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Phospholipid Transport by ABCA1: The Extracellular Translocase or Alternating Access Model?

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Abstract

Purpose of review: ATP-binding cassette transporter A1 (ABCA1) plays a key role in HDL biogenesis and cholesterol export from artery wall cells. Recent evidence challenges the generally accepted model for lipid transport by ABCA1, termed the alternating access mechanism, which proposes that phospholipid moves from the inner leaflet to the outer leaflet of the plasma membrane.

Recent findings: In contrast to the standard model, our computer simulations of ABCA1 indicate that ABCA1 extracts phospholipid from the plasma membrane's outer leaflet. The lipid then diffuses into the interior of ABCA1 to contact a structure termed the 'gateway'. A conformational change opens the gateway and forces the lipid through a ring-shaped domain, the 'annulus orifice', into the base of an elongated hydrophobic tunnel in the transporter's extracellular domain. Engineered mutations in the gateway and annulus strongly inhibited lipid export by ABCA1 without affecting cell-surface expression levels of the transporter, strongly supporting the proposed model.

Summary: Our demonstration that ABCA1 extracts lipid from the outer face of the plasma membrane and forces it into an elongated hydrophobic tunnel contrasts with the alternating access model, which flops phospholipid from the membrane's inner leaflet to its outer leaflet. These results suggest that ABCA1 is a phospholipid translocase that transports lipids by a mechanism distinct from that of other ABC transporters.

Keywords

HDL; cholesterol efflux; atherosclerosis

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Conflicts of interest.

None.

Introduction

Atherogenic lipoproteins promote the unregulated accumulation of cholesterol in artery wall cells, promoting both the initiation and progression of atherosclerosis. In contrast, high-density lipoprotein (HDL) may be cardioprotective, in part because it stimulates cholesterol export from macrophages[1,2]. The underlying mechanism involves phospholipid transport by ABCA1, an ATP-binding cassette (ABC) transporter that is integral to the plasma membrane and uses energy derived from ATP to rid the membrane of excess lipid.

Most ABC transporters translocate substrates across cellular membranes[3]. The ABC membrane transporter superfamily has five subfamilies[4,5]. The medical importance of subfamily A, which includes ABCA1, is demonstrated by its association with a wide variety of inherited human diseases. For example, mutations in ABCA1 cause Tangier disease, a rare genetic disorder that impairs phospholipid and cholesterol export from cells, causing cholesterol accumulation in macrophages and very low levels of HDL[5,6].

Apolipoprotein A-I (APOA1), the major HDL protein, binds directly to ABCA1 in the plasma membrane, promoting the export of phospholipid and cholesterol from cells, though the underlying mechanisms remain unclear[5]. Phillips proposed that ABCA1 translocates phospholipid from the inner to the outer leaflet of the plasma membrane, where cholesterol accumulates to promote the formation of nascent HDL particles[7].

The generally accepted model for substrate export by ABC transporters, called the alternating access mechanism, envisions a switch between a inward-open cavity that opens to the interior of the plasma membrane which transitions to an outward-open cavity that faces the exterior of the plasma membrane[8]. For ABCA1, the inward-facing cavity is proposed to accept phospholipid from the inner leaflet of the plasma membrane[7], which closes when ABCA1 binds ATP, vectorially transporting the substrate into the outer leaflet of the membrane. In this model, phospholipids rotate through 180° to assume the orientation of outer leaflet lipids. However, such reorientation would be energetically costly, and it is unclear how inner leaflet phospholipids could undergo such a rotation[7]. Moreover, in the cryo-electron microscopy (cryo-EM) structure of ATP-free ABCA1[9], the transmembrane domains do not form an inward-facing transmembrane cavity, which would be required for the alternating access mechanism[7,8]. In contrast, the dimeric complex of the half-transporters ABCG5/ABCG8, which play key roles in sterol secretion into the bile and gut by promoting the transport of substrate across the membrane[10], do have an inward-facing transmembrane cavity similar to that of other nucleotide-free ABC exporters.

Recent observations

The key structural features of ABCA1—the outward-facing transmembrane cavity, extracellular domains, transmembrane domains, and elongated hydrophobic tunnel[13]—are shown in Fig. 1. To determine how ABCA1 transports phospholipid, we inserted the cryo-EM structure of the protein[9] into a phospholipid membrane bilayer made of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (phosphatidylcholine, POPC)[11]. We then used both coarse-grained molecular dynamics (CGMD) and steered molecular dynamics

(SMD) to study interactions between phospholipid and protein. This approach allowed us to monitor both the movements of phospholipids and alterations in ABCA1's structure during simulations of wild-type and mutated ABCA1 structures.

The final CGMD simulations, converted to all-atom models, demonstrated that 3–5 phosphatidylcholine molecules extracted from the outer leaflet of the membrane had diffused through an opening in the outward-facing transmembrane cavity of ABCA1 into the interior of the protein[11]. The extracted phospholipids initially formed a complex, termed the **membrane mound,** that resided above the plane of the lipid bilayer (Fig. 1). At the end of the simulations, the mound collapsed into a single phospholipid molecule, indicating the transient nature of the mound.

Inspection of the cryo-EM structure revealed a loop of ABCA1 at the base of the elongated hydrophobic tunnel[11]. This loop was contiguous with the outward-facing transmembrane cavity on one side and the hydrophobic tunnel on the other[13]. The large open loop contained 29 amino acid residues. Multiple charged residues in the loop formed salt bridges with the head groups of the phospholipids that formed the membrane mound.

We term the loop formed by the residues the **gateway** (Fig. 1) for three reasons[11]. First, phospholipid molecules from the membrane diffused through the loop toward the elongated hydrophobic tunnel of ABCA1. Second, four of the point mutations that cause Tangier disease are in that loop (D571G, R587W, W590S/L). Third, mutating charged amino acid side chains in the loop blocked the movement of membrane phospholipid into the interior of ABCA1 in our simulations. They also inhibited ABCA1-dependent phospholipid and cholesterol export by cultured cells without affecting the protein's cell-surface expression. These observations suggest that the gateway regulates the movement of phospholipid into the elongated hydrophobic tunnel.

Another structure, termed the **annulus** (Fig. 1) residues 69, 71–80, 363, 368–379), formed a compact domain at the elongated hydrophobic tunnel's base[11]. We defined the annulus domain as all residues at the base of the elongated hydrophobic tunnel within 10 Å of gateway residues. Mutations of key residues in the central portion of this domain, called the **annulus orifice**, impaired ABCA1-dependent phospholipid and cholesterol export by cultured cells, indicating that the annulus regulates phospholipid export by ABCA1.

Three potential energy barriers would likely prevent the spontaneous movement of POPC through the annulus orifice into the elongated hydrophobic tunnel[11]. First, exposing the acyl chains of POPC to solvent as they move up the gateway to the annulus would be energetically costly. Second, movement of the charged headgroup of POPC into the hydrophobic outward-open transmembrane cavity and into the annulus orifice would also be unfavorable. Third, Van der Waals forces would make it difficult for POPC to pass through the initially narrow annulus orifice. Overcoming these possible energy barriers would likely require some type of ATP-dependent power stroke.

We used SMD to simulate the movement of a POPC molecule from the membrane mound to the annulus and elongated hydrophobic tunnel[11]. A single POPC molecule initially interacted with the gateway and was then translocated through the orifice as its $N(CH)_3^+$

headgroup made Van der Waals contact with the annulus. Passage of POPC through the annulus orifice required input of force. As the POPC molecule moved through the orifice, the helical region in the annulus partly unwound[11]. These observations suggest that phospholipid can move from the outward-facing transmembrane cavity into the elongated hydrophobic tunnel if the annulus changes its conformation by using an energy source such as ATP. To test this hypothesis, we generated three different double mutations in ABCA1 that were predicted to prevent the annulus from opening because of disulfide crosslinking or salt bridge formation. We then expressed the wild-type and mutated ABCA1s in cells, and quantified phospholipid and cholesterol efflux. All three double mutations reduced lipid efflux by ABCA1 by >80% compared with wild-type ABCA1. Importantly, none of the three mutations affected ABCA1's cell-surface expression.

Our simulations suggest that ABCA1 transports phospholipid by a mechanism distinct from the alternating-access mechanism and that of other known ABC transporters. To further explore that mechanism, we constructed a homology model of ATP-bound ABCA1 based on the cryo-EM structures of ABCA4[12,13] (such structures are not yet available for ABCA1-ATP). The amino acid sequence of ABCA1 is 51% identical to and 66% similar to the sequence of ABCA4[14]. Moreover, phylogenetic modeling[15] has shown that the two proteins are among the most closely related ABCA transporters[16]. Finally, ABCA4 extracts its substrate from the outer monolayer of the plasma membrane[13,14], in excellent agreement with our proposed model of phospholipid transport by ABCA1.

In the cryo-EM structure of ATP-free ABCA1, the transmembrane cavity is outward-facing[10]. In contrast, our homology model of ATP-bound ABCA1 resembles the cryo-EM structure of ABCA4 because there is little evidence of a substrate-binding transmembrane cavity on either side of the membrane leaflet[11]. This homology model suggests that ATP binding changes the conformation of ABCA1 while providing the energy for translocating phosphatidylcholine through the annulus orifice and into the elongated hydrophobic tunnel.

We hypothesized that the change from the outward-open to outward-closed conformation of ABCA1 might also open the annulus orifice of the gateway. Inspection of the two conformations revealed that the orifice's two helices moved 6 Å farther apart, providing space for phospholipid molecules to pass through[1]. Our observations suggest that ATP-driven changes in the conformation of ABCA1 from the outward-open to the outward-closed form provides the force for translocating substrate through the gateway, and into the elongated hydrophobic tunnel (Fig 2).

Our findings contrast with the alternating access mechanism of substrate export used by ABC transporters with floppase activity. Those transporters switch between an open, inward-facing transmembrane cavity and an open, outward-facing transmembrane cavity **7,8**. Moreover, most of their structures reveal multiple basic amino acid residues in the middle of the transmembrane domain. In contrast, the cryo-EM structure of ABCA1 has only hydrophobic amino acids in the middle of the transmembrane domains[11]. These observations are inconsistent with the alternating access model for phospholipid transport by ABCA1.

Conclusion

Our simulations suggest that a key early step in phospholipid export by ABCA1 is extraction of lipid from the outer leaflet of the plasma membrane into the transporter's outward-open transmembrane cavity (Fig. 2) to provide an orifice into the protein's interior. Homology modeling using the ABCA4-ATP cryo-EM structure suggested that closure of the outward-open transmembrane cavity drives lipid out of the cavity. Our model (Fig. 2) support the assertion that ABCA1 is an extracellular translocase that transports phospholipid from the outer leaflet of the plasma membrane through the gateway-annulus complex and into the elongated hydrophobic tunnel[11].

Importantly, this hypothesis is consistent with the outward-open transmembrane cavity of nucleotide-free ABCA1 determined by cryo-EM[13] and of the outward-closed ATP-bound ABCA1 we produced by homology modeling of ABCA4-ATP. In striking contrast, the alternating access model predicts that nucleotide-free ABCA1 should exhibit an inward-facing transmembrane cavity and that ATP-bound ABCA1 should have an outward-facing one[7].

It is important to note that our studies used a simple phospholipid membrane as a first approximation to plasma membranes. It will be important to extend our observations to lipid mixtures that more closely mimic the plasma membrane.

ABCA1 expressed by cultured macrophages promotes the formation of three classes of extracellular lipid particles[17]. In the presence of APOA1, two of the classes (9 nm and 12 nm in diameter) contain APOA1, HDL's major protein. The 9 nm particles are similar in size to nascent HDL generated by ABCA1 in humans. The composition of the 9 nm particles generated by macrophages was 66% phosphatidylcholine, 9% sphingomyelin, and 26% acidic phospholipids. Phosphatidylcholine and sphingomyelin are located largely in the extracellular side of the plasma membranes of mammalian cells while acidic phospholipids are in the inner leaflet[18]. The finding that ~75% of the phospholipids in the small HDL-like particles were outer leaflet lipids supports our proposed model.

ABCA1 reconstituted into liposomes was proposed to use ATP to actively transport fluorescently labeled phospholipid from the cytosolic (inner) to exocytoplasmic (outer) leaflets of the membrane[19]. If the alternating access mechanism were involved, phospholipid would have to rotate through 180° inside the transporter's transmembrane domains. ABC transporters that use the alternating access mechanism to flop charged substrates tend to have counter-charged amino acids in the middle of the transmembrane domain[11]. This property is critical for moving charged substrates across membrane bilayers because opposing charges on a transporter reduce the energy barrier to moving an amphipathic substrate across the bilayer[20]. Because the middle of the transmembrane domain of monomeric ABCA1 lacks charged groups, such a rotation would likely be energetically unfavorable. Collectively, these observations argue against the alternating access mechanism for ABCA1 and, by extension, the widely held view that it is an intracellular lipid floppase. Instead, we argue that it is an extracellular phospholipid translocase.

We identified the gateway as a 29-residue loop of ABCA1 between the outward-open transmembrane cavity and the elongated hydrophobic tunnel[11]. CGMD simulations of human ABCA1 embedded in a phosphatidylcholine bilayer showed that the gateway extracts the membrane mound—a 3–5-molecule cluster of phosphatidylcholine—by lifting it above the extrafacial surface of the plasma membrane. The amino acid residues lining the elongated hydrophobic tunnel that are in Van der Waals contact with the gateway form the annulus. The orifice of the annulus is situated to accommodate a phosphatidylcholine molecule during translocation into the elongated hydrophobic tunnel[11].

The physiological relevance of our results is exemplified by the molecular defects in Tangier disease. In this disorder, any one of eight mutations in extracellular domain-1, which forms the large exocytoplasmic domain of ABCA1, impairs cholesterol export from cells[11]. Four of these mutations—D571G, R587W, W590S, and W590L[21]—reside in the gateway. Of the other four, one is near the gateway and three line the elongated hydrophobic tunnel[11]. Consistent with the hypothesis that the gateway plays an important role in phospholipid export, 25 of its 29 residues are 100% conserved in 34 species of ABCA1, from mammals to bony fish[11]. Of the 53 amino acid residues that make up the gateway–annulus complex, 46 are highly conserved[11].

Although our gateway–annulus model provides strong evidence that phospholipid is translocated into the elongated hydrophobic tunnel of ABCA1, it does not address how translocation subsequently induces cholesterol efflux and the formation of nascent HDL. One hypothesis is that cholesterol efflux is driven by a concentration gradient: free cholesterol in the plasma membrane migrates into cholesterol-free phosphatidylcholine exported by ABCA1[6,22]. Our demonstration that phosphatidylcholine moves from the outer leaflet of the plasma membrane into the elongated hydrophobic tunnel is consistent with the hypothesis that the lipid subsequently migrates into the extracellular milieu, where it could play a role both in cholesterol export and the biogenesis of HDL. It should be noted that our model is also consistent with the proposal that lipid buildup in ABCA1's monomeric extracellular domain triggers the transporter to dimerize and bind to APOA1 to produce nascent HDL[23]. However, our model does not explain how ABCA1 could create an active cholesterol-rich plasma membrane surface where lipid-free APOA1 could be transformed into a nascent HDL particle[24] by micro-solubilization. In future studies, it will be important to determine how phospholipid translocation into the elongated hydrophobic tunnel of ABCA1 feeds distal events that promote cholesterol efflux and HDL formation.

Our model of ABCA1 as an extracellular phospholipid translocase suggests a distinct transport mechanism that differs substantially from mechanisms described for the other members of the ABC transporter superfamily. This surprising finding highlights the remarkable diversity in substrate transport within the that family.

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Key Points

- Our findings demonstrate that ABCA1 transports phospholipid from the outer leaflet of the plasma through the interior of the protein and then into the elongated hydrophobic tunnel in the extracellular compartment of ABCA1.
- Phospholipid movement initially involves the formation of a membrane mound in the outer leaflet of the plasma membrane, followed by lipid diffusion into the gateway in the interior of the transmembrane domain, which then undergoes an ATP-dependent conformational change propeling the phospholipid through the annulus and into the elongated hydrophobic tunnel.
- Homology modeling of ATP-ABCA1 suggests that the mechanism involves the closing of an outward-open to outward-closed conformation of the protein, which is distinct from the inward-open to outward-open mechanism of the alternating access model.

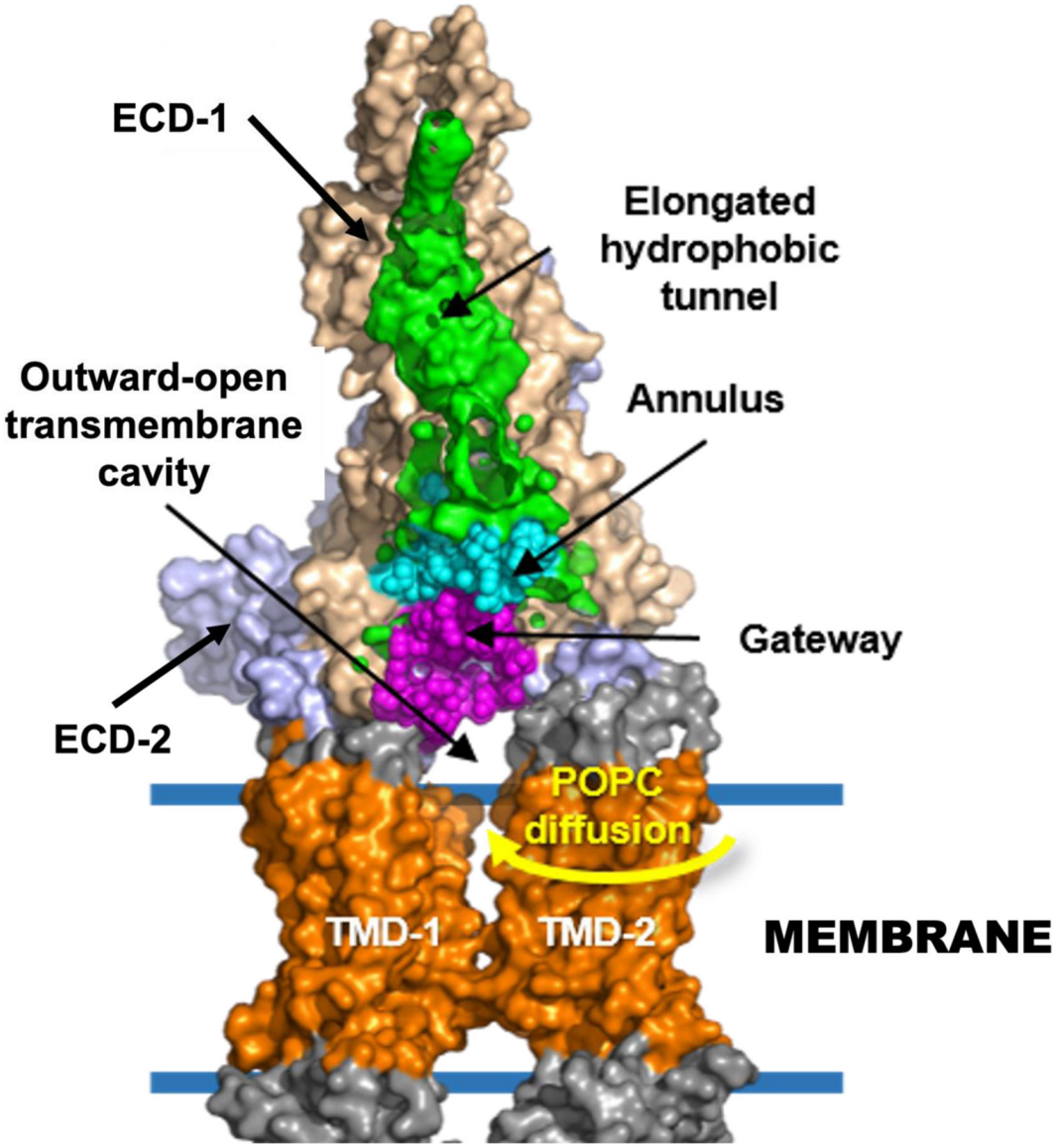

Figure 1. Key structural features of ABCA1.

The outward-facing transmembrane cavity, transmembrane domains (TMD-1 and TMD-2 (orange)), extracellular domains (ECD-1 (peach) and ECD-2 (light blue)), and the elongated hydrophobic tunnel (green) were identified in the structure of ABCA1[9,11]. The elongated hydrophobic tunnel in ECD-1 was demarcated using the PyMol cavity algorithm. The gateway (residues 564–592, magenta) and annulus (residues 69, 71–80, 363, 368–379, cyan) are also shown. The annulus forms the bottom of the elongated hydrophobic tunnel. Also shown is the pathway for diffusion of POPC from the outer leaflet of the membrane bilayer (blue lines) into the outward-facing transmembrane cavity (yellow arrow).

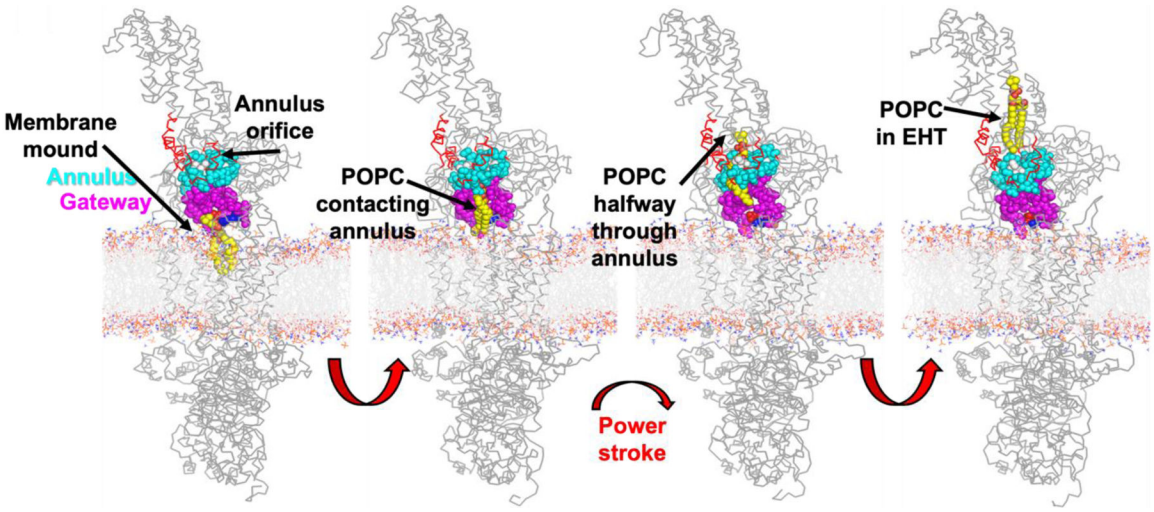

Figure 2. Proposed steps in the pathway for phospholipid transport by ABCA1.

The starting structure was a simulation of ABCA1 embedded in a POPC bilayer. The gateway and annulus are colored magenta and cyan, respectively. **First,** outer leaflet POPC molecules of the plasma membrane form a membrane mound that resides outside the outer leaflet of the membrane. **Second**, a single POPC molecule (yellow) contacts the gateway. **Third**, a conformational change in the gateway positions the lipid for movement through a ring-shaped domain, termed the annulus orifice, into the base of an elongated hydrophobic tunnel (EHT). **Fourth**, POPC extracted by the gateway/annulus complex migrates into the EHT. To be energetically favorable in vivo, we propose that the gateway conformational change and the movement of POPC into the ETH is mediated by an ATP-dependent process —the power stroke. The fate of the POPC in the ETH, which is in the interior of the extracellular domain, is unclear. We hypothesize that POPC migrates from that location into the extracellular environment, where is plays a key role in promoting cholesterol export and the biogenesis of nascent HDL.