MsbA-dependent translocation of lipids across the inner membrane of Escherichia coli

William T. Doerrler  
*Duke University Medical Center*

Henry S. Gibbons  
*Duke University Medical Center*

R. Christian  
*Duke University Medical Center*

H. Raetz  
*Duke University Medical Center*

Follow this and additional works at: [https://digitalcommons.lsu.edu/biosci_pubs](https://digitalcommons.lsu.edu/biosci_pubs)

**Recommended Citation**
[https://doi.org/10.1074/jbc.M408106200](https://doi.org/10.1074/jbc.M408106200)

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.
MsBA-dependent Translocation of Lipids across the Inner Membrane of Escherichia coli

MsBA is an essential ABC transporter in Escherichia coli required for exporting newly synthesized lipids from the inner to the outer membrane. It remains uncertain whether or not MsBA catalyzes trans-bilayer lipid movement (i.e. flip-flop) within the inner membrane. We now show that newly synthesized lipid A accumulates on the cytoplasmic side of the inner membrane after shifting an E. coli msbA missense mutant to the non-permissive temperature. This conclusion is based on the selective inhibition of periplasmic, but not cytoplasmic, covalent modifications of lipid A that occur in polymyxin-resistant strains of E. coli. The accessibility of newly synthesized phosphatidylethanolamine to membrane impermeable reagents, like 2,4,6-trinitrobenzenesulfonic acid, is also reduced severalfold. Our data showed that MsBA facilitates the rapid translocation of some lipids from the cytoplasmic to the periplasmic side of the inner membrane in living cells.

The envelope of Gram-negative bacteria contains two distinct lipid bilayers. The inner membrane lipids consist largely of phosphatidylethanolamine (PE),1 phosphatidylglycerol, and cardiolipin (1–3). The outer membrane is an asymmetric bilayer with glycerophospholipids on its outer surface and the lipid A moiety of lipopolysaccharide (LPS) on the outside (1–3). Lipid A biosynthesis is required for the growth of Escherichia coli and other Gram-negative bacteria (1). It is an excellent target for novel antibiotics (4, 5). Lipid A is also a very potent stimulant of the human innate immune system via Toll-like receptor 4 (6, 7).

The constitutive enzymes of phospholipid and lipid A biosynthesis are well characterized in E. coli (1, 3, 8, 9). They are localized in the cytoplasm or on the cytoplasmic side of the inner membrane. It remains unclear how newly synthesized lipids are moved across the inner membrane and are exported to the outer membrane (1, 10, 11). Trans-bilayer phospholipid movement is kinetically unfavorable in model systems and therefore is thought to be protein-mediated in biological membranes (12–16).

The transport of newly synthesized phospholipids and LPS from the inner to the outer membrane of E. coli is dependent upon the ABC transporter MsBA (10, 17). This protein is essential for E. coli growth and is highly conserved in Gram-negative bacteria (18). It is closely related to the mammalian P-glycoproteins, especially those that confer multidrug resistance (18–20). X-ray structures of MsBA are available (20, 21) and support the view that MsBA might function as a lipid flippase. We previously isolated a temperature-sensitive msbA mutant, in which newly synthesized phospholipids and LPS accumulate in the inner membrane under non-permissive conditions (10). Our work demonstrated a critical role for MsBA in lipid export (10, 17) but left unanswered the question whether or not MsBA is an inner membrane lipid flippase.

There is considerable disagreement in the literature concerning the mechanism of trans-bilayer lipid movement. In some biological membranes, lipid flip-flop appears to be ATP-independent, arguing against the involvement of an ABC transporter (22–24). Kol et al. (15, 24) have shown that lipid flip-flop in phospholipid vesicles is stimulated by almost any membrane-spanning helical peptide. Hrafnssottir et al. (14) have reported ATP-independent flip-flop activity in Gram-positive membrane vesicles but have not isolated a transport protein or established a mechanism. On the other hand, Margolles et al. (25) have demonstrated ATP-dependent lipid flip-flop activity associated with the LmrA protein, an MsBA homologue from the Gram-positive bacterium Lactococcus lactis, which confers resistance to a variety of amphiphilic drugs. The ATPase activity that is associated with purified MsBA is greatly stimulated by lipids (26), especially by Kdo₂-lipid A, but reconstitution of ATP-dependent lipid flip-flop with pure MsBA in phospholipid vesicles has not been successful (27).

Most studies of in vitro lipid flip-flop have employed short chain or relatively hydrophilic lipid analogues, modified with chromophores, spin labels, or radioactive moieties to measure transport (14, 24, 28, 29). Because in vitro flip-flop assays may not accurately reflect what is taking place inside E. coli, we have examined MsBA function in living cells. Upon inactivation of MsBA in the msbA2 temperature-sensitive mutant WD2 (10), newly made LPS, and possibly PE, accumulate preferentially on the inner face of the inner membrane as judged by the accessibility to enzymatic modification and labeling with membrane-impermeable reagents. These results provided the first direct evidence that rapid movement of some lipids across the inner membrane of E. coli is dependent upon MsBA.

EXPERIMENTAL PROCEDURES

Materials—Tryptone and yeast extract were from Difco. Radioisotopes were purchased from PerkinElmer Life Sciences. Protein concentrations were determined with the BCA protein assay reagent from Pierce using bovine serum albumin as the standard (30). Sulfo-N-hydroxysuccinimidobiotin (sulfo-NHS)-biotin was purchased from Pierce, and 2,4,6-trinitrobenzenesulfonic acid (TNBS) was from Sigma.
All other chemicals were reagent-grade and purchased from either Sigma or Mallinckrodt.

**Strain Construction**—Strain WD2 harboring the temperature-sensitive msha2 mutation has been described previously (10) (see Table I). It is derived from strain W3110 (E. coli Genetic Stock Center). A P1 lysate of the polymyxin resistant E. coli mutant WD101 (31, 32), harboring a mutation (designated pmra<sup>Δ</sup>) that constitutively activates the PmrA transcription factor, was used to transduce WD2 to polymyxin resistance. P1<sub>lys</sub> transduction was carried out as described (33, 34), except that the transduced cells were allowed to grow overnight in the absence of polymyxin to express the resistance phenotype prior to selection with polymyxin (10 μg/ml in LB plates). A polymyxin-resistant colony was repurified and tested for temperature-sensitive growth. The desired strain was designated WD201. A marked derivative of the wild-type E. coli K12 strain W3110, designated W3110A (aroA::Tn10), was prepared by P1<sub>lys</sub> transduction using the same araBAD::Tn10 donor strain as was used to generate WD2 (see Table I).

The Salmonella lipX gene on plasmid pSHG1 (35) was introduced into both W3110 and WD2 by electroporation. The transformants were selected based on their resistance to ampicillin. LipX is an inner membrane hydroxylase (35, 36) that converts the secondary myristate chain at the lipid A 3′-position to S-2-hydroxy-myristate in a reaction that requires molecular oxygen, iron, ascorbate, and κ-ketoglutarate.

**Cell Labeling and Isopyknic Sucrose Gradient Centrifugation**—Strains WD101 and WD201 were grown at 30 °C, shifted to 44 °C for 30 min, and labeled for 10 min with <sup>32</sup>Pi (1 Ci/ml, 900 Ci/mmol), as described previously (10). Inner and outer membranes were separated by sucrose gradient ultracentrifugation (17). Fractions from the sucrose gradient were subjected to mild acid hydrolysis at pH 4.5 in the presence of SDS to release the lipid A from the LPS core sugars prior to lipid A and phospholipid extraction with chloroform/methanol (10, 17). Labeled lipids were resolved by thin layer chromatography (TLC) in the solvent system chloroform, pyridine, 88% formic acid, H<sub>2</sub>O (50:50:16.5, v/v/v), and radioactive phospholipids were visualized and quantified on the plate using a PhosphorImager equipped with IQMac software.

The marker enzymes phospholipase A (outer membrane) and NADH oxidase (inner membrane) were measured (17) and expressed as the percent radioactivity per fraction across the gradient. Total protein was measured with the BCA assay (30). The radioactivity of each fraction was determined using a liquid scintillation counter.

**Sulfo-NHS-biotin and TNBS Modification of Newly Synthesized PE**—Overnight cultures of W3110A and mutant WD2 cells were diluted 1:100 into LB broth, pH 7.0. Cells were grown with shaking at 30 °C until a cell density (A<sub>600</sub>) of 0.5–1.0 was reached. Cells were then diluted 1:4 into 10 ml of LB broth, pre-warmed to 44 °C, and grown with shaking for another 30 min. Next, 1 μCi/ml <sup>32</sup>Pi was added, and the cells were grown for an additional 10 min. Flasks were cooled on ice, and the cells were collected by centrifugation for 10 min at 4000 × g at 2 °C.

For sulfo-NHS-biotin modification of PE, the cells were washed twice with ice-cold 10 mM Tris chloride, pH 8.6, containing 140 mM NaCl. The washed cell pellet was then resuspended in 4 ml of the same buffer at 0 °C. Sulfo-NHS-biotin was added at a final concentration of 0–2 mg/ml, as indicated, and the cells were incubated on ice for 0–4 h. Reactions were quenched by the addition of 50 mM glycine, and the cells were collected in a microcentrifuge.

For the TNBS modification of PE, the cell pellet was washed twice with ice-cold phosphate-buffered saline. The washed pellet was resuspended in 10 ml of cold 50 mM NaHCO<sub>3</sub>, pH 8.5, containing 100 mM NaCl. The TNBS was added to give a final concentration of 3 mM by dilution from a fresh 5% commercial stock solution (Sigma) in water, and the cells were incubated on ice. The reaction was stopped at indicated times by addition of 50 μl of 5% bovine serum albumin and 200 μl of 30% trichloroacetic acid/ml of cells. The cells were collected using a microcentrifuge. For the separation of TNBS-modified membranes by isopycnic sucrose density gradient ultracentrifugation, <sup>32</sup>P-labeled cells were incubated in the presence of 3 mM TNBS on ice for 90 min. The reaction was stopped by the addition of an equal volume of 20 mM Tris chloride, pH 8.0, in 1% bovine serum albumin without trichloroacetic acid, and the membranes were prepared (10, 17) and separated into inner and outer fractions.

Glycerophospholipids present in cell pellets or sucrose density gradient fractions were extracted using a single phase Bligh-Dyer mixture consisting of chloroform:methanol:H<sub>2</sub>O (1:2:0.8, v/v). Following a brief centrifugation to separate the phases, the upper phase was removed and discarded, and the lower phase was washed with a fresh pre-equilibrated upper phase. The lower phase was dried, redissolved in a small volume of chloroform/methanol (4:1, v/v), and spotted onto a Silica Gel 60 TLC (Merck). Sulfo-NHS-biotin-modified lipids were resolved in chloroform:methanol:acetic acid:H<sub>2</sub>O (65:25:4, v/v), and TNBS-modified lipids were resolved in chloroform:methanol:H<sub>2</sub>O (65:25:4, v/v). Radioactive phospholipids were detected using a PhosphorImager and quantified using IQMac software.

**RESULTS**

**Lipid A Modification with Phosphoethanolamine and Aminoarabinose Requires MshaA**—Earlier studies of the temperature-sensitive MshaA mutant WD2 demonstrated the accumulation of newly synthesized phospholipids and lipid A in the inner membrane following inactivation of MshaA at 44 °C (10). These findings are consistent with two alternative mechanisms. In one scenario, MshaA catalyzes the trans-bilayer movement of newly synthesized core-lipid A and glycerophospholipids within the inner membrane. In this case, newly made lipids would accumulate at the cytoplasmic face of the inner membrane upon loss of MshaA function. In the “ejection” model, MshaA catalyzes the ATP-dependent movement of newly made lipids from the outer surface of the inner membrane into the periplasm and/or outer membrane. In this case, newly made lipids would accumulate at the periplasmic surface of the inner membrane following MshaA inactivation.

![FIG. 1. Possible functions of MshaA in the export of newly synthesized lipids in E. coli. A. In the “flip-flop” model, MshaA catalyzes the ATP-dependent trans-bilayer movement of newly synthesized core-lipid A and glycerophospholipids within the inner membrane. In this case, newly made lipids would accumulate at the cytoplasmic face of the inner membrane upon loss of MshaA function. B. In the “ejection” model, MshaA catalyzes the ATP-dependent movement of newly made lipids from the outer surface of the inner membrane into the periplasm and/or outer membrane. In this case, newly made lipids would accumulate at the periplasmic surface of the inner membrane following MshaA inactivation.](338x487 to 542x737)
groups to lipid A (Fig. 2, blue substituents). These amine-containing substituents reduce the net negative charge of the cell surface and decrease its affinity for cationic anti-microbial peptides, including polymyxin (31, 32). As explained in the legend to Fig. 2, the addition of aminoarabinose and phosphoethanolamine groups are catalyzed by distinct enzymes (ArnT and EptA/PmrC, respectively)², which face the periplasmic surface of the inner membrane (31, 32, 38, 39). These modifications should require the flip-flop of lipid A from the cytoplasmic to the periplasmic surface of the inner membrane and therefore are markers for the trans-membrane movement of newly synthesized lipid A.

To examine the accessibility of newly made lipid A to ArnT and EptA/PmrC, we constructed strain WD201, which carries the temperature-sensitive msbA allele of WD2 together with the polymyxin resistance gene pmrAc of WD101 (Table I). WD101 and WD201 were pulse-labeled for 10 min with ³²P, following 30 min of growth at 44 °C to inactivate MsbA in WD201. Cells were harvested, and inner and outer membranes were separated by isopyknic sucrose gradient ultracentrifugation. Glycerophospholipids and lipid A molecules present in each fraction were extracted, separated by TLC, and visualized by the analysis of PhoA fusions (39). LpxO has only two transmembrane domains (one at the N terminus and one near the C terminus) with a large, central active site region that faces the cytoplasm (35). LpxO requires Fe²⁺, ascorbate, and α-ketoglutarate as co-substrates, which are present in the cytoplasm (35).

As shown in Fig. 3, A and B, sucrose gradient ultracentrifugation efficiently separated the outer and inner membranes of both strains as judged by marker enzyme assays. As seen in Fig. 3, C and E, the analysis of fractions from the polymyxin-resistant strain WD101(pmrAc) revealed that glycerophospholipids are evenly distributed between the outer and inner membranes, similar to what is seen in wild-type cells, like the parental strain W3110 (10). Lipid A from the latter strain migrates just below PE in this system (10). However, the lipid A molecules from the polymyxin-resistant strain WD101 are displayed as a group of slowly migrating bands (Fig. 3E, left arrows 1–4). This pattern is because of the presence of one or two hydrophilic phosphoethanolamine and/or aminoarabinose

² PmrC is an older designation for the enzyme EptA and should not be confused with the constitutively active version of the transcription factor, PmrA.²
groups (31, 32), as explained in the legend. These modified lipid A species are present mainly in the outer membrane when MsbA is functional (Fig. 3E).

Fig. 3, D and F show the analysis of the sucrose gradient fractions from the polymyxin-resistant strain WD201, which in addition to pmrA2C contains the temperature-sensitive msbA2 mutation. WD201 accumulates newly made phospholipids and lipid A within its inner membrane after a shift to 44 °C (Fig. 3, D and F), similar to what is seen in the polymyxin-sensitive parental strain WD2 (10). However, the lipid A that accumulates in the inner membrane of WD201 is largely unmodified, as judged by its migration just below PE (Fig. 3F, right arrow). These data indicate that following the inactivation of MsbA newly made lipid A does not gain access to ArnT and EptA/PmrC (Fig. 2), supporting the view that MsbA plays a critical role in the trans-bilayer movement of lipid A.

Hydroxylation of Newly Made Lipid A by LpxO Is MsbA-independent—To make certain that newly synthesized lipid A is not sequestered from all enzymes involved in lipid A modification when msbA is inactivated, we constructed a derivative of WD2 and its parent W3110 expressing the Salmonella lpxO gene (Fig. 2), an inner membrane hydroxylase that can function only when the biosynthesis of hexa-acylated lipid A is completed (35). The active site of LpxO must face the cytoplasm given that it utilizes several cytoplasmic substrates (Fig. 2).

Inactivation of the MsbA mutant protein in WD2/pHSG1 did not interfere with lipid A hydroxylation when compared with W3110/pHSG1 (Fig. 4), consistent with the unrestricted acces-
bility of newly made lipid A to LpxO on the inner surface of the inner membrane under conditions that block lipid export and lipid A modification with phosphoethanolamine or aminoarabinose (Fig. 4). As an additional control the concurrent formation of the lipid A diphosphate variant (Fig. 4A), which was previously shown to be MsbA-dependent (17), was determined in this construct. As shown in Fig. 4B, formation of the lipid A diphosphate variant during the 10-min labeling with $^{32}$P$_i$ following MsbA inactivation in WD2/pHSG1 is reduced by more than 85%.

Reduced Accessibility of Newly Synthesized PE to Membrane-impermeable Reagents following MsbA Inactivation—We next asked the question of whether or not newly synthesized PE, the major glycerophospholipid of E. coli, accumulates on the inner or outer face of the inner membrane following inactivation of MsbA in WD2. To address this question, we first utilized the cell-impermeable, amine-reactive reagent, sulfo-NHS-biotin (41). W3110A and WD2 cells were grown for 30 min at 44 °C, labeled for 10 min with $3 \mu$Ci/ml $^{32}$P$_i$, then incubated on ice in the presence of 0, 1, or 2 mg/ml sulfo-NHS-biotin. Portions were removed at different times, and the reactions were quenched by the addition of excess glycine. Phospholipids were extracted, separated by TLC, and quantified with a PhosphorImager. Fig. 5A shows a time course of biotinylation of the $^{32}$P-labeled PE in the parental strain W3110A. Covalent modification of the newly synthesized PE with sulfo-NHS-biotin is very efficient. Up to 25% of the total cellular PE is modified. The migration of the biotinyl-PE in this TLC system was confirmed by biotinyl-PE labeling of a commercial PE standard (data not shown).

PE biotinylation in W3110A was compared with that in WD2 following MsbA inactivation by growth at 44 °C for 30 min. The biotinylation of newly synthesized PE was much less efficient in WD2 than in W3110A (Fig. 5, B versus A). When quantified by PhosphorImager analysis, the extent of newly synthesized PE modification was reduced 2–3-fold in WD2 compared with W3110A (Fig. 5C). Some of the modified PE seen in WD2 (Fig. 5) might be attributed to the leakiness of the msbA2 point mutation or by other processes facilitating PE flip-flop at a slower rate. It could also be caused by a limited penetration of sulfo-NHS-biotin into the cytoplasmic compartment during the prolonged incubation of the cells with the reagent (Fig. 5).

The relative inaccessibility of newly synthesized PE to chemical modification in WD2 was confirmed using TNBS, a different reagent that has been used to label free amine groups of lipids in many classical studies of membrane topography (13, 42, 43). W3110A and WD2 cells were grown for 30 min at 44 °C, labeled with $^{32}$P$_i$ for 10 min, and then incubated on ice in the presence of 0 or 3 mM TNBS. Portions were removed at different times, and the reaction was quenched by the addition of Tris buffer, albumin, and trichloroacetic acid. Phospholipids were extracted, separated by TLC, and quantified. As shown in Fig. 6A, newly synthesized PE in W3110A is efficiently modified under these conditions. ~30% of the $^{32}$P-labeled PE is converted to [trinitrophenyl]TNP-PE after 90 min of incubation with 3 mM TNBS (Fig. 6C). In contrast, newly synthesized PE in WD2 cells is much less susceptible to modification by TNBS (Fig. 6, B and C). Only 10–12% of the newly synthe-
and WD2 cells were grown for 30 min at 44 °C, labeled with TNBS modification as in wild-type cells. Accordingly, W3110A::Tn10 (msbA2 araoA44 °C for 30 min and then labeled with 32Pi for 10 min. Washed cells in ice-cold phosphate-buffered saline were treated with 3 mM TNBS on ice for the indicated times. Reactions were quenched with albumin and trichloroacetic acid. The phospholipids were extracted and separated by TLC using the solvent chloroform:methanol:H2O (65:25:4, v/v). PE, TNP-PE, and radioactive phospholipids were extracted and separated by TLC using the solvent chloroform:methanol:H2O (65:25:4, v/v). A and B, PE and TNP-PE are indicated with arrows. C, the percent conversion of PE to TNP-PE with time was calculated.

Membrane in WD2 Is Unaffected by Inactivation of MsbA—If MsbA is required mainly for the translocation of newly synthesized PE across the inner membrane, one would predict that any PE exported to the outer membrane in WD2 at 44 °C due to leakiness of the msbA2 mutation would be as susceptible to TNBS modification as in wild-type cells. Accordingly, W3110A and WD2 cells were grown for 30 min at 44 °C, labeled with 32P, and treated with 3 mM TNBS on ice for 90 min. The reactions were quenched, as described above, with Tris buffer and albumin but without trichloroacetic acid. Membranes were prepared and separated into inner and outer fractions by isopycnic sucrose gradient ultracentrifugation. A and B, outer and inner membrane marker enzymes, phospholipase A (red x), and NADH oxidase (blue squares), respectively, and protein (black circles) are expressed as the percent of the total, as described in the legend to Fig. 3. C and D, radioactivity (32P) in each fraction was determined by counting 50–μl samples. E and F, the radioactive phospholipids were extracted and separated by TLC using the solvent chloroform:methanol:H2O (65:25:4, v/v). PE, TNP-PE, and phosphatidylglycerol (PG) are indicated. G and H, the percent of the total PE in each sucrose gradient fraction that was converted to TNP-PE was calculated for E and F.

W3110A. The results indicated that some of the modified PE seen in WD2 (Figs. 5 and 6) originates from newly synthesized PE that reached the outer membrane, most likely because of the leakiness of the msbA2 point mutation. Newly synthesized inner membrane PE in WD2 is much less accessible to TNBS than in the wild-type suggesting a predominant cytoplasmic orientation of this lipid.

DISCUSSION

The inactivation of MsbA in WD2, or in related strains harboring the msbA2 mutation, leads to rapid accumulation of newly synthesized core-lipid A and glycerophospholipid molecules within the inner membrane (Figs. 3 and 7) (10). We have now demonstrated that the modification of newly synthesized lipid A with phosphoethanolamine and aminophosphonate groups in polymyxin-resistant mutants (31, 32) is also MsbA-depend-
ent (Fig. 3). However, LpxO-catalyzed hydroxylation of lipid A (35) is MsbA-independent (Fig. 4). Given the orientation of these inner membrane enzymes (Fig. 2), we concluded that MsbA must participate in the flipping of newly synthesized core-lipid A molecules from the cytoplasmic to the periplasmic surface of the inner membrane (Fig. 1A). The case is especially compelling for EptAP/PrmC (Fig. 2), because a large pool of PE is always present on the outer surface of the inner membrane to serve as a phosphoethanolamine donor substrate for the modification of a newly synthesized lipid A (23). Recent data with PhoA fusions of EptAP/PrmC have established conclusively the periplasmic localization of the large conserved C-terminal domain of this enzyme, which contains the active site (39).

The possibility that MsbA carries out additional functions on the periplasmic side of the inner membrane must be considered. ATP hydrolysis might be required for periplasmic lipid release from MsbA, or for lipid ejection from the outer surface of the inner membrane in coordination with other envelope proteins (Fig. 1). Whatever the case may be, the fact that MsbA was originally identified as a multicopy suppressor of E. coli LpxL(HtrB) mutants (18, 44, 45), which accumulate tetra-acylated lipid A precursors in their inner membranes unless MsbA is overproduced (17) supports a direct role for MsbA in LPS export.

The reduced reactivity of newly synthesized PE toward sulfo-NHS-biotin (Fig. 5) and TNBS (Fig. 6) following MsbA inactivation in WD2 is intriguing, as it suggests a significant function for MsbA in catalyzing the trans-bilayer movement of glycerophospholipids (Fig. 1A). We cannot, however, exclude the possibility that the accumulation of core-lipid A on the inner surface of the inner membrane interferes with glycerophospholipid flip-flop catalyzed by some other protein. E. coli mutants with a reduced accessibility of newly synthesized PE release from MsbA, or for lipid ejection from the outer surface (50, 51), but otherwise in an operon downstream of msbA (50, 51). Like MsbA (17), LpxK is essential for growth (51), but point mutations in lpxK have not been characterized. A possible role for LpxK in lipid translocation might be established by searching for temperature-sensitive lpxK alleles that block lipid export without compromising 4'-kinase activity. A more general search for additional E. coli mutants defective in lipid export, based on selectable phenotypes of WD2, might also prove informative.

The large periplasmic extend of MsbA homologues are present in eukaryotic organisms (19, 52). Some of these proteins have been reported to catalyze phospholipid flip-flop when reconstituted in vitro (53). Unlike what is seen with E. coli MsbA, however, no single eukaryotic ABC transporter has emerged as being essential for generalized lipid transport (52). The best characterized eukaryotic ABC transport proteins are implicated in the trafficking of specific lipids. For instance, ABCA1 is required for reverse cholesterol transport (54, 55), whereas mouse Mdr2 is needed specifically for biliary phosphatidylcholine secretion (56, 57). The presence of so many MsbA homologues in eukaryotic cells suggests that there may be redundancy of function. Mutations in multiple selected ABC transporters may be required to cause generalized lipid transport defects, such as those associated with the mba2 mutation in WD2.

REFERENCES