 2020). The diphosphate group of Und-PP interacts specifically with positively charged residues at the substrate tunnel entrance, initiating binding of the WTA precursor and guiding it up a vertical path through the cell membrane (Chen et al. 2020, Perez et al. 2015). Upon ATP binding, the nucleotide-binding domains move toward each other, clamping two ATP molecules at the interface. The conformational change in the nucleotide-binding domains is transmitted through the transmembrane domains, leading to an outward-facing state (Chen et al. 2020). The closed nucleotide-binding domains hydrolyze the bound ATPs, providing the energy to mediate WTA precursor movement through the transporter, after which TarGH reverts back to its resting state (Chen et al. 2020). Multiple rounds of ATP binding and hydrolysis likely drive the processive pumping of the WTA polymer until the entire substrate has been translocated across the membrane (**Figure 2*d***).

3.3. LtaA

LtaA flips a precursor across the plasma membrane for incorporation into LTA.

3.3.1. Function. LTA is a membrane glycolipid on the surface of gram-positive bacteria (Percy & Gründling 2014). The core lipid–linked disaccharide precursor of LTA, gentiobiosyl-diacylglycerol, is synthesized in the inner leaflet of the membrane and is translocated across the membrane to the outer leaflet by LtaA, where LTA biosynthesis is completed (Gründling & Schneewind 2007, Jorasch et al. 1998, Kiriukhin et al. 2001, B. Zhang et al. 2020).

3.3.2. Components.

This system consists of a single component:

- LtaA: flippase, plasma membrane

3.3.3. Proposed transport mechanism. LtaA is a pseudodimeric, proton-driven member of the major facilitator superfamily, and likely uses an alternating-access mechanism (**Figure 2*a***) to flip gentiobiosyl-diacylglycerol across the plasma membrane (B. Zhang et al. 2020) (**Figure 3*c***). Thus far, the structure of LtaA has only been trapped in a single state (B. Zhang et al. 2020), but based upon homology to other major facilitator superfamily transporters, a transport cycle can be proposed (Drew et al. 2021, B. Zhang et al. 2020). Gentiobiosyl-diacylglycerol is likely loaded into an inward-facing state of LtaA, and computational docking suggests that the gentiobiosyl headgroup binds a hydrophilic pocket near the pseudodimer interface (B. Zhang et al. 2020). Substrate binding may trigger conformational changes in LtaA, transitioning the complex to an outward-facing state. A structure of the outward-facing state reveals an amphiphilic central cavity, from which the substrate is released (B. Zhang et al. 2020). The outward flipping of substrate by LtaA is driven by the flux of protons in the opposite direction, from the environment into the cell interior.

3.4. MprF

MprF both synthesizes aminoacyl-PG in the cytoplasm and transports it across the plasma membrane.

3.4.1. Function. Aminoacyl-PG is a class of common lipids found in both leaflets of the plasma membrane. Its presence in the outer leaflet has been implicated in pathogenicity and increased resistance to cationic antimicrobial peptides (Ernst et al. 2009, Kilelee et al. 2010, Slavetinsky et al. 2017). Aminoacyl-PG is synthesized at the inner leaflet of the plasma membrane and flipped

across the membrane by the bifunctional protein multiple peptide resistance factor (MprF) (Oku et al. 2004).

3.4.2. Components.

This system consists of a single component:

- MprF: flippase, plasma membrane

3.4.3. Proposed transport mechanism. MprF is a homo-oligomeric synthase and flippase that uses aminoacyl-tRNAs (transfer RNAs) to link amino acid residues such as lysine or alanine to PG (Lennarz et al. 1966, Peschel et al. 2001). In addition to lipid synthesis, MprF flips aminoacyl-PG from the inner to the outer leaflet of the plasma membrane (**Figure 3*d***). MprF is structurally similar to other members of the major facilitator superfamily, but whether its transport mechanism is passive or active is unclear (Song et al. 2021, Wang et al. 2020). The structure of MprF shows it to be a dimer, but higher-order oligomers such as tetramers may also exist (Ernst et al. 2015, Song et al. 2021). There are two substrate-binding cavities located at the cytoplasmic and extracytoplasmic sides of the membrane in the flippase domain, connected by an ionic gate. Nascent aminoacyl-PG enters the cytoplasmic cavity, and the headgroup is bound in a polar pocket that orients it toward the outer leaflet, in preparation for flipping and translocation (Song et al. 2021). The lipid is then thought to diffuse through the ionic gate to the extracytoplasmic cavity and ultimately into the outer leaflet of the plasma membrane (Song et al. 2021) (**Figure 2*e***).

3.5. Open Questions

- What is the power source for MurJ and how does it facilitate lipid flipping? Membrane potential from the proton motive force contributes to conformational changes in MurJ. The cation dependence of similar transporters suggests that MurJ transport may also be driven by ion gradients, though it remains unclear exactly which ion may be required in flipping and how it influences MurJ conformational states.
- How is the substrate extracted from the membrane environment and released from LtaA? The outward-facing structure of LtaA reveals an optimal binding site for its substrate, but other conformational states of LtaA are still unknown. How the substrate is recognized by LtaA, extracted from the membrane, and released into the outer leaflet of the membrane also remains unknown. Snapshots of LtaA in different states will provide insights into its transport mechanism.
- How does TarGH mediate the flipping of such a large substrate across the plasma membrane? While most other lipid transporters discussed here use alternating-access mechanisms, TarGH is thought to undergo multiple ATPase cycles to pump long polysaccharides across the membrane. How an ABC transporter might function as a linear motor to translocate a polymer across a membrane remains to be discovered. Future studies of the apo- and ATP-bound TarGH with substrate bound in intermediate states will build a clearer picture of what conformational changes facilitate WTA precursor flipping and pumping across the plasma membrane.
- How is the oligomeric state of MprF related to its function? MprF has been shown biochemically to be capable of forming both homodimers and homotetramers (Ernst et al. 2015, Song et al. 2021). However, each protomer appears to contain all of the elements required to mediate aminoacyl-PG synthesis and flipping. Oligomerization may be functionally advantageous to MprF transport by concentrating multiple flippase domains in a local region where aminoacyl-PG is produced, to enhance the coupling of aminoacyl-PG synthesis to transport or perhaps be involved in the allosteric regulation of MprF.

4. LIPOPOLYSACCHARIDE EXPORT TO THE OUTER MEMBRANE

Found in the OM of gram-negative bacteria, LPS is made up of three parts: a lipid A moiety, the core oligosaccharide, and a long-chain O-antigen (**Figure 1*c***). Fully mature LPS molecules (~10 kDa) are much larger than conventional phospholipids (~0.75 kDa), due to the large glycan polymer that forms the lipid headgroup. Translocating LPS across the plasma membrane and aqueous periplasm presents challenges, including stabilizing the lipidic portion of LPS while simultaneously accommodating the large polar headgroup. Perhaps in part due to this complexity, the steps of LPS synthesis and transport are intermingled: first, the key precursors, the LPS core (lipid A + the core oligosaccharide) and the lipid-linked O-antigen, are synthesized in the inner leaflet of the plasma membrane; second, the LPS core and O-antigen are flipped to the outer leaflet of the plasma membrane by MsbA and WzmWzt, respectively; third, LPS assembly is completed; fourth, mature LPS is transported across the periplasm and inserted into the OM by the lipopolysaccharide transport pathway (Lpt) (Konovalova et al. 2017, Raetz et al. 2007, Ruiz et al. 2009).

4.1. MsbA

MsbA flips the LPS core across the plasma membrane, from the inner leaflet to the outer leaflet.

4.1.1. Function. The formation of the LPS core, one of two major precursors of mature LPS, occurs at the inner leaflet of the plasma membrane in gram-negative bacteria. The essential type IV ABC transporter, MsbA (Thomas et al. 2020), is responsible for flipping the LPS core across the plasma membrane (Doerrler et al. 2004, Karow & Georgopoulos 1993, Mi et al. 2017, Raetz et al. 2007, Zhou et al. 1998). Once flipped to the outer leaflet of the plasma membrane, LPS synthesis can be completed by coupling O-antigen, via the ligase WaaL, to the LPS core (Ashraf et al. 2022, Whitfield & Trent 2014).

4.1.2. Components.

This system consists of a single component:

- MsbA: ABC transporter, plasma membrane

4.1.3. Proposed transport mechanism. MsbA uses an alternating-access mechanism of transport (Dong et al. 2005, Mi et al. 2017, Ward et al. 2007, Zou & McHaourab 2009), powered by ATP (**Figure 2*b***). In the absence of nucleotides, or when bound to ADP, MsbA is in an inward-facing state with the transmembrane domains open to the cytoplasm, allowing for the LPS core to enter the inner cavity of the transporter (**Figure 4*a***). The size of the cavity is tuned for the preferential binding of the shorter, 12- to 14-carbon acyl chain LPS core, relative to the ~16-carbon chains of most phospholipids (Ho et al. 2018, Mi et al. 2017). Once the LPS core is bound, MsbA closes around the substrate. In a concerted process, ATP binding triggers conformational changes that break the extensive interactions between MsbA and the LPS core, enabling it to be flipped across the plasma membrane. The LPS core exits through a lateral gate in MsbA such that its acyl chains can then enter the outer leaflet. Following ATP hydrolysis and release, the transporter reverts from its closed state back to its inward-facing state to capture a new LPS core molecule for the next transport cycle (Liston & Willis 2021, Mi et al. 2017, Padayatti et al. 2019, Voss & Trent 2018).

4.2. WzmWzt

WzmWzt exports O-antigen polysaccharide and its lipid carrier across the plasma membrane, from the inner to the outer leaflet.

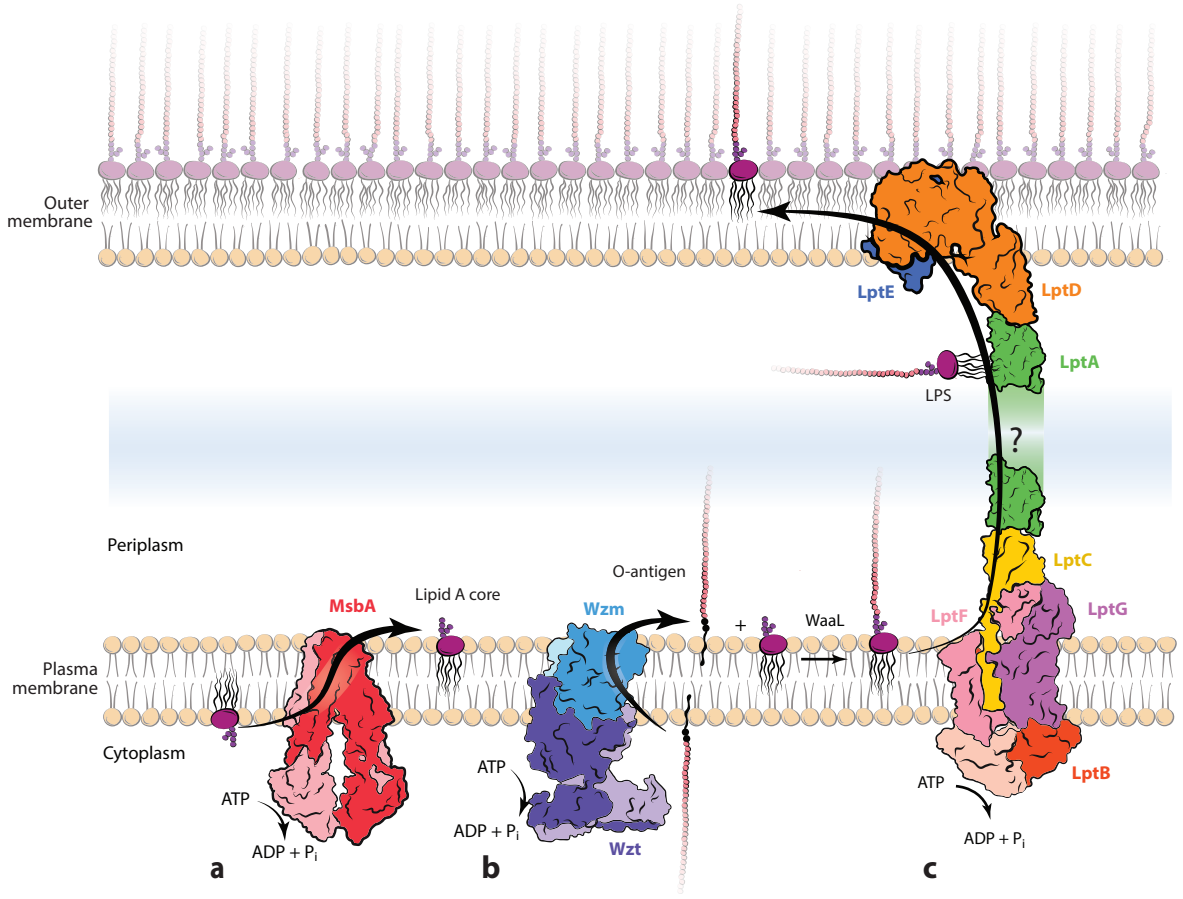


Figure 4

Transporters responsible for lipopolysaccharide (LPS) transport across the cell envelope. (*a*) Flippase MsbA in an LPS-bound, inward state [PDB (Protein Data Bank) 5TV4], responsible for flipping the LPS core moiety from the inner leaflet to the outer leaflet of the plasma membrane. (*b*) ATP-binding cassette transporter WzmWzt in an ATP-bound, closed resting state (PDB 7K2T), responsible for exporting lipid-anchored O-antigen polysaccharide from the inner to the outer leaflet of the plasma membrane. The O-antigen can then be coupled to the LPS core by the enzyme WaaL. (*c*) The Lpt system, which transports LPS from the plasma membrane to the outer leaflet of the outer membrane. LptBFGC (PDB 6MJP), LptA (PDB 2R19), and LptDE (PDB 5IV9) proteins are shown; the question mark indicates the unknown number of LptA subunits.

4.2.1. Function. O-antigen polysaccharide is the second major precursor of mature LPS. O-antigen is synthesized at the inner leaflet of the plasma membrane atop the lipid anchor Und-PP (Caffalette et al. 2020). The type V ABC transporter WzmWzt (Thomas et al. 2020) mediates the flipping of the lipid anchor and the pumping of the O-antigen polysaccharide chain across the plasma membrane in gram-negative bacteria.

4.2.2. Components.

The components of this system are as follows:

- Wzm: transmembrane component of the ABC transporter, plasma membrane
- Wzt: ATPase, cytoplasm

4.2.3. Proposed transport mechanism. The trigger for initiation of transport is likely the binding of the O-antigen cap to the Wzt carbohydrate-binding domain (Cuthbertson et al. 2005, Mann et al. 2019), which may in turn help facilitate the binding of the substrate to the transmembrane domain entrance (Bi & Zimmer 2020, Bi et al. 2018, Caffalette et al. 2019). The Und-PP lipid tail is proposed to flip spontaneously across the membrane via a continuous transmembrane tunnel between the Wzm subunits (**Figure 4*b***). In flipping Und-PP across the membrane, its associated polysaccharide headgroup is threaded through the central tunnel, priming the transporter for O-antigen translocation. Multiple rounds of ATP hydrolysis may be required to translocate the long polysaccharide chain through the tunnel, perhaps involving the asymmetry in the transmembrane subunits and ATP hydrolysis, potentially leading to alternating staggered power strokes promoting processive movement (Caffalette & Zimmer 2021) (**Figure 2*d***). Once translocation is completed, Und-PP-anchored O-antigen is released into the outer leaflet of the plasma membrane, likely through a lateral gate, and WzmWzt then resets back to its inward-facing resting state until the next O-antigen molecule is loaded (Caffalette & Zimmer 2021).

4.3. Lpt

The Lpt system uses a bridge-like mechanism for the translocation of mature LPS from the plasma membrane to the OM.

4.3.1. Function. Once LPS biosynthesis is completed, this large glycolipid must be extracted from the outer leaflet of the plasma membrane and transported across the periplasm to the cell surface (Bertani & Ruiz 2018, Rietschel et al. 1994). The Lpt system is formed by a multiprotein complex that spans the length of the periplasm and mediates the translocation of LPS and its assembly at the cell surface in gram-negative bacteria (Okuda et al. 2016). The seven proteins of the Lpt system, LptA–LptG, are all essential for LPS transport in *E. coli* (Sperandeo et al. 2008).

4.3.2. Components.

The components of this system are as follows:

- LptB: ATPase component, cytoplasm
- LptG: transmembrane component of the ABC transporter, plasma membrane
- LptF: transmembrane component of the ABC transporter, plasma membrane
- LptC: membrane-anchored bridge subunit, plasma membrane
- LptA: soluble bridge subunit, periplasm
- LptD: β-barrel protein, OM
- LptE: lipoprotein-plugging LptD barrel, OM

4.3.3. Proposed transport mechanism. The LPS exporter consists of three main parts: (*a*) a plasma membrane–embedded complex, (*b*) a periplasmic bridge, and (*c*) an OM complex (**Figure 4*c***). Transport is driven by the type VI ABC transporter complex in the plasma membrane, $LptB_2FG$ (Thomas et al. 2020), powered by the cytoplasmic ATPase LptB (Okuda et al. 2012). In the resting state, $LptB_2FG$ adopts an outward-open state, with a periplasmic cavity formed at the interface between LptF and LptG (Dong et al. 2017, Li et al. 2019, Luo et al. 2017, Owens et al. 2019, Tang et al. 2019). A lateral gate connecting the cavity to the outer leaflet of the plasma membrane likely allows LPS entry. After substrate binding, ATP binding triggers the closing of the two LptB subunits, collapsing the LPS-binding cavity and extruding LPS upward into the periplasm-spanning bridge (Li et al. 2019, Lundstedt et al. 2020) (**Figure 2*c***). ATP hydrolysis and the release of ADP and P_i trigger the reopening of the LptB dimer and consequently the cavity, which resets the transporter to its initial state (Simpson et al. 2019).

The periplasmic bridge, across which LPS is transported, is formed by the oligomerization of several homologous rod-shaped protein domains from LptF, LptC, LptA, and LptD. A hydrophobic groove runs along the length of each bridge subunit. These hydrophobic grooves come together to create a continuous pathway from $LptB_2FG$ in the plasma membrane through LptC and LptA to the OM complex of LptDE (Freinkman et al. 2012, Owens et al. 2019, Ruiz et al. 2009, Suits et al. 2008). The amount of LPS bound to LptC remains constant throughout the transport process, implying that a continuous stream of LPS likely flows across the periplasmic bridge while cells are growing (Okuda et al. 2012). LptA can polymerize in vitro, raising the possibility that multiple LptA subunits may assemble to form the middle of the bridge (Suits et al. 2008) (**Figure 4*c***).

At the end of the periplasmic bridge, LPS is delivered to an OM complex formed by the β-barrel protein LptD and the OM lipoprotein LptE, which plugs the pore through LptD. The polysaccharide headgroup of LPS is translocated across the OM through a channel formed by LptD and LptE (Dong et al. 2014, Gu et al. 2015, Li et al. 2015, Qiao et al. 2014). The hydrophobic, lipid A moiety of LPS is selectively released into the outer leaflet of the OM though a lateral gate between two β-strands of the LptD barrel (Bos et al. 2004, Chng et al. 2010, Dong et al. 2014, Lundquist & Gumbart 2020, Qiao et al. 2014).

4.4. Open Questions

- How does the asymmetric transporter WzmWzt translocate substrate across the plasma membrane? While WzmWzt is an ABC transporter, it does not use the classical alternating-access mechanism to flip its substrate across the plasma membrane. Future structural studies examining the transporter in a stalled O-antigen translocation intermediate state would provide insight into how this substrate is pumped across the lipid bilayer.
- How does the bridge of the LPS transporter accommodate variable periplasmic width? While LptA can oligomerize and has been shown to assemble in a head-to-tail fashion in the presence of LPS (Suits et al. 2008), how many LptA molecules form the periplasmic bridge is unknown. Given the variation in periplasmic width observed in individual cells, as well as across species, incorporating more or fewer LptA subunits may be tailored to the size of the gap between membranes.
- How does LptDE translocate a molecule with up to ~200 sugars across the OM? LptDE faces the challenge of translocating large LPS molecules across a lipid bilayer while maintaining a tight barrier against the entry of small molecules such as antibiotics. It is unclear how this large entity is pushed through the LptDE translocon, whether conformational changes occur due to ATP binding and hydrolysis in the cytoplasm or by spontaneous breathing of the barrel (as seen in FadL, described in Section 5.5).

5. TRANSPORT OF PHOSPHOLIPIDS AND OTHER LIPIDS BETWEEN THE PLASMA MEMBRANE AND OUTER MEMBRANE

In contrast to the LPS-rich outer leaflet of the OM, the inner leaflet of the OM and the plasma membrane consist predominantly of phospholipids. Bidirectional transport of phospholipids occurs between the plasma membrane and OM in *E. coli*, but the molecular mechanisms that underlie transport remain poorly understood. In recent years, a few systems have been implicated in phospholipid transport across the cell envelope, though the details of their exact functions are still emerging. Best understood is an active transport system called the maintenance of lipid asymmetry (Mla) pathway that is proposed to be involved in phospholipid trafficking and the fine-tuning of OM properties. Two distantly related systems in *E. coli*, called lipophilic envelope-spanning tunnel (Let) and paraquat inducible (Pqi), have also been proposed to act as lipid-transport

tunnels between the plasma membrane and OM. Most recently, a putative passive transport pathway involving YhdP and related proteins was proposed to allow equilibration of phospholipids in bulk between the plasma membrane and OM. In this section, we discuss our current understanding of phospholipid transport as well as the transport of cholesterol-like molecules called hopanoids (by HpnN), which populate both the plasma membrane and the OM. Finally, we cover how bacteria may import nutrients such as hydrophobic fatty acids across the OM barrier into the cell (by FadL).

5.1. Mla

The Mla system uses a ferry-like mechanism to shuttle phospholipids across the periplasm.

5.1.1. Function. Phospholipids need to be transported between the plasma membrane and the OM. The Mla pathway is a multicomponent system that is involved in the process of phospholipid transport (Chong et al. 2015, Ekiert et al. 2017, Malinverni & Silhavy 2009, Thong et al. 2016). The direction of lipid transport by the Mla system is controversial, with data that support import (Chong et al. 2015, Low et al. 2021, Malinverni & Silhavy 2009, Powers et al. 2020, Sutterlin et al. 2016, Yeow et al. 2018), export (Hughes et al. 2019, Kamischke et al. 2019), and bidirectional transport (Tang et al. 2021). Mla was originally proposed to help maintain the asymmetric distribution of phospholipids and LPS in the OM by removing mislocalized phospholipids from the outer leaflet of the OM and importing them back to the plasma membrane (retrograde transport). Alternatively, Mla has been suggested to play a role in phospholipid export from the plasma membrane to the OM (anterograde transport), perhaps exporting particular subsets of OM lipids.

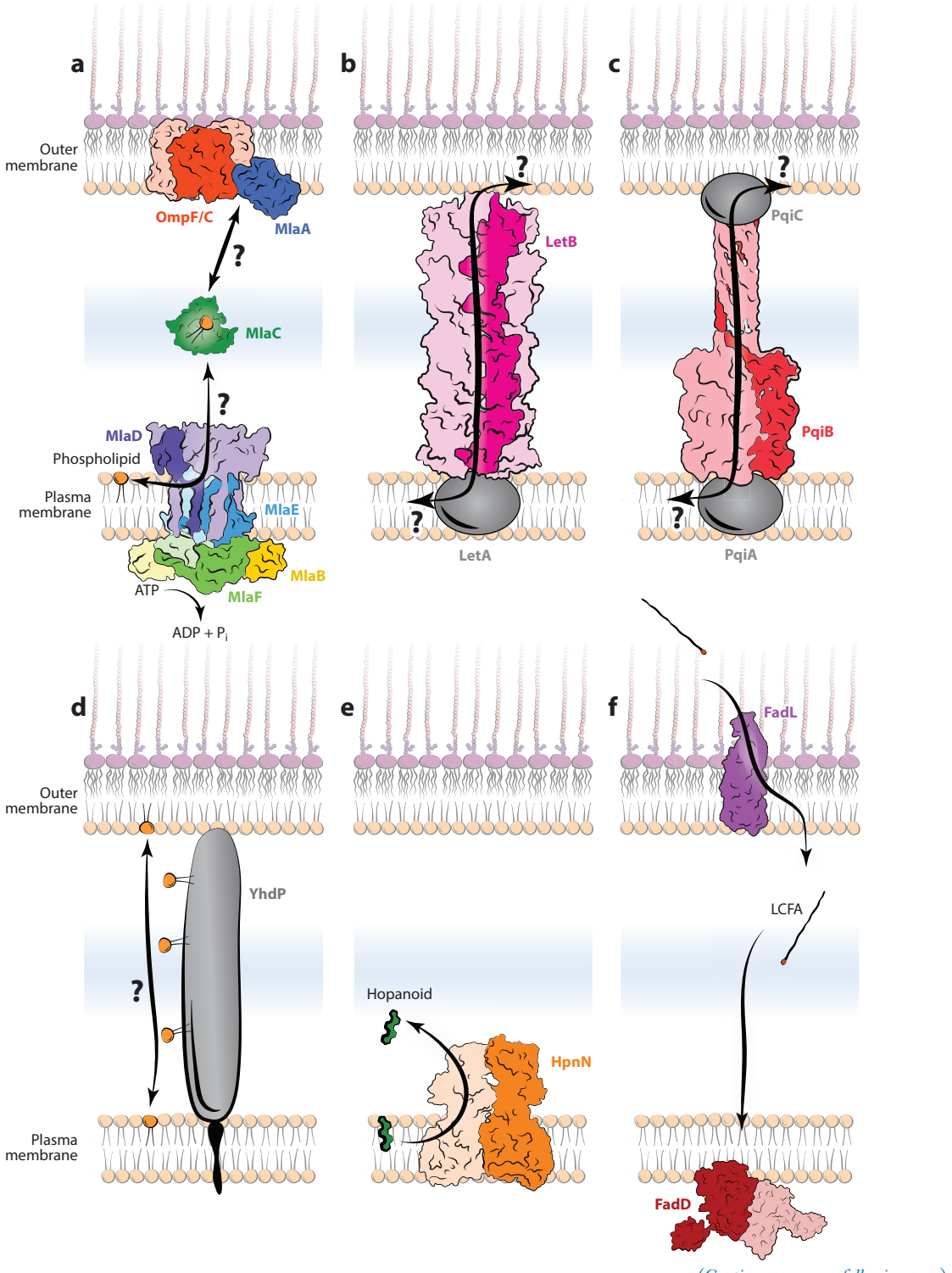
5.1.2. Components.

The components of this system are as follows:

- MlaF: ATPase, cytoplasm
- MlaE: transmembrane component of the ABC transporter, plasma membrane
- MlaD: MCE-domain protein, periplasmic, anchored in plasma membrane
- MlaB: STAS-domain protein, cytoplasm
- MlaC: soluble lipid-binding protein, periplasm
- MlaA: lipoprotein, OM
- OmpF/C: OM porin, scaffold for MlaA; OM

5.1.3. Proposed transport mechanism. The Mla system consists of three main parts (**Figure 5*a***): (*a*) MlaA in complex with OmpF/C, located in the outer membrane; (*b*) a soluble lipid-binding protein, MlaC, located in the periplasm; and (*c*) MlaFEDB, an ABC transporter complex in the plasma membrane, which drives transport (Low et al. 2021, Tang et al. 2021). While the direction of transport by Mla is still a subject of debate, for simplicity, we only describe the proposed mechanism here in the context of phospholipid import; however, reversing the described steps could instead result in lipid export, as has also been proposed (Hughes et al. 2019, Kamischke et al. 2019).

MlaA is almost entirely embedded within the OM as part of a complex with OmpF/C and forms a channel partway across the membrane (Abellón-Ruiz et al. 2017). Phospholipids from the outer leaflet can traverse the channel and be transferred to MlaC (Tang et al. 2021), which has a high affinity for lipids and has been shown to interact directly with MlaA (Ekiert et al. 2017, Ercan et al. 2019). MlaC is thought to deliver lipids to MlaFEDB, a type VIII ABC transporter (Thomas et al. 2020). MlaFEDB consists of the transmembrane component MlaE and the ATPase domain MlaF as well as MlaD in the periplasm and MlaB in the cytoplasm (Ekiert et al. 2017, Kamischke et al.



(*Caption appears on following page*)

Figure 5 (*Figure appears on preceding page*)

Transporters involved in phospholipid and other lipid transport. (*a*) The Mla system, which is responsible for phospholipid transport across the gram-negative cell envelope. The MlaFEDB complex (PDB 6XBD), MlaC (PDB 5UWA), and MlaA-OmpF (PDB 5NUO) are shown. Bidirectional arrows between the plasma membrane and OM components and MlaC indicate uncertainty in the direction of transport. (*b*) The Let system, which is proposed to transport lipids or other substrates across the cell envelope. A single monomer of the homohexameric LetB (PDB 6V0C) is highlighted in the darker purple. LetA is shown as a gray oval, as the structure is currently unknown. Question marks indicate uncertainty in substrate and directionality. (*c*) The Pqi system, which is proposed to transport lipids or other substrates across the cell envelope. A single monomer of the homohexameric PqiB (PDB 5UVN) [data from Ekiert et al. (2017)] is shown in the darker red. PqiA and PqiC are shown as gray ovals, as the structures are currently unknown. Question marks indicate uncertainty in substrate and directionality. (*d*) YhdP is proposed to mediate passive diffusion of phospholipids in bulk across the cell envelope. Although no structures are available for the protein, it is thought to be anchored in the plasma membrane and span across the periplasm. Question marks indicate uncertainty in substrate and directionality. (*e*) HpnN (PDB 5KHN) is responsible for trafficking hopanoids from the outer leaflet of the plasma membrane to the periplasm. (*f*) FadL (PDB 1T16) is responsible for importing LCFAs from the extracellular space across the OM to the periplasm. FadD (PDB 3G7S) is responsible for activating the fatty acid to prevent its diffusion out of the cell. Abbreviations: Hpn, hopanoid; LCFA, long-chain fatty acid; Let, lipophilic envelope-spanning tunnel; Mla, maintenance of lipid asymmetry; OM, outer membrane; PDB, Protein Data Bank; Pqi, paraquat inducible.

2019, Thong et al. 2016). The MlaD subunits each contain a single MCE domain [mammalian cell entry (Arruda et al. 1993)], which assembles to form a hexameric ring surrounding a central hydrophobic tunnel for lipid transport. MlaC binds directly to MlaD (Ekiert et al. 2017, Ercan et al. 2019) and likely transfers the bound lipid into a continuous tunnel running through MlaD to an outward-facing pocket MlaE (Chi et al. 2020, Coudray et al. 2020, Mann et al. 2021, Tang et al. 2021, Y. Zhang et al. 2020, Zhou et al. 2021). Upon lipid binding in the MlaE pocket, ATP binding triggers a conformational change, leading to the collapse of the lipid-binding pocket (Chi et al. 2020) and the extrusion of the lipids into one of the plasma membrane leaflets (**Figure 2*c***). MlaB has been proposed to play a regulatory role in the transport process (Kolich et al. 2020, Thong et al. 2016).

5.2. The Let and Pqi Systems

The Let and Pqi systems form tunnel-like structures proposed to facilitate substrate transport across the periplasm.

5.2.1. Function. Like MlaD, the Let and Pqi systems in *E. coli* form hydrophobic tunnels built from MCE domains (Ekiert et al. 2017, Isom et al. 2020). Based on structures (Ekiert et al. 2017, Isom et al. 2020, Liu et al. 2020, Vieni et al. 2022), homology to Mla, and in vitro substrate-binding data (Ekiert et al. 2017, Isom et al. 2020), these systems are hypothesized to transport phospholipids or other hydrophobic molecules between the plasma membrane and OM. Their cellular functions, substrates, and transport mechanisms are still emerging.

5.2.2. Components.

The components of the Let system are as follows:

- LetA: putative transmembrane protein, plasma membrane
- LetB: MCE-domain protein; periplasm, anchored in plasma membrane

The components of the Pqi system are as follows:

- PqiA: putative transmembrane protein, plasma membrane
- PqiB: MCE-domain protein; periplasm, anchored in plasma membrane
- PqiC: lipoprotein, OM

5.2.3. Proposed transport mechanism. LetB and PqiB are both homohexameric MCE domain proteins that form tunnels of different architectures that are long enough to span the periplasm (Ekiert et al. 2017, Isom et al. 2020) (**Figure 5*b*,*c***). Substrates have been trapped in the LetB tunnel via site-specific cross-linking, suggesting that substrates are translocated between the plasma membrane and OM through the LetB tunnel, shielded from the periplasm. LetA and PqiA are plasma membrane–embedded proteins that are proposed to interact with LetB and PqiB, respectively. Additionally, the Pqi system includes PqiC, an OM lipoprotein that interacts with PqiB (Nakayama & Zhang-Akiyama 2017). Whether the Let and Pqi systems are powered by an energy source or are passive transporters is as yet unknown.

5.3. YhdP

YhdP is proposed to use a bridge-like mechanism to transport phospholipids across the periplasm.

5.3.1. Function. YhdP has been proposed to form a bridge across the periplasm in *E. coli*, potentially creating a hydrophobic pathway to allow lipids to move directly between the plasma membrane and the OM (Grimm et al. 2020, Ruiz et al. 2021).

5.3.2. Components.

This system is composed of a single component:

- YhdP: putative lipid-binding protein; periplasm, anchored in plasma membrane

5.3.3. Proposed transport mechanism. YhdP has recently been proposed as a candidate for phospholipid transport between the plasma membrane and OM in *E. coli* (**Figure 5*d***). The gene *yhdP* from *E. coli* was first identified as playing a role in OM maintenance (Mitchell et al. 2017), and more recent studies (Douglass et al. 2022, Grimm et al. 2020, Ruiz et al. 2021) support a role in lipid transport. YhdP and related proteins share homology with the eukaryotic phospholipid-transport protein Vps13, which forms a bridge with a hydrophobic groove that transports lipids between the endoplasmic reticulum and adjacent organelles (Kumar et al. 2018, Levine 2019, Li et al. 2020). Predictions of the YhdP structure using AlphaFold (Jumper et al. 2021) support a Vps13-like transport mechanism, suggesting that YhdP may form a long protein bridge between the plasma membrane and the OM, similar to that formed by the Lpt proteins (Douglass et al. 2022, Ruiz et al. 2021). Intriguingly, YhdP is one of six AsmA-like proteins found in the *E. coli* genome (AsmA, TamB, YdbH, YicH, YhjG, and YhdP), and recent work (Douglass et al. 2022, Ruiz et al. 2021) has suggested that this group of proteins may function together.

5.4. HpnN

HpnN mediates the export of hopanoids from the plasma membrane into the periplasm.

5.4.1. Function. Hopanoids can be present in the plasma membrane of some gram-positive and gram-negative bacteria and the OM of some gram-negative bacteria (Belin et al. 2018, Sohlenkamp & Geiger 2016, Willdigg & Helmann 2021). Hopanoids are involved in promoting stress tolerance in conditions such as high temperature or low pH (Kumar et al. 2017; Malott et al. 2012, 2014; Schmerk et al. 2011; Welander et al. 2009). HpnN, the archetype of the hopanoid biosynthesis-associated resistance-nodulation-division subfamily of bacterial transporters, has emerged as the facilitator of hopanoid trafficking from the plasma membrane to the OM in gram-negative bacteria (Daligault et al. 2014, Doughty et al. 2011, Tseng et al. 1999).

5.4.2. Components.

This system consists of a single component:

- HpnN: transmembrane protein, plasma membrane

5.4.3. Proposed transport mechanism. The HpnN transporter is powered by proton motive force (Perrin et al. 2013, Sáenz et al. 2015). Each HpnN protomer is embedded in the plasma membrane and has a large periplasmic domain (**Figure 5*e***). A hydrophobic tunnel was identified that runs from the outer leaflet of the plasma membrane up through the periplasmic domain and seems likely to serve as the beginning of the pathway for trafficking hopanoids toward the OM (Doughty et al. 2011, Kumar et al. 2017). A trio of conserved residues found in HpnN's transmembrane domain have been implicated in a possible proton relay that couples hopanoid export to proton translocation across the plasma membrane (Takatsuka & Nikaido 2006). However, how hopanoids traverse the large gap between the end of the HpnN tunnel and the OM remains unclear.

5.5. FadL

FadL imports exogenous long-chain fatty acids (LCFAs) across the OM.

5.5.1. Function. Long-chain fatty acids (LCFAs) are imported into the cell as sources of metabolic energy and carbon (Hearn et al. 2009, Nunn et al. 1986, van den Berg 2005). *E. coli* FadL is the prototypical member of a family of proteins that facilitates the passive diffusion of hydrophobic LCFAs across the OM in gram-negative bacteria (Black et al. 1985, 1987; Ginsburgh et al. 1984; Hearn et al. 2009; Mangroo & Gerber 1993; Nunn et al. 1986; van den Berg 2005).

5.5.2. Components.

The components of this system are as follows:

- FadL: β-barrel transporter, OM
- FadD: long-chain fatty acyl–coenzyme A (CoA) synthetase, associated with plasma membrane

5.5.3. Proposed transport mechanism. FadL does not require exogenous energy to power LCFA import (Azizan et al. 1999, van den Berg 2005). Instead, FadL most likely mediates LCFA transport by spontaneous breathing of its structure. This allows an LCFA molecule to move through a series of binding sites along a diffusive pathway from the extracellular matrix through the OM (**Figure 5*f***), where it is ultimately released into the periplasm (Lepore et al. 2011, van den Berg 2005). LCFA is captured from the extracellular space by binding to a hydrophobic groove of the FadL barrel. It then diffuses into a high-affinity binding pocket inside the barrel. Conformational changes release the substrate and allow LCFA to diffuse through the lateral opening in the FadL β-barrel wall to the outer leaflet of the OM (Hearn et al. 2009, Lepore et al. 2011, van den Berg 2010). LCFA can then move to the inner leaflet of the OM and diffuse into the periplasm, creating a pathway that bypasses the need to move through an aqueous channel of the hydrophilic LPS layer. Once LCFAs traverse the periplasm, they spontaneously enter and flip across the plasma membrane, where they are extracted and activated to long-chain acyl–coenzyme A by the plasma membrane–associated fatty acyl–CoA synthetase, FadD (Black & DiRusso 2003, Hamilton 2003, van den Berg 2005).

5.6. Open Questions

- What is the direction of lipid transport in the Mla system in vivo? Several biochemical experiments have provided insights into the direction of lipid transport, but the results are

Table 1 Summary of lipid transporters in the bacterial cell envelope

Transport system	Transport location	Protein components	Substrate	Function	Proposed mechanism
MurJ	Across the plasma membrane	MurJ pseudodimer	Lipid II	Flipping Lipid II for incorporation into the cell wall	Alternating access; possibly powered by ion gradients
TarGH	Across the plasma membrane	TarG: transmembrane protein TarH: ATPase	Und-PP-WTA	Flipping WTA precursor for incorporation into the cell wall	Processive pumping of substrate; powered by ATP hydrolysis
LtaA	Across the plasma membrane	LtaA pseudodimer	Gentiobiosyl-diacylglycerol	Flipping LTA precursor for completion of LTA biosynthesis	Putative alternating access; powered by proton gradient
MprF	Across the plasma membrane	MprF homo-oligomer	Aminoacyl-PG	Synthesizing and flipping aminoacyl-PG for incorporation into the outer leaflet of the plasma membrane	Putative diffusive translocation through an ionic gate; unclear if transport is active or passive
MsbA	Across the plasma membrane	MsbA homodimer	LPS core	Flipping LPS-core across the plasma membrane for completion of LPS biosynthesis	Alternating access; powered by ATP hydrolysis
WzmWzt	Across the plasma membrane	Wzm: transmembrane protein Wzt: ATPase	Lipid-anchored O-antigen polysaccharide	Flipping the lipid-anchored O-antigen across the plasma membrane for completion of LPS biosynthesis	Flipping and putative processive pumping of substrate; powered by ATP hydrolysis
Lpt	Between the plasma membrane and OM	LptB: ATPase LptG: transmembrane protein LptF: transmembrane protein LptC: membrane-anchored bridge protein LptA: soluble bridge protein LptD: OM β-barrel protein LptE: OM lipoprotein	LPS	Transporting LPS from the plasma membrane to the outer leaflet of the OM	Bridge mechanism moving LPS across the periplasm; powered by ATP hydrolysis
Mla	Between the plasma membrane and OM	MlaF: ATPase MlaE: transmembrane protein MlaD: MCE-domain protein MlaB: STAS-domain protein MlaC: lipid-binding protein MlaA: OM lipoprotein OmpF/C: OM porin	Phospholipids	Transporting phospholipids between the plasma membrane and OM for OM maintenance	Shuttle mechanism moving lipids across the periplasm; powered by ATP hydrolysis
Let	Between the plasma membrane and OM	LetA: putative transmembrane protein LetB: MCE-domain protein	Lipids	Transporting lipids across the periplasm	Tunnel mechanism moving lipids across the periplasm; unclear how transport is powered
Pqi	Between the plasma membrane and OM	PqiA: putative transmembrane protein PqiB: MCE-domain protein PqiC: OM lipoprotein	Lipids	Transporting lipids across the periplasm	Tunnel mechanism moving lipids across the periplasm; unclear how transport is powered
YhdP	Between the plasma membrane and OM	YhdP: putative lipid-binding protein	Phospholipids	Transporting phospholipids between the plasma membrane and OM for OM maintenance	Putative bridge mechanism moving lipids across the periplasm; unclear how transport is powered

(*Continued*)

Table 1 (*Continued*)

Transport system	Transport location	Protein components	Substrate	Function	Proposed mechanism
HpnN	Export from the plasma membrane to the periplasm	HpnN: transmembrane homodimer	Hopanoids	Exporting hopanoids from the plasma membrane	Translocation through a hydrophobic tunnel; powered by proton motive force
FadL	Across the OM	FadL: β-barrel protein FadD: long-chain fatty acyl–coenzyme A synthetase	Long-chain fatty acids	Transporting long-chain fatty acids into the cell for metabolic consumption	Diffusive translocation through spontaneous breathing

Abbreviations: LPS, lipopolysaccharide; LTA, lipoteichoic acid; MCE, mammalian cell entry; OM, outer membrane; PG, phosphatidylglycerol; UND-PP, undecaprenyl pyrophosphate; WTA, wall teichoic acid.

conflicting. Assays that directly detect lipid transport in vivo will be invaluable in revealing the answer to this important question.

- What are the substrates of Let, Pqi, and YhdP? The Let, Pqi, and YhdP systems share homology with known lipid transporters, suggesting they carry out similar functions. However, the functions and substrates of each of these systems remain key open questions.
- In gram-negative bacteria, how do hopanoids and LCFAs cross the periplasm and arrive at their final destination? It is unclear whether hopanoids and LCFAs diffuse across the periplasm unassisted or whether their transport is protein mediated. Additionally, whether there are additional components that mediate hopanoid insertion into the OM or whether this process is spontaneous is unknown.
- How do transport systems that assemble in the periplasm function in coordination with the peptidoglycan cell wall? The cell wall must be rigid enough to withstand turgor pressure yet porous enough to allow transport proteins to diffuse through and transenvelope complexes to be assembled. How the cell wall influences the movement of proteins such as MlaC, as well as whether local remodeling occurs around proteins such as PqiB and LetB, is unclear.

6. FUTURE OUTLOOK

Numerous mechanisms have evolved to support the assembly and maintenance of the lipid components of the bacterial cell envelope. Several systems, and open questions relating to those systems, have been described in this review and summarized in **Table 1**. In the bigger picture, several frontiers remain open for discovery, of which we highlight three. (*a*) While phospholipids form the bulk of the lipids in the bacterial cell envelope, how they are trafficked and what the main phospholipid transporter is remain unknown. The genes encoding the putative phospholipid-transport systems previously described are all nonessential genes, and knocking these out individually does not significantly impact cell fitness under standard culture conditions (Ekiert et al. 2017, Grimm et al. 2020, Malinverni & Silhavy 2009), suggesting that multiple redundant systems may work together in this process. (*b*) Cellular localization of the lipid-transport machines is not well understood. Recent studies have described membrane domains or compartments, reminiscent of lipid rafts, where particular proteins may localize (Benn et al. 2021, Puffal et al. 2022, Rokicki et al. 2021, Zhu et al. 2021). Whether there are major sites of lipid synthesis and trafficking in the cells or whether this is a delocalized, less regulated process is unknown. (*c*) Many bacteria live inside, or together with, their host cells. The exchange of lipids between bacteria and their hosts, the machinery specialized for this purpose, and whether bacteria may even modulate host cell signaling in this way are all open questions. As such, the study of lipid-transport mechanisms in many contexts, from the level of atomic mechanism to cell biology, is ripe for discovery.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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