Review

Function of prokaryotic and eukaryotic ABC proteins in lipid transport

Antje Pohl, Philippe F. Devaux, Andreas Herrmann*

A Humboldt-University Berlin, Institute of Biology, Invalidenstr. 42, D-10115 Berlin, Germany
B Institut de Biologie Physico-Chimique, UMR CNRS 7099, 13 rue Pierre et Marie Curie, 75005 Paris, France

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Abstract

ATP binding cassette (ABC) proteins of both eukaryotic and prokaryotic origins are implicated in the transport of lipids. In humans, members of the ABC protein families A, B, C, D and G are mutated in a number of lipid transport and metabolism disorders, such as Tangier disease, Stargardt syndrome, progressive familial intrahepatic cholestasis, pseudoxanthoma elasticum, adrenoleukodystrophy or sitosterolemia. Studies employing transfection, overexpression, reconstitution, deletion and inhibition indicate the transbilayer transport of endogenous lipids and their analogs by some of these proteins, modulating lipid transbilayer asymmetry. Other proteins appear to be involved in the exposure of specific lipids on the exoplasmic leaflet, allowing their uptake by acceptors and further transport to specific sites.

Additionally, lipid transport by ABC proteins is currently being studied in non-human eukaryotes, e.g. in sea urchin, trypanosomatides, arabidopsis and yeast, as well as in prokaryotes such as *Escherichia coli* and *Lactococcus lactis*. Here, we review current information about the (putative) role of both pro- and eukaryotic ABC proteins in the various phenomena associated with lipid transport. Besides providing a better understanding of phenomena like lipid metabolism, circulation, multidrug resistance, hormonal processes, fertilization, vision and signalling, studies on pro- and eukaryotic ABC proteins might eventually enable us to put a name on some of the proteins mediating transbilayer lipid transport in various membranes of cells and organelles.

It must be emphasized, however, that there are still many uncertainties concerning the functions and mechanisms of ABC proteins interacting with lipids. In particular, further purification and reconstitution experiments with an unambiguous role of ATP hydrolysis are needed to demonstrate a clear involvement of ABC proteins in lipid transbilayer asymmetry.

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Keywords: ABC protein superfamily; Flippase; Cholesterol; Lipid asymmetry; Lipid exposure; Molecular mechanism

1. Introduction

The ATP binding cassette (ABC) protein superfamily comprises transporters for a whole variety of organic and inorganic compounds. In 1992, Higgins and Gottesmann [1] pointed out that ABCB1 (MDR1 Pgp) behaved like a “flippase” which would be able to transport amphiphilic molecules (potentially also lipids) from the inner to the outer leaflet of the plasma membrane. Since then, there have been many indications for lipid transport mediated by ABC proteins in cellular membranes, and a few reports on lipid transport by purified ABC proteins in reconstituted systems. Lipid transport by human ABC proteins has been the subject of several review articles in the past [2–5]. In the following, we will first give a general introduction on lipid transbilayer movement and assays used for its determination, before reviewing data accumulated on the involvement of eukaryotic and prokaryotic ABC proteins in lipid transport. We will then discuss their putative role in lipid transbilayer transport and exposure, and show models proposed for mechanisms of lipid transbilayer transport by ABC proteins,
before relating some concluding remarks. Considering the broad span of disciplines contributing to this topic, it appears to be important in this article to draw a clear line between the transport of endogenous lipids and lipid analogs carrying a reporter group or other modifications. Similarly, phenomena which appear to be related to the action of a particular protein need to be distinguished from those for which a specific transport has been proven unequivocally. On the one hand, most attempts in reconstituted systems have revealed only very small effects of ABC proteins on lipid transport so far, with an influence of ATP hydrolysis hardly significant.

In experiments on whole cells, on the other hand, the involvement of various transport steps must be taken into account, complicating transport rate quantification, and hence the evaluation of their physiological significance.

2. Lipid transbilayer movement

Lipids form a vast group of chemically different amphiphilic or hydrophobic substances containing a substantial portion of aliphatic or cyclic hydrocarbon. Abundant lipid classes are (glycero-) phospholipids, sphingolipids, steroids, lipopolysaccharides and triacylglycerols (for some examples see Fig. 1). The phospholipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyserine (PS) and phosphatidylinositol (PI), the sphingolipid sphingomyelin (SM) and the steroid cholesterol are the major lipids found in mammalian membranes [6,7].

The lipid transbilayer distribution in the eukaryotic plasma membrane is asymmetrical, generally with the majority of PC and sphingolipids in the exoplasmic leaflet, and the aminophospholipids PE and PS mainly in the cytoplasmic leaflet (reviewed in [8]). This asymmetry raises several questions about the biological mechanisms by which it is established (Fig. 2A), and about the putative biological functions coupled to it.

2.1. Spontaneous transbilayer movement

The rate of spontaneous transbilayer movement (flip-flop) in a pure lipid membrane differs for each lipid, depending on its structure (headgroup, backbone) and its environment (reviewed in [9]). Small, uncharged lipids (including cholesterol) and negatively charged lipids in their

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Fig. 1. Lipid substrates of ABC proteins (examples). Hydrophilic parts are indicated by blue clouds. Examples for the phospholipid moiety X are ethanolamine (in PE), choline (in PC) or serine (in PS). Examples for the sphingolipid moiety Y are hydrogen (in ceramide), phosphorylcholine (in SM), glucose (in GlcCer), galactose (in GalCer) or lactose (in LacCer). The fatty acid shown is oleic acid, the steroid shown is cholesterol. Lipid A structure as predominant in E. coli [218].
protonated form can flip across pure lipid bilayers within seconds or minutes. In contrast, lipids with highly polar headgroups move only slowly from one leaflet of a lipid bilayer to the other (half-times of the order of hours to days \[10,11\]). Cholesterol has been shown to decrease the transbilayer movement of phospholipids \[12,13\].

2.2. Energy-independent and energy-dependent flippases

In eukaryotic cells, most lipids are synthesized asymmetrically in the membranes of the endoplasmic reticulum (ER) and the Golgi system from which they reach, e.g. via vesicle traffic, the plasma membrane or other organelles (reviewed in \[9\]). Because lipid movement across cellular membranes is essential for cell growth and survival, lipid transporting proteins (flippases) are required for efficient transbilayer lipid movement. Although some proteins have been identified as candidate lipid transporters over the last years (reviewed in \[9,14,15\]), the protein vehicles responsible for many lipid transport phenomena have not been identified yet (Table 1).

In the mammalian ER, energy-independent flippases were shown to mediate rapid bidirectional, rather unspecific phospholipid flip-flop (half times of the order of minutes or less) to ensure balanced growth of this membrane \[16–18\]. Similarly, rapid protein-mediated flip-flop has been demonstrated for phospholipids and glucosylceramide (GlcCer, a precursor for complex glycosphingolipids) in the Golgi \[19\]. Lipid flippase activity was also found in the bacterial plasma membrane \[20,21\].

Already in 1980, it was shown that the mere presence of membrane proteins facilitates lipid flip-flop \[22\]. Thus, it is not clear whether a dedicated flippase or a family of selective flippases is involved in bidirectional lipid movement across ER and Golgi membranes \[23,24\].

Energy-independent, bi-directional transbilayer movement of all major phospholipids has equally been shown in the eukaryotic plasma membrane. This flip-flop (half time of the order of 1 min) \[25,26\], activated by cell stimulation and the subsequent increase in intracellular calcium, has been ascribed to the lipid scramblase protein. Effort has been made to isolate and clone the potential scramblase PLSCR1 \[27\].

Experiments with fluorescent (nbd1) phospholipids in the rabbit intestine brush border have indicated ATP- and Ca\(^{2+}\)-independent transbilayer movement of monoacyl phospholipids and lipids with short chains, supposedly constituting a mechanism for the intestinal uptake of phospholipid digestion products as lysolipids \[28\].

While energy-independent flippases allow lipids to equilibrate rapidly between the two bilayer leaflets, energy-dependent flippases are responsible for a net transfer of specific lipids to one leaflet of a membrane. In the plasma membrane of eukaryotic cells, spontaneous flip-flop of phospholipids is limited, possibly due to the high cholesterol content, allowing the generation of a stable asymmetric lipid
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distribution between the two leaflets. The depletion of cholesterol leads to an enhanced spontaneous flip-flop of phospholipid analogs in the red blood cell membrane [12].

PE and PS are subject to an efficient and rather rapid energy-dependent inward transport from the outer to the inner leaflet mediated by a protein, the aminophospholipid translocase [29,12]. Some cell types display similar transport of PC across the plasma membrane and may contain either a PC-specific translocase in addition to the aminophospholipid translocase, or an inward translocase of different specificity which transports both aminophospholipids and PC (see [15]). The aminophospholipid translocase, not yet identified on the molecular level, very likely belongs to the novel Drs2p P-type-ATPase subfamily with over a dozen members in eukaryotes from yeast to plant cells [30,31,15]. Two members of this family, Dnf1p and Dnf2p, have been shown to be essential for ATP-dependent transport of fluorescent analogs of PS, PE, and PC from the outer to the inner leaflet of the yeast plasma membrane [32].

Recently, it was reported that the fluorescent analog nbd PS is a preferred substrate of Drs2p localizing to the trans-Golgi-network [33].

However, it is still unclear what permits the accumulation of the majority of PC and sphingolipids in the mammalian outer plasma membrane leaflet. ABC proteins were suggested to be involved in the outward transport of phospholipids [34]. Alternatively, it was proposed on theoretical grounds that the inward transport of aminophospholipids, together with passive fluxes, would be sufficient to accumulate choline containing lipids in the outer leaflet [35].

Changes in the distribution of a lipid species between the membrane leaflets can influence membrane curvature and fusion competence, protein association and activity, as well as various biochemical pathways (reviewed in [9]). Because of the low compressibility of lipid monolayers, the inward and outward transport of lipids requires a subtle balance. In the absence of a compensatory flux, unidirectional lipid transport by energy-coupled transporters might create an area imbalance between the two membrane leaflets, building up surface tension eventually relaxing by membrane budding or invagination [36,37] (see also Section 6). This phenomenon was suggested to be a molecular motor for the first stage of endocytosis [38,39]. The balance between inner and outer membrane leaflet has been generally overlooked when the activity of potential lipid transporters was assessed in large unilamellar vesicles (LUVs), where surface tension generated by lipid transport could be sufficient to eventually block the transporter itself (discussed in [37,39,40]).

2.3. Determination of lipid transbilayer distribution

The techniques used to determine the transbilayer distribution of lipids have been described and critically evaluated in the literature [7,41–46]. In Fig. 3, some assays for lipid analogs as well as for endogenous lipids are shown schematically. Since methods for the rapid quantification of endogenous lipids are still very limited, spin-labeled (SL) or fluorescent lipid analogs are frequently employed to determine lipid transbilayer distribution. The relevance of such probes has been discussed by Devaux et al. [42]: Briefly, bulky reporter moieties and a short fatty acid chain replacing one of the natural long chains to facilitate membrane incorporation may affect lipid polarity and cause perturbations, somewhat modifying the absolute values of the transbilayer distribution of a lipid. However, compar-

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Substrates confirmed in reconstitution experiments are in bold print. Question marks indicate functions or substrates for which an involvement has not been proven experimentally.
isons between lipids with different headgroups, but with the same fatty acid chains, can be very revealing about the specificity of the lipid transport. Recently, various cholesterol analogs have been studied showing large variations in the potential to mimic endogenous cholesterol [44]. Nevertheless, new assays will have to be developed to rapidly determine the transbilayer distribution of endogenous lipids.

3. General features of the ABC protein superfamily

The ATP-binding cassette (ABC) protein superfamily comprises a large number of transporters, channels and regulators in pro- and eukaryotes [reviewed in [47–49]]. Their functions range from the acquisition of nutrients and the excretion of waste products to the regulation of various cellular processes. Generally, ABC proteins are low capacity, but high affinity transporters, able to transport substrates against a concentration gradient of up to 10 000 fold. Hydrolysis of ATP is required for substrate transport. ABC proteins are mainly either import or export pumps, bidirectional ABC proteins appear to be rare exceptions [50]. Import pumps seem to be limited almost exclusively to prokaryotes. Typically, ABC proteins are relatively specific for a particular set of substrates (the multispecific ABCB1 (MDR1 Pgp) probably represents an exception in this respect). Substrates can be amino acids, sugars, inorganic ions, peptides, proteins, lipids and various organic and inorganic compounds. ABC proteins consist of nucleotide binding domains (NBDs), and of transmembrane (TM) domains of usually six alpha-helices. NBDs and TM domains can occur as separate proteins (frequently found in prokaryotes) or fused together. The transmembrane domains vary considerably between different ABC proteins, whereas the nucleotide binding domains are highly conserved (e.g. Walker motifs). The substrate specificity is believed to be determined by the transmembrane domains, including the loops connecting the individual helices [47].

4. Eukaryotic ABC proteins

Structurally, eukaryotic ABC proteins typically possess two nucleotide binding domains and two TM domains, probably representing the minimal functional unit (full-size protein). In some ABC proteins, deviating organizations of the domains can prevail [49] (Fig. 4), e.g. half-size proteins with one NBD and one TM domain each, thought to require
dimerization in order to be functional. ABC proteins with nucleotide binding domains only (families E, F) are likely not directly involved in membrane transport, and are thought to have regulatory functions.

4.1. Human ABC proteins

The 49 human ABC proteins currently known can be classified into 7 families (A–G) according to sequence similarity [49, 51]. An overview can be found on Michael Müller’s website (http://nutrigene.4t.com/humanabc.htm). Several human ABC proteins found to be mutated in lipid-linked diseases (families A, B, C, D, and G) were suggested to be involved in lipid transport. Although diseases due to complete loss of function of single ABC proteins do not appear to have a very high incidence in the population, polymorphisms in ABC genes are likely to play a role in medically relevant phenomena. At present, direct transport of lipid substrates has only been shown for a small number of human ABC proteins. The ABC proteins identified in mammals so far (e.g. in mouse, rat, pig) are highly similar to members of the human ABC protein families, and will therefore not be discussed separately in this work.

4.1.1. Human ABCA (ABC1) family

Out of the 12 ABCA family members, all of which are full-size proteins, 8 are assumed to transport lipophilic substrates. For a number of these, an involvement in lipid transport was deduced from lipid dependent expression, and homology to ABCA1, but has not yet been confirmed experimentally.

ABCA1 (ABC1), studied intensely with regards to lipid transport, is expressed in numerous tissues such as the trachea, lung, adrenal gland, spleen and uterus [52], in some of which its expression is steroid-dependent [53]. ABCA1-GFP chimeras localize to the plasma membrane and to intracellular vesicles in transfected HeLa cells [54]. ABCA1 has been implicated with the transport of cholesterol and phospholipids (see Section 6).

In mammals, the majority of cholesterol is synthesized de novo in the liver, and delivered to peripheral cells by lipoproteins. Since peripheral cells are unable to degrade cholesterol, any surplus of cholesterol must either be stored in the cytosol in the form of esters, or released from the cell. ABCA1 mutations can be responsible for some cases of familial high density lipoprotein (HDL) deficiency, e.g. Tangier disease [55–57], characterized by impaired efflux of cholesterol and phospholipids from peripheral cells onto apolipoproteins such as apoA-1. Cholesterol accumulation in macrophages and apolipoprotein degradation lead to tissue deposition of cholesterol esters and increase the risk of arteriosclerosis in the patients. While Tangier cells typically fail to bind nascent apoA-1 [58, 59], the expression of ABCA1 in cultured cells has been found to enhance the binding of apoA-1 to the plasma membrane [60], and to increase the efflux of cellular phospholipid and cholesterol to this apolipoprotein [61]. Both sequential and parallel mechanisms have been proposed for the transport of phospholipids and cholesterol by ABCA1 (see also Section 6).

ABCA1 has been suggested to directly transport the aminophospholipid PS (typically restricted to the cytoplasmic leaflet of mammalian plasma membranes) to the

![Diagram](image)
exoplasmic leaflet [61], where the presence of PS facilitates apoA-1 binding, leading thus indirectly to cholesterol efflux [61–63]. Indeed, the exposure of endogenous PS (detected via prothrombinase activity and binding of Annexin V) upon Ca2+ induced stimulation was found to be low in red blood cells, resp. thymocytes, derived from mice lacking ABCA1, and could be partially restored upon ABCA1 transfection [61]. A recent study on apoptotic murine cells confirmed elevated levels of exposed PS to cause a strong increase in apoA-1 binding, although not sufficient to trigger phospholipid and cholesterol efflux to apoA-I [64].

In contrast, Wang et al. [65] recently proposed direct transbilayer transport of both cholesterol and phospholipids via ABCA1, following experiments in which phospholipid/apoA-I particles made by ABCA1 were unable to stimulate passive cholesterol efflux when added to a second set of cells.

In addition to a role in lipid loading of apolipoproteins, ABCA1 has been implicated with PS exposure on the outer plasma membrane leaflet of apoptotic cells and phagocytizing macrophages [66]. In the absence of apoliposomes, Ca2+ induced externalization of spin-labeled (SL) analogs of PS, but not of PC, was reduced in mice lacking ABCA1 [61].

The large ABC protein ABCA2, found in high levels in human brain (possibly in oligodendrocytes), colocalizes with a lysosomal/Endosomal marker [67]. Vulevic et al. [67] have associated ABCA2, which contains a signature motif for lipocalins (protein family binding small hydrophobic molecules), with the transport of steroids and lipids due to colocalization with an analog of the steroid estramustine, and increased estramustine resistance upon ABCA2 gene overexpression.

Kaminski et al. suggested a role for ABCA2 in macrophage lipid metabolism and neural development, after having found an induction of ABCA2 mRNA during steroid loading [68]. Based on these observations and the unique expression profile, Schmitz and Kaminski [69] inferred a role of ABCA2 in transbilayer lipid transport of neural cells.

ABCA3 is expressed exclusively in type II epithelial lung cells expressing the gene surfactant protein A. It is hypothesized to play a role in the formation of pulmonary surfactant [70], a mixture containing PC and various surfactant proteins, which reduce surface tension on the surface of the alveoli. Mutations in ABCA3 were found in several cases of infants with fatal surfactant deficiencies [71]. ABCA3 is localized in the plasma membrane and in the limiting membrane of lamellar bodies, in which surfactant is stored prior to exocytotic delivery into the alveolar space. Mulugeta et al. have therefore speculated that ABCA3 might transport PC into or other lipids out of lamellar bodies, which are highly enriched in PC [72].

ABCA4 (ABCR or Rim protein) [73] is localized in the photoreceptor outer segment disc membranes of the retina [74,75]. Reconstitution studies, in which its ATPase activity was stimulated by retinal, lead to the hypothesis that ABCA4 may function as an active retinoid transporter [76]. ABCA4 appears to be highly substrate-specific, being involved in dark-adaptation through the transport of the lipid product all-trans-N-retinylidene-PE across the disc membrane following the photobleaching of rhodopsin. This transport allows all-trans-retinal to be reduced to all-trans-retinol on the surface of the disc membrane. Thus, ABCA4 mediated transport is an important step in the recycling of all-trans-retinal to 11-cis-retinal for the regeneration of rhodopsin. Studies on the ATPase activity of reconstituted ABCA4 strongly suggested PE to be required to couple the binding of retinoids to ABCA4 ATPase activity [76]. Defective ABCA4 can cause the degeneration of the macula lutea and consequent deterioration of vision (Stargardt disease), presumably through the accumulation of the lipofuscin fluorophore N-retinylidene-N-retinyl ethanolamine (A2E) [77,78].

ABCA6 shows expression in the lung, heart, brain, liver and ovaries. As ABCA6 expression is suppressed by cholesterol loading [79], the protein was attributed a potential role in macrophage lipid homeostasis.

ABCA7 is expressed mainly in myelo-lymphatic tissues. The protein is localized either in the plasma membrane or the endoplasmic reticulum [80], and was implicated with the specification of hematopoietic cell lineages during development [81]. As the loading of macrophages with steroids increases, and unloading decreases the expression of the ABCA7 gene, Kaminski et al. proposed ABCA7 to be involved in macrophage transbilayer lipid transport [82]. ABCA7 overexpression in HeLa cells increased the exposure of endogenous ceramide (Cer) on the outer plasma membrane leaflet, and raised levels of endogenous PS [83]. As ABCA7 is upregulated during keratinocyte differentiation, this led to speculations about a regulator role for ABCA7 in lipid transport during terminal keratinocyte differentiation. Very recently, apolipoprotein-mediated release of cellular cholesterol and phospholipids was reported from HEK293 cells transiently or stably expressing ABCA7 [80,84]. Furthermore, the transfection of GFP-tagged ABCA7 of L929 cells triggered apolipoprotein-mediated assembly of cholesterol-containing HDL. In contrast, Wang et al. [65] reported ABCA7-mediated efflux of phospholipid and SM, but in contrast to ABCA1 not of cholesterol, from HEK293 cells.

ABCA9, highly homologous to ABCA6, shows ubiquitously expression, the highest levels being found in the heart, brain and fetal tissues. ABCA9 is induced during macrophage differentiation. Different from ABCA7, the expression of ABCA9 in macrophages decreases upon steroid loading of the cells. Pihler et al. suggested that ABCA9 might act on monocyte differentiation and macrophage lipid homeostasis [85].

ABCA10, as ABCA9 highly homologous to ABCA6, is ubiquitously expressed, with high gene expression levels in the heart, brain, and the gastrointestinal tract. As its gene expression in macrophages is suppressed by cholesterol loading, Wenzel et al. hypothesized on the involvement of ABCA7 in macrophage lipid homeostasis [86].
4.1.2. Human ABCB (MDR/TAP) family

The 4 full-size and 7 half-size proteins of the ABCB family show highly varied specificities (e.g. amphiphilic compounds, peptides, iron, phospholipids, bile salts) [51]. The proteins associated with antigen processing ABCB2, 3 (TAP1, 2), and the bile salt export pump ABCB11 (BSEP) are ABCB protein family members.

ABCB1 (Multidrug Resistance 1 P-glycoprotein, MDR1 Pgp) [87] is a full-size protein with two transmembrane domains and two nucleotide binding domains, which appears to be functional as a monomer [88]. It occurs in domains and two nucleotide binding domains, which appears to be functional as a monomer [88]. It occurs in intestinal and secretory endocrine extract (TAP1, 2), and the bile salt export pump ABCB11 (BSEP) are ABCB protein family members.

ABCB1 has a surprisingly broad spectrum of (mainly cationic or electrically neutral) amphiphilic substrates [92]. One of its important physiological roles appears to be the protection of the organism against toxins by exporting these into the bile, urine, or gut. In tumors, the overexpression of ABCB1 is one of the principal factors responsible for multidrug resistance (MDR) against a variety of structurally unrelated drugs. ABCB1 is involved in other phenomena as well, in which, interestingly, lipid transport often seems to be implicated: It was found to mediate the secretion of the steroid aldosterone by the adrenals [93], and its inhibition blocked the migration of dendritic immune cells [94], possibly related to the outward transport of the lipid platelet activating factor (PAF, see below). Ueda et al. reported ABCB1 mediated transport of the steroids cortisol and dexamethasone, but not of progesterone in ABCB1 transfected cells [95]. Inhibition studies have also led to speculations about the transport of cholesterol by ABCB1 [96] (see also Section 6). Short-chain and long-chain (nbd and radiolabeled) analogs of PC, PE, PS, SM, and GlCer were found to be expelled from ABCB1 overexpressing cells [97–99]. Among the endogenous lipids, the short-chain PC PAF [100] is an ABCB1 substrate (ABCB1 antisense oligonucleotides blocking PAF secretion in human mesangial cells). Other potential substrates are GlCer (rescued from cytosolic hydrolysis in the presence ABCB1, and strongly reduced in the absence of ABCB1) [101] and PS (as found by Annexin V binding experiments in ABCB1 overexpressing human gastric carcinoma cells) [99]. Interestingly, the exposure of endogenous SM to the outer plasma membrane leaflet of human myeloblastic cells was reduced upon ABCB1 inhibition, a possible hint for ABCB1 mediated transport of this lipid involved in signalling [102]. While the inability of the ABCB1 mouse homologs Mdr1a/1b to restore the transport of PC into the bile of mice lacking Mdr2 (homologous to human ABCB4) [103] could suggest that natural long-chain PC is not an ABCB1 substrate, it is also conceivable that Mdr1a/1b activity was too low in this system. Multispecific transport of diverse endogenous lipids via ABCB1 could affect the transbilayer distribution of lipids, in particular of species normally predominant on the inner plasma membrane leaflet, such as PS and PE. Reconstitution experiments with ABCB1 have thus far yielded ambiguous results: While Romsicki and Sharom [104] found a low ATP dependent increase in reoriented short-chain nbd analogs of PC, PE, PS and SM, Rothnie et al. [40] observed low reorientation of short-chain nbd analogs of PC, PE, Cer and of short-chain SL analogs of PC, PE, GlCer, and SM which was ATP independent. Interestingly, the activity of ABCB1 was dependent on cholesterol. Due to the small size of the vesicles containing the reconstituted protein, lateral pressure (surface tension) might have prevented substantial transport of lipids via ABCB1 [105] (see Section 2). The structure and potential mechanisms of ABCB1 will be discussed in Section 7.

ABCB4 (MDR2/3 Pgp), a full-size protein, is a close relative of ABCB1 (MDR1 Pgp), sharing 75% of its amino acid sequence [49]. Unlike ABCB1, ABCB4 is highly substrate specific, exclusively transporting (short-chain nbd analogs of) PC, as observed in ABCB4 transfected porcine cells [97]. The secretion of PC into the bile appears to be the physiological function of ABCB4 [103] (see also Section 6), the protein being present in high amounts in the canalicular membranes of hepatocytes. In mice lacking ABCB4, PC secretion into the bile is abolished [103], and the transbilayer movement of radioactively labeled endogenous PC appears to be slightly enhanced in fibroblasts from mice overexpressing the ABCB4 gene [106]. In some cases of progressive familiar intrahepatic cholestasis (type III) in humans, ABCB4 has indeed been found to be defective [107].

4.1.3. Human ABCC (CFTR/MRP) family

All 13 ABCC family members are full-size proteins. However, they differ in the number of transmembrane domains (two for ABCC4, ABCC5, ABCC11 and ABCC12, while all others possess a third TM domain, TMD0 (Fig. 4)). The major functions of the ABCC proteins are, among others, the protection against toxic compounds and the secretion of organic anions [108]. Examples for ABCC family members are the cystic fibrosis transmembrane conductance regulator ABCC7 (CFTR), defective in mucoviscidosis, and the sulfonylurea receptors ABCC8,9 (SUR 1, 2), regulating associated potassium channels. ABCC8 is defective in patients with familial persistent hyperinsulinemic hypoglycemia of infancy. The ABCC family members differ in substrate specificity, tissue and organelle distribution [109].

ABCC1 (MRP1) [110] is located in the basolateral domain of polarized cells [111], and displays wide tissue distribution [109]. ABCC1 transports a large variety of toxins across the plasma membrane, either unconjugated or conjugated with glutathione, sulfate or glucuronate [112]. While it is unclear whether ABCC1 contributes significantly to multidrug resistance in tumor cells [109], it protects particularly sensitive organs by expelling toxins into the blood (the internal environment [113], in contrast to the apically located...
ABC1 (MDR1 Pgp) which exports toxins into the external environment. Additionally, ABCC1 mediates the leuco-
triene C (LTC) dependent inflammatory response by the
transport of the arachidonic acid derivative LTC4 [114].
ABCC1 transfected pig kidney epithelial cells showed
increased outward transport of short-chain nbd analogs of
SM, and GlecCer [115], and the transport of short-chain (PS,
PC) and long-chain PC nbd analogs in erythrocytes was
equally attributed to ABCC1 in an inhibition study [116].
Upon reconstitution, ABCC1 transported an nbd PC analog
[117]. However, thus far, no endogenous lipids have been
found to be ABCC1 substrates.

Despite similarities in the substrate spectrum of ABCC1
and several other ABCC proteins, Ragers et al. did not
observe translocation of short-chain nbd lipid analogs by
other ABCC proteins tested besides ABCC1 [101].

ABCC6 (MRP6) is expressed exclusively in the liver and
kidney, where the gene product is localized on the
basolateral domain of the plasma membrane [118]. ABC6
defects result in pseudo-xanthoma elasticum (PXE)
[119,120], a disorder characterized by a calcification of
the elastic fibers of the eye, skin, and vasculature, leading
to decreased visual acuity, characteristic skin lesions, and
peripheral vascular disease. Additionally, frequently found
high plasma triglyceride levels and low plasma HDL
cholesterol in PXE patients suggest an involvement of
ABCC6 in lipid transport and metabolism [121].

The molecular basis of this disease is not solved, and
PXE might be a primary metabolic disorder with secondary
involvement of elastic fibers [122].

The transport of glutathione conjugates upon ABCC6
gene expression in Sf9 insect cells point to a role for ABCC6
in the transport of organic anions [123], thought to confer
low levels of resistance to certain anticancer agents [124].

4.1.4. Human ABCD (ALD) family

All four known members of the ABCD family are half-size
proteins found in peroxisomes, single-membrane organelles
involved in beta-oxidation of long and very long chain fatty
acids, synthesis of bile acids, cholesterol plasmalogens and
metabolism of amino acids and purines. ABCD proteins have
been implicated with the transport of fatty acids (FA),
coenzyme A (CoA), or FA-CoA, although ATP independent
transport of very long chain fatty acids (VLCFA) into
peroxisomes [125] might argue against direct VLCFA trans-
port via ABCD proteins. ABCD half-size proteins can form
not only homodimers (ABCD1–ABCD1), but also hetero-
dimers (ABCD1–ABCD2, ABCD1–ABCD3, ABCD2–
ABCD3) [126]. Although the heterodimers might be func-
tional, the differing tissue gene expression of ABCD
members argues against obligatory heterodimerization [127].

ABCD1 (ALDP) mRNA is highly expressed in human
liver, heart, skeletal muscle, lung and intestine [128].
Defects in ABCD1 result in the inherited neurometabolic
disorder X-linked adrenoleukodystrophy (X-ALD) [129],
characterized by elevated levels of VLCFA in nervous
system white matter and the adrenal cortex [130], leading
to neuron demyelinization, renal insufficiency and testicular
dysfunction. Increased VLCFA levels have been attributed
to impaired peroxisomal beta-oxidation, and transfection
with ABCD1 cDNA was shown to restore beta-oxidation in
X-ALD fibroblasts [131] (see also yeast homolog Pat1p; 
Section 4.5). Recently, mitochondrial beta-oxidation was
reported to affect peroxisomal beta-oxidation, and a role of
ABCD1 in the interaction of peroxisomes with mitochon-
dria was suggested [132]. ABCD1 has been implicated with
peroxisome biogenesis, restored upon ABCD1 overexpres-
sion in Zellweger cells (having a defect in the peroxisomal
protein Pex2p) [133].

ABCD2 (ALDRP) is closely related to ABCD1 (63% 
amino acid identity). Its mRNA is highly expressed in human
brain and heart [128]. Similar to ABCD1, transfection with
ABCD2 cDNA restored beta-oxidation in X-ALD (ABCD1
defect) fibroblasts [134], and peroxisome proliferation could
be induced upon pharmacologically increased ABCD2
expression in X-ALD cells [135]. In X-ALD fibroblasts,
ABCD2 induction by steroid depletion was found to reduce
the accumulation of VLCFA [136]. While defects in ABCD2
as a cause for X-ALD were considered to be unlikely,
ABCD2 could be a heterodimeric partner for ABCD1 in some
issues, acting as a modifier gene accounting for the high
phenotypic variability of X-ALD [127].

ABCD3 (PMP70, PXMP1) shows 36% amino acid
identity with ABCD1. The mRNA of its mouse homolog
is highly expressed in the liver, kidney, heart, lung and
intestine [128]. The overexpression of ABCD3 was found to
restore beta-oxidation in X-ALD fibroblasts and to normal-
ize peroxisome biogenesis in Zellweger cells [133] (see also
yeast homolog Pat2p, Section 4.5).

ABCD4 (PXMP1L, P70R, PMP69) shares 25% of
ABCD1 amino acid sequence. ABCD4 mRNA is highly
expressed in human kidney, spleen and testis [128]. As
ABCD2 and 3, it has been suggested to act as a modifier
genre contributing to X-ALD phenotypic variability, possibly
heterodimerizing with the other members of this protein
family [137].

4.1.5. Human ABCG (WHITE) family

The five characterized ABCG proteins are half-size
proteins. In contrast to other proteins, the ABC-domain is
N-terminal, followed by the transmembrane domain (Fig. 4)
[138]. A number of ABCG members are thought to be
involved in the transport of steroids (ABCG1, 5, 8),
additionally, some appear to transport phospholipids
(ABCG1) and toxins (ABCG2).

ABCG1 (WHITE) [139] is thought to be active either as a
homo- or a heterodimer (possibly with ABCG2) [140]. It
shows an ubiquitous gene expression pattern [138] and
localizes primarily to ER and Golgi [140]. ABCG1 derives
its trivial name from its homology to the Drosophila
white protein, which transports guanine and tryptophane as
precursors for eye pigments [141]. ABCG1 itself appears
to serve a different function, presumably in the transport of phospholipids and steroids out of macrophages, as its gene expression in macrophages was found to be induced during cholesterol influx, and suppressed by lipid efflux via HDL. The inhibition of ABCG1 expression resulted in reduced HDL-dependent efflux of cholesterol and PC from these cells [140].

ABCG2 (BCRP, MXR, ABCP) [142] is found in the placenta, intestinal epithelium, liver canaliculi, breast ducts and lobules, as well as in venous and capillary endothelium [143]. Recent studies suggest the protein to be active as a homotetramer [144] in the plasma membrane [145]. It is assumed to prevent the tissue uptake of xenobiotics [143], transporting various drugs across the plasma membrane in an ATP dependent process [146]. Transfection studies proved the overexpression of ABCG2 to induce multidrug resistance in a previously drug sensitive cell line [147]. In addition to drug transport, the reduced accumulation of a short-chain Bodipy analog of ceramide in ABCG2 overexpressing cells has given hints for a transport of lipid analogs [146]. Recently, we have found increased outward movement of short-chain nbd analogs of PS and PC, but not of PE, and increased exposure of endogenous PS in a human gastric carcinoma cell line overexpressing ABCG2 [148]. In Lactococcus lactis transfected with human ABCG2, ABCG2-associated ATPase activity was significantly stimulated by cholesterol and estradiol, pointing to a possible role of ABCG2 in the transport of steroids [149].

ABCG5 (WHITE3) and ABCG8 (WHITE4) are encoded by neighbouring genes [150]. Their mouse homologues are expressed in the liver and intestine [151]. Very recently, ABCG5 and ABCG8 have been demonstrated to function as an obligate heterodimer to promote steroid secretion into the bile [152–154]. Mutations in either ABCG5 or ABCG8 result in an identical clinical phenotype; and the expression of both genes is required for either protein to be transported to the plasma membrane. Defects in ABCG5 and 8 can result in sitosterolemia, a disorder characterized by increased uptake of steroids (among them the plant steroid beta-sitosterol) in the intestine, combined with decreased biliary excretion, resulting in cholesterol deposits in skin and tendons, and in premature coronary artery disease [150,155,156].

The overexpression of human ABCG5 and 8 in mice promoted biliary cholesterol secretion, reduced cholesterol absorption, and increased hepatic cholesterol synthesis [157]. Furthermore, no significant sitosterol transport by ABCB1 (MDR1 Pgp), ABCC1 (MRP1), and ABCG2 (BCRP) was found [158]. Taken together, these data demonstrate a central role of ABCG5 and ABCG8 in vivo cholesterol excretion [159] (see also Section 6).

4.2. Sea urchin ABC proteins

SuABCA is a full-size ABC protein found abundantly in the sea urchin sperm plasma membrane, and is possibly implicated in the sperm acrosome reaction during fertilization. Due to its close homology to human ABCA3, Mengerink and Vacquier suggested SuABCA to be involved in phospholipid or cholesterol transport [160].

4.3. Eukaryotic parasite ABC proteins

ABC proteins found in parasites are of particular clinical relevance due to the occurrence of multidrug resistant strains. Specific attention will be given here to ABC proteins found in unicellular eukaryotes of the Trypanosomatidae family, causing Leishmaniasis and Trypanosomiasis, major and globally widespread parasitic diseases.

In Leishmania spp., three different families of ABC proteins are known (reviewed in [161,162]), homologous to the mammalian ABC families ABCA, ABCB, and ABCC, respectively.

4.3.1. Leishmania ABCA family

LtrABC1.1 [161], a full-size protein containing two transmembrane domains and two nucleotide binding domains, is found mainly in the plasma membrane and flagellar pocket of Leishmania [163]. LtrABC1.1 expression appears to be uncorrelated with multidrug resistance. In a Leishmania tropica cell line overexpressing LtrABC1.1, the accumulation of short-chain nbd analogs of PC, PE, and PS was found to be reduced [163], regardless of the nature of the phospholipid head group. Furthermore, LtrABC1.1 overexpression reduced vesicular transport. Parodi-Talice et al. have suggested a role for LtrABC1.1 in lipid movement across the plasma membrane and in vesicle trafficking. Finally, Legare et al. noted the potential importance of members of the Leishmania ABCA family in the interaction of the parasite with its host, an engulfing macrophage cell [162].

4.3.2. Leishmania ABCB family

Leishmania Pgp-like proteins (LPgp-lp), about 37% identical to mammalian ABCB1s, are full-size ABC proteins [161]. They have been shown to transfer multidrug resistance upon transfection [164]. In Leishmania enriettii, Leishmania Pgp-like protein is located mainly in different intracellular vesicles [165]. Upon the overexpression of the LPgp-lp gene in L. tropica, the parasite cells accumulated lower amounts of a short-chain Bodipy analog of PC and the anti proliferative alkyl-lysophospholipids miltefosine and edelfosine than did the controls [166].

4.4. Plant ABC proteins

Ped3p (peroxisome defective protein 3, also designated COMATOSE, CTS, PXA1), predicted to be a full-size protein [167,168], is found in arabidopsis glyoxysomes, specialized peroxisomes occurring in cells of storage organs (endospersms, cotyledons) and senescent organs [168]. Both halves of Ped3p show significant sequence homology to the human
half-size protein ABCD1 (ALDP) [169]. In Ped3p defective plants, fatty acid beta-oxidation is impaired [168,169], causing a defect in gluconeogenesis that severely inhibits seedling germination in the absence of sucrose [170]. As Ped3p mutants accumulate fatty acyl CoAs, Footitt et al. proposed the protein to be a transporter of fatty acyl CoAs with little specificity concerning chain length [169].

4.5. Yeast ABC Proteins

In yeast, the 31 ABC proteins identified so far have been classified into 6 clusters, including 10 subclusters, according to predicted topology, binary amino acid sequence comparison and phylogenetic classification. Both half-size and full-size proteins exist in yeast. Besides I.1 and VI, all subclusters seem to have human homologues [171].

4.5.1. PDR/CDR family

Pdr5p is a full-size protein located in the plasma membrane [172] of Saccharomyces cerevisiae, mediating multidrug resistance. It confers resistance to progesterone and deoxycorticosterone, which are also inhibitors of Pdr5p drug transport, suggesting these steroids to be direct transport substrates of Pdr5p [173]. Pdr5p deletion in S. cerevisiae leads to an increased accumulation of short-chain nbd PE [32]. Notably, the depletion of Yor1p and Pdr5p causes reduced surface exposure of endogenous PE [32].

Cdr1p and Cdr2p, candidate drug resistance proteins of the human pathogenic yeast C. albicans, are full-size proteins composed of two homologous halves, each comprising a TM domain and a nucleotide binding domain [175]. Cdr3p, highly homologous to Cdr1p and Cdr2p, shows the same domain organization, but appears not to be involved in drug resistance [176]. In C. albicans strains with a disrupted CDR1 gene, the (already low) exposure of endogenous PE on the outer plasma membrane leaflet, revealed via TNBS labeling, was reported to be further reduced [177]. However, taking into account the limitations of the TNBS labeling approach and the very low percentage of labeled PE, the results have to be taken with caution. Furthermore, gene disruption may also affect other processes involved in PE exposure, for example, intracellular lipid trafficking. In transfected S. cerevisiae cells, Cdr1p and Cdr2p elicited in-to-out transport of short-chain nbd analogs of PE, PC and PS. In contrast and rather exceptional, Cdr3p mediated the out-to-in transport of these analogs [178].

4.5.2. MDR family (ABCB homolog)

Ste6p, a S. cerevisiae full-size ABC protein transporting the farnesylated dodecapeptide mating pheromone a-factor [179], was reported to confer resistance against the lyso-PC analog ET-18-OCH3 (Edelfosine) in transfected yeast cells, presumably by outward transport of the drug [180]. However, failure to reproduce these results led to retraction of the original article [181].

4.5.3. MRP/CFTR family (ABCC homolog)

Yor1p is a plasma membrane located [182] full-size protein in S. cerevisiae. It is related to the human ABCB family [183]. Besides conferring drug resistance, Yor1p has been suggested to be involved in the efflux of organic anions. The deletion of Yor1p in S. cerevisiae resulted in increased accumulation of a short-chain nbd analog of PE [174].

4.5.4. ALDp family (ABCD homolog)

Patlp (also designated Pxl2p) and Pat2p (also designated Pxl1p) [184,185] are S. cerevisiae half-size proteins related to human ABCD3 (PMP70) and ABCD1 (ALDP), respectively. Pat1 and Pat2 are thought to heterodimerize [185] in the peroxisomal membrane, and are required for the import of long-chain fatty acids into peroxisomes, Pat1 and Pat2 deletion causing a partial deficiency in long-chain fatty acid beta-oxidation [184]. These data also support the hypothesis that ABCD1 and ABCD3 are involved in VLCFA transport (see Section 4.1).

5. Prokaryotic ABC proteins

ABC proteins exist in both Gram-positive and Gram-negative bacteria (reviewed in [48]). Most of them are concerned with the import of small solutes, depending on specific binding proteins, but exporters exist as well. In prokaryotes, ABC proteins can possess either separate NBD and TM domains, or fused domains.

5.1. Prokaryotic ABCB family

MsBA is a half-size ABC protein [186,187] active as a homodimer [188] in the Escherichia coli inner membrane. It is a close bacterial homolog of ABCB1 (MDR1 Pgp) as was deduced from protein sequence homology [189,190]. It is an essential ABC protein in prokaryotes, conserved in all bacteria, with more than 30 orthologs identified today [191]. MsBA plays an important role in the transport of lipid A from the inner to the outer membrane of Gram-negative bacteria. Lipid A, a hexa-acylated disaccharide of glucosamine unique to Gram-negative bacteria, is a major component of the outer membrane, representing the hydrophobic anchor of lipopolysaccharides on the outside of the outer membrane. MsBA defects cause the accumulation of lipid A and phospholipids in the inner membrane, lethal to E. coli [187,192]. The stimulation of the ATPase activity of MsBA reconstituted into liposomes by lipid A [193], but not by short-chain nbd phosphatidylglycerol [23], provides further indications for specific transport of lipid A by MsBA [23]. Very recently, newly synthesized lipid A, and possibly PE, has been shown to accumulate on the cytoplasmic half of the E. coli inner membrane upon the inactivation of MsBA in a temperature-sensitive mutant [194] arguing for acceleration of transbilayer movement by MsBA.
The structure and potential mechanisms of MsbA will be discussed in Section 7.

LmrA, a half-size protein in \textit{L. lactis} forming homodimers, extrudes various drugs \cite{195}. Like MsbA, LmrA is homologous to both halves of human ABCB1 (MDR1 Pgp) \cite{196,189}, which it can complement functionally in human lung fibroblasts, causing multidrug resistance \cite{189}. Recently, Reuter et al. could show functional substitution of temperature-sensitive mutant MsbA in \textit{E. coli}, suggesting transport of lipid A by LmrA \cite{190}.

Reconstituted LmrA has been found to mediate ATP-dependent transport of fluorescent short-chain nbd PE \cite{197}. However, short-chain nbd PC was not recognized, suggesting headgroup-specificity, different from the low substrate specificity of ABCB1 despite the high sequence conservation. It is not known whether LmrA also transports endogenous phospholipids of \textit{L. lactis}. In the light of the rapid, ATP-independent lipid flip-flop mediated by proteins in the inner membrane of bacteria \cite{20,21,198–200} with half-times of the order of one minute, it remains open whether lipid transport by LmrA is of physiological relevance for \textit{L. lactis}.

5.2. Val A

\textit{ValA} is an ABC protein in \textit{Francisella novicida} with high homology to \textit{E. coli} MsbA, able to rescue MsbA defective \textit{E. coli} \cite{201}. Due to this finding and decreased cell surface exposure of a lipopolysaccharide epitope in the absence of functional ValA, McDonald et al. suggested ValA to be required for the transport of lipid A molecules linked to core polysaccharide across the inner membrane.

6. Putative functions in lipid transbilayer transport and exposure

The studies summarized above document the physiological importance of ABC proteins in lipid transport, their malfunction being able to cause severe diseases. This raises the question of the specific function of ABC proteins in lipid transport (Fig. 2B).

Protein-mediated lipid transport can serve essentially two functions, which may not exclude each other:

(i) Lipid transbilayer transport, establishing, preserving or perturbing a distinct asymmetric transbilayer lipid distribution (asymmetric distribution of lipid species between the two leaflets, or asymmetry in the number of lipid molecules per leaflet, resulting in an area difference between the two leaflets)

(ii) Exposure of lipids to acceptors: Besides being membrane constituents, some phospholipids are constituents of bile and of pulmonary surfactant, and various steroids act as hormones. In addition to movement from one membrane leaflet to the other, some lipids require therefore transport to other membranes, onto lipoproteins, or into the extracellular lumen.

So far, there is no indication for an involvement of ABC proteins in the generation of an asymmetric transbilayer distribution of abundant lipids such as phospholipids or cholesterol. All available quantitative data indicate that the activity of ABC transporters in cellular membranes cannot compete with the inward-directed activity of the flippases described in Section 2 to affect transbilayer distribution on a qualitative level. However, the activity of ABC proteins may not be negligible. Several studies indicate that outward-directed lipid transport mediated by ABC proteins can modulate the transbilayer distribution even of abundant phospholipids inward transported by energy-dependent flippases in the mammalian plasma membrane. An example is the enhanced exposure of aminophospholipids on the outer plasma membrane leaflet of ABCB1 (MDR1 Pgp), Yor1p, Pdr5p and ABCA1 expressing cells. ABC proteins may thus affect physiological functions associated with asymmetric transbilayer lipid distribution. Their outward-directed lipid transporting activity may also counteract vesicle budding driven by energy-coupled flippases (see Section 2.2): While the overexpression of ABC genes in yeast can lead to endocytosis defects \cite{202,174}, the loss of ABCA1 function in Tangier fibroblasts is associated with enhanced endocytosis \cite{203}, supporting a functional link between lipid transport and vesicle biogenesis.

Furthermore, ABC proteins may play a role in the transbilayer distribution of marginal, but physiologically relevant lipids exhibiting slow passive transbilayer movement. In Section 7, models for lipid transbilayer transport by ABC proteins will be discussed.

Most examples of ABC proteins implicated in lipid transport indicate a role not primarily in lipid asymmetry, but rather in the exposure of specific lipids on the exoplasmic leaflet, allowing their uptake by acceptors and further transport to specific sites: The lipopolysaccharide precursor lipid A has to be delivered from the outer leaflet of the inner membrane to the outer membrane of bacterial cells, and PC transported across the canalicular membrane by ABCB 4 (MDR3 Pgp) is taken up by bile salts in the bile canalicular lumen. The necessity of appropriate exposure of membrane-bound lipid to a cognate acceptor can maybe best be illustrated for cholesterol: Deeply buried in the membrane with the polar OH group facing the aqueous phase and the alkyl chain oriented toward the bilayer center, cholesterol has recently been shown to experience rapid flip-flop in PC vesicles, and membranes of erythrocytes and presumably most other cells (reviewed in \cite{204}). The primary function of ABCG5/8 may therefore very likely not be cholesterol transbilayer transport across the canalicular membrane, but rather facilitation of lumenal cholesterol uptake (e.g. by mixed bile salt and PC micelles), possibly by pushing it partly into the aqueous phase, as suggested recently \cite{205}.
A similar mechanism may be relevant for ABCA1 in the delivery of cholesterol to apoA-1, although this seems to be more complicated: Here, PC is involved in the release of cholesterol from the respective membranes, which has also been suggested for ABCG5/8 [205].

Taking into account the opening of the central pore of ABCB1 (MDR1 Pgp) to the lipid phase in the presence of nucleotide (see Section 7), one could even ask whether ABC proteins may take up substrates from the same leaflet from which they are delivered to an acceptor.

While being hypothetical at present, different modes of lipid exposure by ABC proteins could be imagined, which may or may not include the transport of these lipids from the opposite leaflet of the membrane to the side of acceptor localization (Fig. 2B). In principle, transport and exposure of the lipid to the acceptor could be a two-step process or be combined in one step as proposed in the vacuum cleaner model [1]. In the absence of an acceptor, the lipid might be released to the exoplasmic leaflet (modifying transbilayer lipid distribution as a side effect), while the activity of the ABC protein is likely underestimated in this case.

7. Models for lipid transbilayer transport by ABC proteins

ABC proteins display variable transmembrane domains (TMDs), while the nucleotide binding domains (NBDs) are highly conserved in ABC proteins of diverse origins. It is therefore assumed that all ABC proteins bind and hydrolyze ATP in a similar fashion and use a common mechanism on the NBD level to power the translocation of substrate via the TMDs [206]. In addition to biochemical data, structures obtained by X-ray and electron cryo crystallography have been used to propose models for the mechanism of substrate transport by ABC proteins.

The main models currently discussed are the tilting model and the rotating helix model [207]: In the tilting model (Fig. 5), the TMDs are considered as rigid entities. The protein possesses a chamber open towards the cytoplasmic face. The substrate enters the chamber (e.g. from the cytoplasmic aqueous phase or the inner leaflet of the bilayer). ATP binding and hydrolysis cause conformational changes (tilting) of the TMDs, leading to the release of the substrate into the exoplasmic or periplasmic aqueous phase now accessible from the chamber.

In the rotating helix model (Fig. 6), the individual movement of TM helices is taken into account. Conformational changes lead to the rotation of the TM helices, such as to reorient the substrate binding site from the inner leaflet of the lipid bilayer to the aqueous chamber, from where the substrate is released either into the exoplasmic/periplasmic aqueous phase or to the membrane outer leaflet. As both models focus on different aspects, they may not be mutually exclusive. The order of substrate binding, nucleotide binding and hydrolysis, and conformational changes, as well as the interactions between the protein domains are subject of a current discussion.

In the following, we will summarize information on substrate transbilayer transport on the basis of structures of prokaryotic export (MsbA) and import (BtuCD) ABC proteins, and of the eukaryotic export protein ABCB1 (MDR1 Pgp) in the presence or absence of nucleotide.

7.1. Information derived from MsbA

The X-ray crystal structures of a homodimer of the half-size ABC protein MsbA (see Section 5.1) from E. coli (4.5 Å resolution) and Vibrio cholera (3.8 Å resolution) obtained by Chang and Roth [188] and Chang [208] in the absence of nucleotide have been interpreted to reflect two different conformational states of MsbA involved in transport of lipid A, revealing an open (E. coli) and a closed conformation (V. cholera) [191]. In E. coli MsbA, the TM helices are tilted by 30° to 40° relative to the normal of the membrane, being in intermolecular contact on the outer membrane leaflet side. They form a cone shaped structure with large openings (25 Å) towards the inner membrane leaflet at each of the dimer interfaces. The openings lead into a cone-shaped chamber in the interior of the TMDs. The cytoplasmic side of the chamber contains positively charged residues, while the periplasmic side is essentially hydrophobic. The base of the chamber facing the cytoplasm is up to 45 Å wide. The NBDs do not have intermolecular contact and are separated by about 50 Å. An intracellular domain (ICD) is situated between the TMDs and the NBDs. However, whether the tertiary structure proposed corresponds to a native conformation has been questioned by several authors [207,209,210] (see below).

In V. cholera MsbA, the TM helices are less tilted (10° to 30°), and the openings towards the inner membrane leaflet are more narrow (12 Å) than in the E. coli structure. However, the chamber volume remains large enough to accommodate a lipid A molecule. TMDs and NBDs make intermolecular contact. Compared to E. coli MsbA, the alpha-domain of the NBD is rotated about 120° along the dimer axis and contacts the opposing NBD.

Chang [191] proposed the following transport model (Fig. 5): In the open conformation, the NBDs are not firmly attached to the ICD region connecting TMDs and NBDs, and can rotate freely relative to the TMD. Substrate recognition, perhaps by charge–charge interactions, at the cytoplasmic chamber opening induces nucleotide binding, changing the conformation of the NBDs and promoting their dimerization. Dimerization drives trapping of the substrate in the chamber (closed conformation), from where it spontaneously flips to the periplasmic side due to energetically unfavourable interaction of its hydrophobic chains with the polar part of the chamber. The NBD dimer cooperatively hydrolyzes ATP, leading to conformational changes in the NBDs. Relayed through the ICD, the conformational changes cause the TMDs to open towards...
the periplasmic side, releasing the substrate. Upon the dissociation of ADP and Pi, the NBDs separate from each other and the TMDs are reset to the resting state. While the two MsbA structures favor a tilting mechanism, the data could also be consistent with the rotation of certain helices during the catalytic cycle [191]. The subsequent transfer of lipid A across the periplasmic space and to the outer leaflet of the outer membrane is not fully understood [193].

7.2. Information derived from BtuCD

The ABC protein BtuCD mediates the import of vitamin B12 in *E. coli*. Although BtuCD has not been implicated with lipid transport, its structure (resolved in the absence of nucleotide by X-ray crystallography with a resolution of 3.2 Å [206]) provides important information on the mechanism of substrate transport. The BtuCD complex is a heterotetramer consisting of two copies each of the BtuC (TMD) and BtuD (NBD) subunits. The overall structure has been described to resemble an inverted portal. The two TMDs consist of 10 helices each, unlike the 2\times6 helices predicted for a number of other ABC proteins. At the interface of the TMDs, a cavity opens to the periplasmic space and spans 2/3 of the predicted lipid membrane. The cavity is closed to the cytoplasm by the so-called gate. The cytoplasmic loop between the TM helices 6 and 7 (L loop) provides most of the interface with the NBD. Due to the lack of an ICD between the TMDs and the NBDs, the NBDs are located just below the membrane surface. The two TMDs, as the two NBDs, are in close contact to each other.

The following transport mechanism has been proposed [206]: The substrate (on the periplasmic side) interacts
through the TMD via the connecting L loop with the nucleotide binding site (this step might be particular for importers, in which nucleotide binding site and substrate are located on opposite sides of the membrane). The binding and hydrolysis of ATP at the nucleotide binding site induce close contact of the two NBDs, leading to helix rearrangements which open a gap between two TMDs, enabling the substrate to enter the central pore (2). The substrate moves from the central pore into the outer membrane leaflet or the extracellular milieu (3). ATP hydrolysis resets the transporter into its initial state (4). See Section 7.3 for details. Electron micrographs of ABCB1 were taken from Rosenberg et al. [209] (see Figs. 3a and d of the publication) with permission.

ABC protein to return into its resting state, including reorientation of the NBDs. In contrast to the mechanism proposed for MsbA, NBDs and TMDs remain in contact throughout the transport cycle. BtuCD structure and comparison to the ATP-bound form of Rad50, a DNA double-strand break repair enzyme with an ABC-type ATPase domain [211], suggests a tilting mechanism, with the particularity of describing substrate import. Locher and Borths have speculated on a common mechanism in im- and exporters, considering the binding protein as the substrate in the case of importer proteins [212].
7.3. Information derived from ABCB1 (MDR1 Pgp)

The structure of the full-size ABCB1 (see Section 4.1.2) monomer was determined in the presence and absence of nucleotide using electron cryo crystallography (20 Å resolution) [209]. In the absence of nucleotide, the TMDs are approximately parallel and form a barrel surrounding a central pore appearing to be open at the extracellular face of the membrane, and closed at the intracellular face. Small densities protrude into the pore. The binding of nucleotide leads to substantial reorganization of the TMDs involving the repacking and rotation of the TM helices within the membrane: In the presence of nucleotide, the TMDs consist of three clearly segregated domains, enclosing a central pore less obviously closed towards the intracellular face, and additionally open to the lipid phase along one side with a gap appearing between two domains. This gap, equivalent to most of the depth of the bilayer, could give substrates from the lipid bilayer access to the central pore. Due to the technique employed, data concerning the NBDs are insufficient for exhaustive characterization.

The following transport model was proposed (Fig. 6) [209,213]: Substrate binds to the protein from the inner membrane leaflet. Subsequently, ATP binding leads to conformational changes. The TMDs part in order to enable the substrate to enter the central pore, giving it access to the extracellular milieu. ATP hydrolysis resets the transporter into its initial state as also suggested for BtuCD [212]. This is in agreement with the finding that the binding and hydrolysis of ATP can be independent events associated with distinct conformational states of the transporter [214,215]. Data from the two structures including substantial TM helices repacking upon nucleotide binding is consistent with the helix rotation model, while not being easily reconcilable with the tilting model.

When comparing the different structures and proposed models of transport, important differences are obvious for the ABC proteins discussed here. As it is unclear whether the TMDs of different subfamilies are related to each other either evolutionarily or structurally [209], TMDs might be custom-made for the respective substrate. Obviously, the TMDs for an importer of a hydrophilic molecule (e.g., BtuCD) must differ from that for an exporter of amphiphilic substrates (e.g., MsbA, ABCB1), whereas the mechanism of transport could be a common one. While BtuCD has sequence similarity to ABCB1 only on the level of the NBDs, the ABCB family members ABCB1 and MsbA share sequence similarity in both TMDs and NBDs [210]. However, the lack of a TMD:TMD interface and the large distance between NBDs in the proposed E. coli MsbA structure, incompatible with disulfide cross-linking studies and structural data for ABCB1, lead Stenham et al. [210] to question the validity of the proposed tertiary structure of E. coli MsbA. In contrast, the tertiary structure of BtuCD contains a parallel TMD:TMD interface and an NBD:NBD interface as found in isolated prokaryotic NBDs.

By rotation of the E. coli MsbA NBDs by 150° relative to the cognate TMDs, Stenham et al. [210] have obtained a structural model for ABCB1 with a consensus NBD:NBD interface and a parallel TMD:TMD interface, consistent with crosslinking data and electron crystallography studies. According to this model, the TMDs surround a chamber open at the exoplasmic surface and closed at the cytoplasmic surface. The rotating helix model appears to be particularly suited for lipid transport, as the substrate can access the transport pathway from the membrane, as suggested to be necessary for ABCB1 mediated transport, which might also partially explain ABCB1 substrate multi-specificity [1]. Meanwhile, the various structures discussed here reflect conformational snapshots of ABC proteins during the transport cycle, and may not provide a sufficient basis to unravel the transport mechanism and all involved conformational intermediates.

It also remains to be established whether MsbA and ABCB1 can serve as models for other lipid-transporting ABC proteins not belonging to the ABCB family. Further structural data from other ABC proteins is required to reveal whether the TMDs of lipid-transporting ABC proteins are structurally similar, and function according to the same principle.

8. Conclusions

A long way from being solely a subject for basic research, lipid transport reaches far into the clinical domain. The transport of lipids across a membrane can have a net secretory function for a cell or an organelle, or concern mostly the transbilayer distribution of lipids in a membrane. Cholesterol transport by ABCA1 out of macrophages, PC transport by ABCB4 (MDR3 Pgp), the transport of fatty acids into peroxisomes by members of the ABCD (ALD) family, and steroid secretion by ABCG5 and 8 seem to be examples for secretion events, where rather high quantities of lipids must be efficiently transported to other membranes, onto lipoproteins, or into the extracellular lumen. Here, the exposure to appropriate acceptors appears to be an essential step. In a membrane with an asymmetric distribution of the lipid species across the two leaflets, on the other hand, a comparatively slow transport of relatively few lipid molecules can be sufficient to help increase or break down this asymmetry, having for example potential effects on signalling. This could be the case for the transport of PS by ABCA1 in apoptotic cells, serving as a signal for phagocytosis. Meanwhile, other proteins existing in the same membrane may transport lipids in the same or the opposite direction as ABC proteins. Knock-out studies in mammals on ABC proteins have yielded unexpected results, likely due to functional compensation by other ABC proteins [216]. Similarly, transfection with one ABC gene has been shown to influence the expression of another ABC gene [217].
In addition, ABC proteins have been proposed to be transporters, channels and regulators, meaning they can potentially affect transbilayer transport of lipids directly, or indirectly through regulation of other proteins.

This makes clear why experimental setups are needed which quantify lipid transport in a comparable way, verify its attribution to a particular protein in the absence of a transporter background, determine its specificity and permit the distinction between transport events against or with a concentration gradient, requiring or not the hydrolysis of ATP, and the presence of acceptor molecules.

It should be noted that ABC proteins implicated in the transport of lipids can be localized in the plasma membrane, as well as in intracellular membranes, complicating direct measurements on cells. Now that all ABC proteins in humans, and many in other organisms, have been identified, reconstitution into vesicles large enough to allow lipid redistribution (e.g. giant unilamellar vesicles (GUVs)) appears to be a promising technique to answer many current questions. As the number of unequivocally identified lipid transporter proteins is small, putative lipid bulk transporters will be of particular interest as models.

However, the identification of lipid transport remains difficult. Short-chain lipid analogs offer the advantage of easy integration into and extraction from membranes, but only reach a certain level of accordance with endogenous lipids for these same reasons.

Endogenous lipids can be bound by certain antibodies or lipid binding peptides or proteins such as Annexin V, and they can be chemically modified (e.g. by TNBS), or extracted from membranes via lipid transfer proteins. At the same time, reliable techniques for the quantification of endogenous lipids on a specific leaflet exist for a few lipid species only. The combination of results obtained in model systems with data from cells overexpressing ABC genes upon stimulation, selection or transfection, as well as from cells lacking functional ABC proteins upon mutation, knock-out or inhibition will therefore be indispensable to obtain a clear picture of the role of these proteins in lipid transport.

At the present state, the ABC protein superfamily can already be considered to be of great importance in the transport of lipids in prokaryotic as well as in eukaryotic organisms, while the map of proteins responsible for lipid transport, far from complete today, continues to be drawn.

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Note added in proof

A detailed discussion of the mechanism of ABC protein-mediated transport is presented in the recent view of Higgins and Linton [219]. First biochemical evidence for the binding of N-retinylidene-PE to ABCA4 and for dissociation of this lipids from ABCA4 upon binding and hydrolysis of ATP has been shown recently [220].

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