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REVIEW ARTICLE

An inventory of lysosomal ABC transporters

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ABC transporters fulfill diverse physiological functions in different cellular localizations ranging from the plasma membrane to intracellular membranous compartments. Several ABC transporters have been spotted in the endolysosomal system, which consists of endosomes, autophagosomes, lysosomes, and lysosome-related organelles. In this review, we present an overview of lysosomal ABC transporters including ABCA2, ABCA3, ABCA5, ABCB6, ABCB9, and ABCD4, discussing their trafficking routes, putative substrates, potential physiological functions, and associated diseases. In addition, we offer a critical evaluation of the literature linking ABC transporters to lysosomal drug sequestration, examining pitfalls associated with *in vitro* models of drug resistance.

Keywords: ABC transporters; cholesterol; drug resistance; endolysosomal system; lipid; lysosomes; peptide transport; surfactant; trafficking; vitamin B12

Once considered merely as a garbage bin for unwanted molecules, lysosomes are now recognized as complex and dynamic organelles with a range of crucial physiological functions. Lysosomes are responsible for the recycling of macromolecules including proteins, carbohydrates, nucleic acids, and lipids that are delivered to the lysosomal lumen by endocytosis, phagocytosis, and autophagic pathways [1]. In addition, lysosomes are involved in metabolic signaling, gene regulation, immunity, plasma membrane repair, and cell adhesion and migration [2]. Lysosomes show a large variety in composition, localization, number, and size, as they are continuously shaped by fusion and fission events and extensive interactions with other organelles. In a broader sense, lysosomes are complemented by lysosome-related organelles (LROs), which include melanosomes, lamellar bodies, lytic granules, MHCII compartments, and platelet-dense granules [3]. LROs are heterogeneous in structure, function, and origin. For example, the constituents of melanosomes and lamellar bodies are mainly derived from the endolysosomal compartment, whereas Weibel–Palade bodies storing chemokines and the von Willebrand factor are directly formed at the trans-Golgi of endothelial cells with only a minor contribution from endosomal compartments [3]. In view of this variety and dynamics, it is not surprising that lysosomes are associated not only with rare lysosomal storage diseases but also with neurodegenerative, metabolic diseases and cancer.

Lysosomes contain many constituents, including resident proteins, proteins in transit, and cargo proteins destined for degradation. In addition to the hydrolases that are responsible for the lysosome-mediated degradation and recycling processes, resident proteins

Abbreviations
LMPs, lysosomal membrane proteins; NBD, nucleotide-binding domain; TMD, transmembrane domain; TMH, transmembrane helix.

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include enzyme activators, protective factors, and various lysosomal membrane proteins (LMPs) [4,5]. In line with the complex physiological functions, the lysosomal membrane has roles beyond tightly sealing lysosomes from the cytoplasmic environment. The largest class of LMPs is formed by transporters and channels that enable the exchange of content between lysosomes and the cytosol. Most lysosomal transporters belong to the secondary active transporters, which are energized by the proton gradient established by the V-type ATPase. By controlling the flow of ions and metabolites across the membrane, LMPs play a crucial role in maintaining the unique lysosomal milieu. In addition, LMPs regulate complex processes occurring throughout the endocytic and biosynthetic pathways, influencing diverse cellular functions ranging from phagocytosis, autophagy, cell death, virus infection to membrane repair [6].

Considering the relevance of LMPs along the continuum of the endolysosomal system, remarkably little is known about the molecular machinery responsible for their transport to lysosomes. Sorting and trafficking of LMPs is a complex process. Conventionally, cytosolic sorting motifs are recognized at different trafficking steps by adaptor molecules, which mediate the transport directly from the Golgi or *via* the plasma membrane to lysosomes [7] (Fig. 1). However, there are numerous exceptions where the proteins do not comprise a sorting motif and pursue different trafficking routes. Glycosylated LMPs may follow the constitutive secretory route from the trans-Golgi network to the plasma membrane and then be re-internalized by endocytosis. Alternatively, some LMPs are trafficked directly to the lysosomes *via* endosomal compartments. Clathrin-dependent sorting is mediated by adaptor protein (AP) complexes (AP1, AP2, AP3) or Golgi-localized, gamma-ear containing, ADP-ribosylation factor binding (GGA) proteins, which bind to tyrosine or di-leucine-based sorting motifs located in the cytosolic segments of LMPs [7]. Determinants of the clathrin-independent pathways are less well defined, and it is believed that in the case of multispanning transmembrane proteins composite targeting signals may be formed by the synergy of several motifs. The relative contributions of these pathways depend on physiological conditions, the cell type, and the expression levels of the LMP [8].

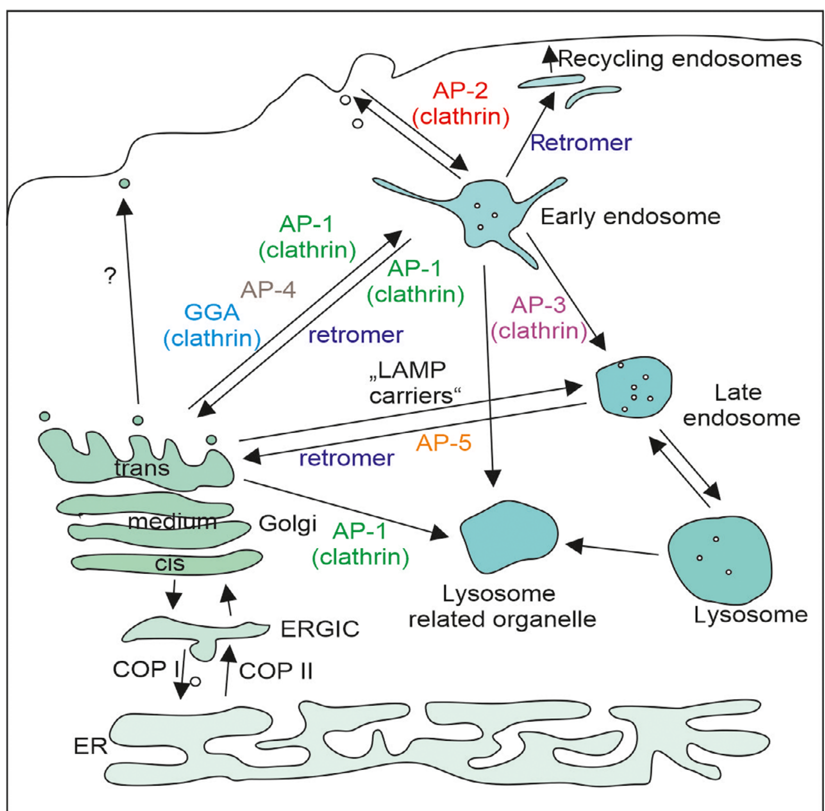

Fig. 1. Intracellular trafficking of transmembrane proteins. Transmembrane proteins synthesized at the ER are transported by COP II vesicles directly or *via* the ER Golgi intermediate compartment (ERGIC) to the cis-Golgi. After reaching the trans-Golgi, transmembrane proteins destined for various compartments are recognized *via* their trafficking signals by respective APs and sorted in clathrin-coated or other vesicles. In the indirect pathway, LMPs are first targeted to the plasma membrane and are subsequently endocytosed and trafficked *via* early and late lysosomes to the limiting membrane of lysosomes. In the direct pathway, the proteins are sorted to endosomal compartments from where they reach the lysosomes. Transmembrane proteins of LROs can reach their final locations by different pathways. Protein complexes involved in sorting of membrane proteins are depicted next to the corresponding routes.

ABC transporters in the continuum of the endolysosomal system

A recent proteomic analysis of native and density-shifted lysosomes has identified more than 100 resident LMPs, excluding many other membrane proteins identified in earlier studies as co-purifying contaminants [9]. Current proteomic datasets classify ABCA2, ABCB6, ABCB9 and ABCD4 as lysosomal proteins [10–12]. Additional lysosomal ABC proteins identified in specific cell types that were not analyzed by proteomic studies include ABCA3 and ABCA5. Our survey of the literature revealed several other ABC transporters annotated as LMPs. However, lysosomal localization of these ABC transporters is only transient, representing an association with the intracellular endocytic pathway leading to recycling or lysosomal degradation. Subcellular distributions of membrane proteins are more volatile than usually appreciated. Sorting is finetuned by post-translational modifications including glycosylation, phosphorylation, or ubiquitylation. The endocytic pathway is a spatio-temporal continuum of early to late endosome intermediates, which continuously exchange their content while undergoing gradual molecular and structural remodeling and functional transformation [1]. Notwithstanding the above, lysosomal targeting of ABC transporters should be delineated from endosomal localizations linked to the ubiquitin-dependent downregulation of plasma membrane transporters. For example, modulation of plasma membrane levels by endocytosis has been established for ABCC2, which can be withdrawn from the apical membrane domain into an intracellular reservoir as a result of PDZ motif-mediated interactions, [13] or ABCA1, which is constitutively recycled depending on the presence of its extracellular ligand ApoA-I [14]. Membrane stability is also crucial for the function of the cystic fibrosis transmembrane conductance regulator (CFTR), which is partly regulated by the balance between the recycling and degradation of endocytosed plasma membrane proteins. Whereas CFTR was reported to play an important role in the acidification of lysosomes by conducting chloride ion for charge balance [15,16], several groups have later shown that lysosomal and phagosomal acidification is independent of CFTR [17,18]. In all fairness, the classification of lysosomal ABC transporters based on subcellular fractionation, affinity purification, or imaging studies is problematic. To some extent, knowledge-based interpretation of the localization data can be helpful; for example, homologs of known lysosomal proteins are particularly likely to reside within this organelle [19]. Unfortunately, in most cases the

Table 1. Lysosomal ABC transporters.

ABC transporter	Localization	Dominant tissue	Function	Substrate	Trafficking signal/domain	Associated disease
ABCA2	Endolysosomes, ER/Golgi	Brain	Intracellular cholesterol & sphingolipidshomeostasis	Putative: Negatively charged phospholipids	xLxxKN (proximal post-Golgi compartment)	Neurological defects Cardiovascular disease Alzheimer
ABCA3	Lamellar Bodies	Lung	Pulmonary surfactant transport	Putative: Phosphatidylcholine Phosphatidylglycerol	xLxxKN (proximal post-Golgi compartment)	Respiratory distress syndrome
ABCA5	Plasma membrane, Lysosome, Late Endosome, Golgi	Heart Brain Testis	Cholesterol efflux	Putative: Cholesterol	xLxxKN (proximal post-Golgi compartment)	Cardiomyopathy, Alzheimer, Parkinson
ABCB6	Endolysosomes, Melanosomes	Ubiquitous Red blood cells Megakaryocytes	Putative: regulation of melanogenesis Heavy metal tolerance	Putative: glutathione-adducts	TMD0	Ocular coloboma pseudohyperkalemia Dyschromatosis universalis hereditaria (DUH)
ABCB9	Lysosomes	Brain, Testis Lymphocytes	Lysosome biogenesis	Peptides	TMD0	Unknown
ABCD4	Lysosomes	Oocyte	Cobalamin metabolism	Putative: Cobalamin	Escorted by LMBD1	Methylmalonic aciduria, hyperhomocysteinemia

physiological function of lysosomal ABC proteins is not known. Given their membrane orientation (the ATP-binding domains are believed to face the cytoplasm), an exporter function is predicted, implying the extrusion of substrates into lysosomes. However, as we will see later, even this simple assumption is incorrect, considering the putative reverse transport function of ABCD4, which promotes the transport of cobalamin from the lumen of lysosomes into the cytosol. Similarly, remarkably little is known about the molecular machinery responsible for the trafficking of ABC transporters to lysosomes. Lysosomal ABC transporters lack known sorting motives and it seems that they reach the lysosomes through different trafficking mechanisms. In this review, we offer an inventory of lysosomal ABC transporters, listing experimental evidence on trafficking, putative substrates, physiological functions, and the diseases that are associated with these proteins (Table 1). We distinguish ABC proteins with established lysosomal targeting and function (ABCD4, ABCA3, ABCB9) from ABC proteins with unknown function, whose lysosomal localization is nevertheless supported by overwhelming experimental evidence (ABCA2, ABCA5, and ABCB6). Finally, we offer a critical evaluation of studies linking ABC transporters to the lysosomal sequestration of drugs, discussing pitfalls associated with the experimental verification of lysosomal localization.

Release of cobalamin from the lysosomes: ABCD4

ABCD4 was named putative peroxisomal membrane protein 69 because of its sequence similarity to the other known members of the ABCD subfamily (ABCD1-3), which are found in peroxisomes [20]. However, ABCD4 was unequivocally localized to the lysosomes and is not found in peroxisomes [21]. ABCD4 is a homodimeric, ubiquitously expressed half transporter [22,23]. ABCD4 belongs to the class IV ABC transporters containing 2×6 transmembrane helices with cytosolic extensions [24]. Recently, the structure of ABCD4 has been solved by single-particle cryogenic electron microscopy in the ATP bound state with a resolution of 3.6 Å [23]. The structure shows a transmembrane domain (TMD) open to the lysosomal lumen and nucleotide-binding domains (NBD) forming head-to-tail dimers with two ATP molecules in the dimer interface. The TMD forms a cavity, which is lined by hydrophobic residues at the entrance, and is

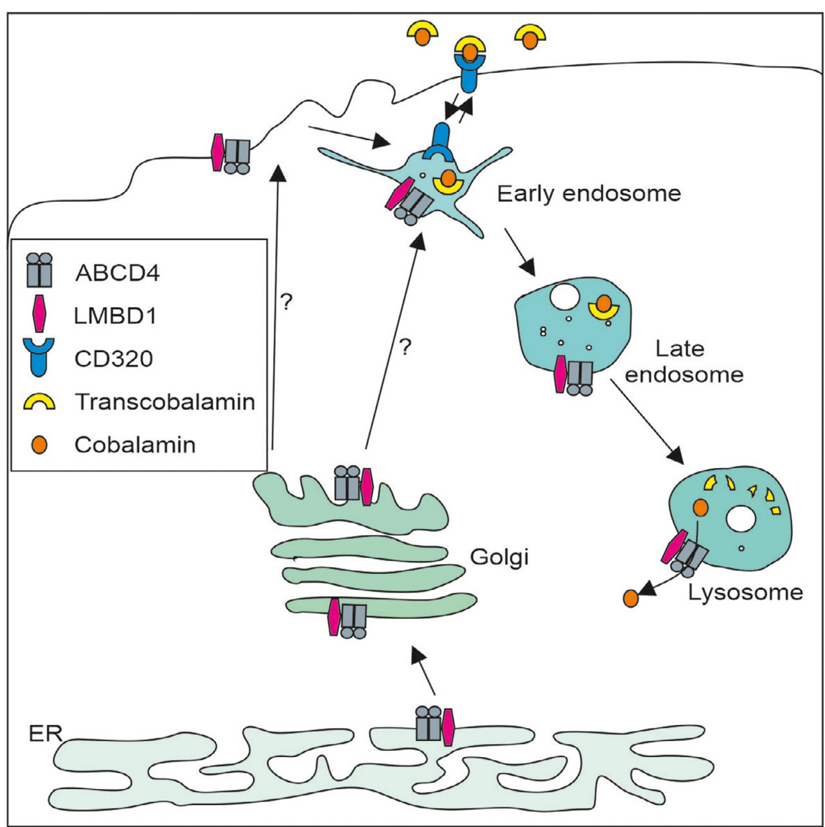

Fig. 2. Role of ABCD4 in intracellular cobalamin shuttling. ABCD4 is guided from the ER to the lysosomes by LMBD1 through a yet unknown intracellular route, believed to involve the plasma membrane. Cobalamin is taken up by the cell in complex with transcobalamin through CD320. The trimeric complex is endocytosed, and the cobalamin/transcobalamin heterodimer dissociates from CD320 in early endosomes. CD320 recycles to the plasma membrane whereas the cobalamin/transcobalamin complex is shuttled to lysosomes. Transcobalamin is degraded by lysosomal proteases, and cobalamin is transported by the ABCD4/LMBD1 complex into the cytosol through a reverse transport mechanism. Methylated and adenosylated cobalamin is an essential cofactor of the methionine synthase and methylmalonyl-CoA mutase, respectively.

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closed at the boundary to the cytosol. Mutations in ABCD4 are linked to a group of nine inherited defects of intracellular cobalamin (vitamin B12) metabolism discovered by somatic complementation assays [21,25]. In the blood, cobalamin is complexed with transcobalamin, which is taken up by CD320-mediated endocytosis (Fig. 2). CD320 dissociates in the endosomes and the cobalamin-transcobalamin complex is translocated to lysosomes, where cobalamin is liberated from the complex. Free cobalamin is released into the cytosol where it is processed to methylcobalamin or adenosylcobalamin, the prosthetic groups of methionine synthase and methylmalonyl-CoA mutase [26]. Defects in this pathway result in the accumulation of the substrates of methionine synthase and methylmalonyl-CoA mutase, resulting in methylmalonic aciduria and hyperhomocysteinemia. Patients within the complementation groups *cblF* and *cblJ* show identical phenotypes including hypotonia, lethargy, poor feeding, bone marrow suppression, macrocytic anemia and heart defects in newborns [21,27]. Fibroblasts from these patients showed elevated levels of free cobalamin but virtually no synthesis of methylcobalamin or adenosylcobalamin. Mutations in LMBRD1 and ABCD4 cause the *cblF* and *cblJ* phenotypes, respectively [21,28]. LMBRD1 encodes a homodimeric lysosomal membrane glycoprotein with nine putative transmembrane helices. Lysosomal cobalamin transport escort protein LMBD1 (LMBD1) interacts with ABCD4 as demonstrated by co-immunoprecipitation and by a fluorescence resonance energy transfer-based live cell assay [29,30]. Based on surface plasmon resonance experiments, the complex is kinetically stable with an affinity in the nanomolar range [22]. ABCD4 is retained in the ER in the absence of LMBD1, but is trafficked to the lysosomes if both proteins are coexpressed. Moreover, ABCD4 is mislocalized to the plasma membrane if coexpressed with a mutant form of LMBD1 containing a modified putative AP-2 binding motif, which traffics to the plasma membrane. Disease-causing mutations in ABCD4 significantly weaken the interaction with LMBD1, resulting in reduced lysosomal targeting and a decrease in cobalamin release from lysosomes [29]. Based on these results it was concluded that LMBD1 functions as an escort protein to overcome ER retention and to guide the lysosomal targeting of ABCD4 [30] (Fig. 2). Expression of wt ABCD4 in fibroblasts of patients with *cblJ* phenotype normalized enzyme-bound cobalamin levels and drastically increased the levels of methylcobalamin and adenosylcobalamin. However, expression of an ABCD4-variant with a mutated Walker B residue (D548N) led to reduced synthesis of both cobalamin cofactors suggesting that ATP hydrolysis is essential for release of free cobalamin from the lysosomes into the cytosol [21]. The exact contribution of ABCD4 remains unclear. Since direct transport of cobalamin by ABCD4 has not been demonstrated, two alternative scenarios may be hypothesized: ABCD4 may be a regulatory protein needed for the function of LMBD1 performing cobalamin transport (a similar collaboration exists between ABCC8 and KIR6.2-SUR). Alternatively, LMBD1 may be an accessory protein needed for the lysosomal trafficking of ABCD4, which may be directly responsible for cobalamin export from the lysosomes to the cytoplasm. However, this scenario would imply that the directionality of transport is reversed, suggesting that in contrast to the majority of eukaryotic ABC transporters, ABCD4 functions as an importer. Future work, based on transport studies performed with reconstituted ABCD4 will determine the validity of this intriguing hypothesis.

Regulation of intracellular lipid transfer in lysosomes and LROs: ABCA2, ABCA3, and ABCA5

ABCA subfamily members are involved in lipid metabolism. Expressed predominantly in the plasma membrane, ABCA1 effluxes cholesterol from cells, while several ABCA family members regulate intracellular lipid homeostasis in the membranes of intracellular organelles [31–35]. ABCA proteins are full transporters, consisting of two TMDs and two NBDs and large hydrophobic loops connecting transmembrane helices. These large luminal loops are highly glycosylated, presumably to protect against degradation. While the participation of ABCA proteins in lipid metabolism and homeostasis is firmly established, their physiological substrates are not known for every member of the subfamily. Also, a rigorous study of the trafficking of ABCA proteins targeted to lysosomes or LROs is lacking. Below we summarize relevant information on the lysosomal localization and function of ABCA2, ABCA3 and ABCA5.

The link between the lysosomal localization and function of ABCA3 is well understood [36]. Predominantly expressed in the alveolar type II (AT2) cells of the lung, ABCA3 is localized to the limiting membrane of LROs called lamellar bodies [37]. Lamellar bodies (also known as lamellar granules) are secretory organelles that fuse with the cell membrane and release pulmonary surfactant into the extracellular space [38]. ABCA3 follows a complex trafficking pathway (Fig. 3). Following synthesis, ABCA3 is routed to the outer membrane of lamellar bodies *via* early

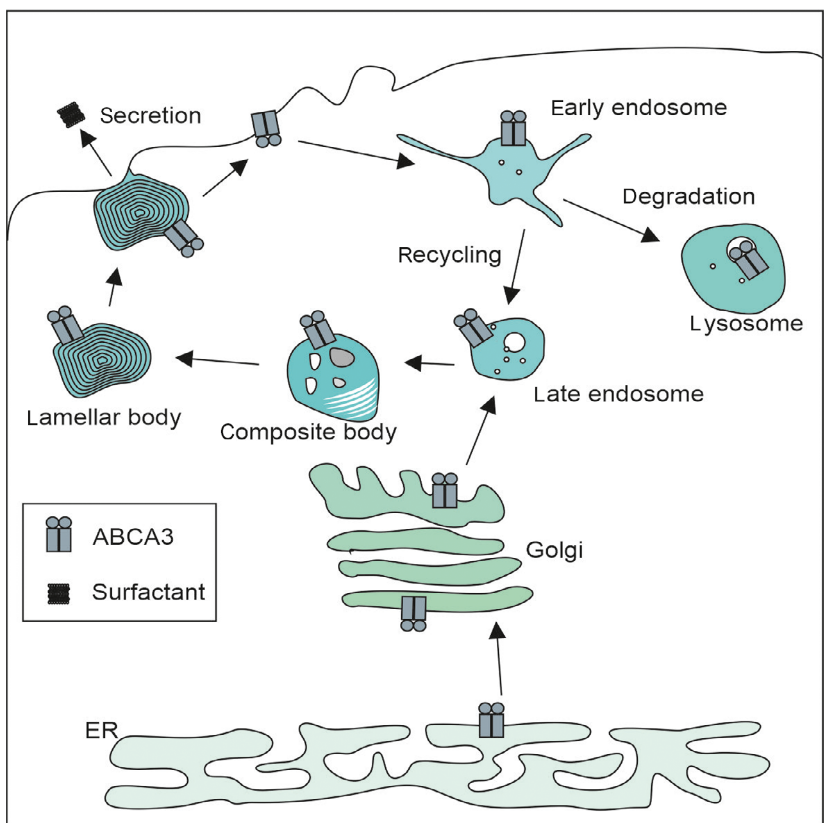

Fig. 3. Role of ABCA3 in lung surfactant production. ABCA3 is trafficked to late endosomes (multivesicular bodies) which fuse to form composite bodies. The composite bodies mature to lamellar bodies, which are filled with surfactant composed of lipids and surfactant proteins. The role of ABCA3 in the production of surfactant is to transport primarily phosphatidylcholine and phosphatidylglycerol into the lumen of lamellar bodies. Surfactants are released by exocytosis. ABCA3 is internalized to early endosomes and subsequently degraded in lysosomes or recycled into late endosomes.

endosomes and late endosomes. The ABCA3 sequence contains the signature motif (xLxxKN or xLxKN) targeting ABCA transporters to the proximal post-Golgi secretory vesicles, but the determinants of targeting to the lamellar body are not known [39]. Subsequently, ABCA3 is trafficked further to the plasma membrane and back, recycling between the endosomal pool and the plasma membrane, or directed to the lysosome for degradation. Through the transport of lipids into the lamellar bodies, ABCA3 is one of the main regulators of lung surfactant metabolism. Functional and trafficking defects in ABCA3 mutants are associated with respiratory distress syndrome [40], and deletion of Abca3 in mice is incompatible with life due to acute respiratory failure after birth. AT2 cells of Abca3-deficient mice contain abnormally dense lamellar body-like organelles with significantly reduced phosphatidylcholine and phosphatidylglycerol levels. These findings indicate that ABCA3 contributes to lamellar body biogenesis by transporting these lipids as substrates [41]. Trafficking of ABCA3 is influenced by post-translational modifications including glycosylation and proteolytic cleavage. Disease-associated mutations result in misfolding, abnormal intracellular trafficking without targeting to lysosomes or catalytic inactivation. Modulation of protein trafficking by chemical chaperones has been suggested as a viable therapeutic approach for disorders caused by ABC transporter mutations affecting folding, and ABCA3 certainly is a prime candidate for trafficking rescue [42].

The role of ABCA2 and ABCA5 in the intracellular lipid homeostasis is less understood. ABCA2 is localized in intracellular vesicles, identified in transfected cells as LROs only partially overlapping with classical endolysosomes [43]. Expression of ABCA2 is correlated with genes involved in cholesterol homeostasis [44], and its function is associated with the intracellular shuttling of free cholesterol delivered from low-density lipoproteins (LDL) to the ER. ABCA2 is highly expressed in the brain and to a lesser extent in other tissues [45]. Overexpression of ABCA2 can enhance the levels of amyloid beta precursor protein and beta amyloid [46], and a polymorphism in exon 14 of ABCA2 was shown to be associated with early and late onset of Alzheimer's disease [47,48]. Abca2 knockout mice exhibit neurological symptoms and a neural accumulation of gangliosides and cerebrosides with reduced sphingomyelin, suggesting an involvement in sphingolipid metabolism [49]. The physiological function of the lysosomal ABCA2 is not known. It is

speculated that ABCA2 flips negatively charged phospholipids to the luminal side of intraluminal vesicles, which results in a high concentration of negative charge attracting the acid ceramidase and glucocerebrosidase catalyzing the degradation of ceramide to sphingosine [50]. Ceramide promotes the displacement of cholesterol onto the soluble Niemann–Pick 2 (NPC2) protein, which transfers cholesterol to NPC1 in the limiting membrane of lysosomes. Cholesterol is distributed from the limiting membrane to other cellular sites including the ER, where the cholesterol biosynthesis is regulated (Fig. 4). In contrast, sphingosine, because of its lipidic structure, is less effective than ceramide in displacing cholesterol on NPC2, resulting in sequestration of free cholesterol in lysosomes [51].

ABCA5 is localized to late endosomes and lysosomes [52] but it was also found in the Golgi [53] and recently in the plasma membrane [54] (Fig. 5). The tissue distribution of ABCA5 varies between species, with predominant expression in the brain, lung and testis. Interestingly, in mouse, rat and human a smaller transcript resembling a half transporter was detected, however at lower expression levels [52,55,53]. ABCA5 knockout mice feature symptoms similar to those of several lysosomal diseases in heart, including dilated cardiomyopathy, leading to death at ~ 10 weeks of age [52]. Macrophages of mice with dysfunctional ABCA5 show reduced cholesterol efflux to high-density lipoproteins (HDL) and an increased cholesterol efflux to ApoA-I [56]. In the mouse macrophage cell line RAW 264.7, expression of ABCA5 was strongly induced in the presence of cholesterol. In contrast to ABCA1, which showed a significantly reduced expression upon exposure to high concentrations of cholesterol, ABCA5 expression was even more induced suggesting that ABCA5 assumes a critical function contributing to cholesterol efflux to HDL in cholesterol-overloaded macrophages [54]. Mutations in ABCA5 are associated with excessive hair overgrowth, accompanied by defects in the autophagy pathway and increased levels of free cholesterol in intraluminal vesicles of lysosomes of keratinocytes [57]. Moreover, ABCA5 was implicated in the neuropathology associated with Alzheimer's and Parkinson disease [58,59]. Alzheimer's disease patients show an increased expression of ABCA5 in hippocampal neurons, and amyloid-β-peptide levels are significantly reduced by ABCA5. Similar changes were observed with α-synuclein in neurons of the amygdala, where Parkinson disease

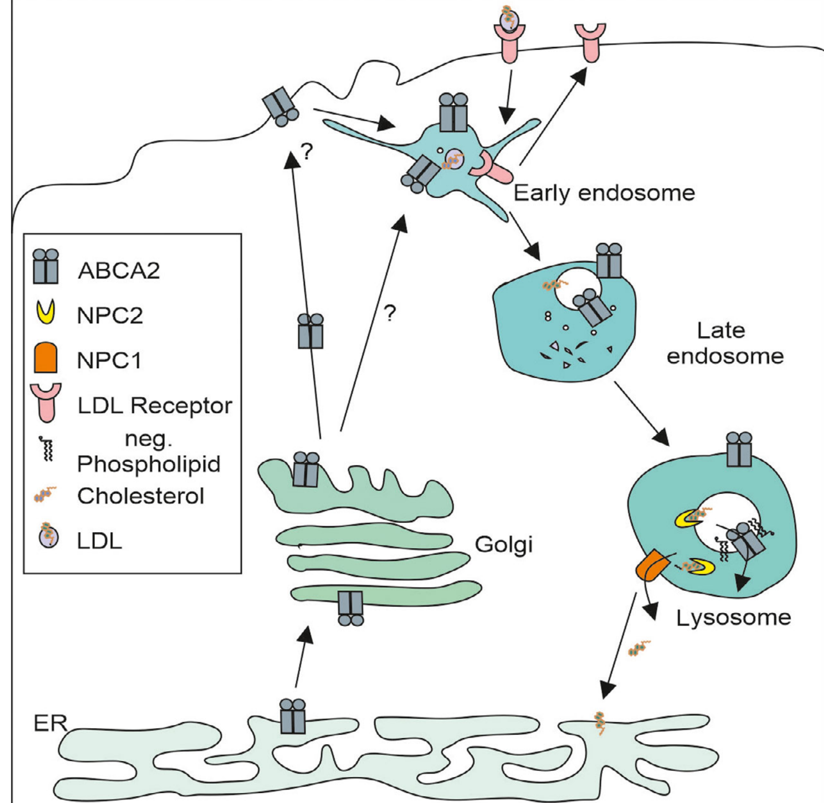

Fig. 4. Presumed function of ABCA2 in cholesterol homeostasis. Free cholesterol, delivered by LDL, is taken up by the cell and delivered to lysosomes where the proteins are hydrolyzed. High levels of ceramide promote the transfer of cholesterol from the membrane of the intraluminal vesicles to Niemann–Pick protein 2 (NPC2). NPC2 delivers cholesterol to NPC1, which transfers cholesterol to the ER by nonvesicular transport. In the ER, the concentration of cholesterol is sensed to regulate cellular cholesterol homeostasis. Since high expression of ABCA2 leads to sequestration of cholesterol into lysosomes, it is speculated that ABCA2 flips negatively charged phospholipids into the luminal leaflet of intraluminal vesicles. The negatively charged surface of the luminal leaflet attracts acid ceramidase and glucocerebrosidase, which leads to a decrease of the intraluminal concentrations of ceramide and therefore a reduced efflux of cholesterol out of the intraluminal vesicles.

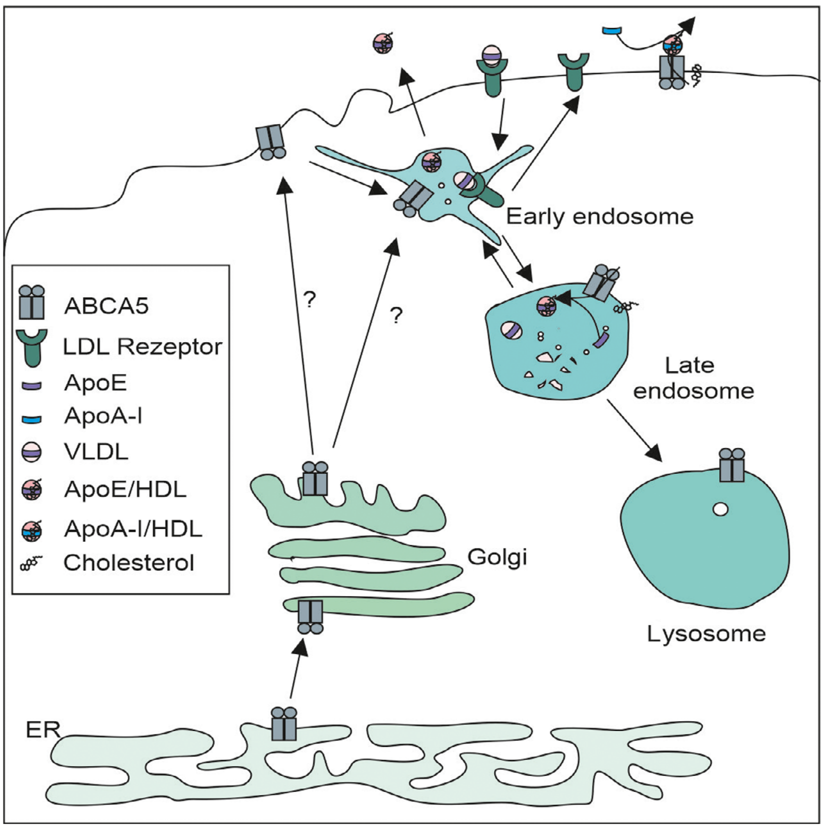

Fig. 5. Hypothetic role of ABCA5 in cholesterol efflux in macrophages. The majority of cholesterol bound to ApoA-1/HDL is exported by ABCA1 and ABCG1. However, ABCA5 seems to be also involved in cholesterol efflux. In the mouse macrophage cell line Raw264.7, ABCA5 is responsible for ApoA-I/HDL formation at the plasma membrane under high cholesterol levels. Since ABCA5 is also found in the endolysosomal system, it may participate in the assembly of ApoE/HDL where ApoE is delivered by endocytosis of VLDL. The trafficking route of ABCA5 is not known.

patients showed higher levels of ABCA5. It is assumed that the overexpression of ABCA5 is a protective response in these conditions.

Lysosomal peptide transport: ABCB9

ABCB9 forms a homodimeric ABC transporter, shuttling peptides into the lumen of lysosomes. Due to a ~ 40% sequence identity with the subunits of the heterodimeric transporter associated with antigen processing (TAP, ABCB2/3), ABCB9 is also named TAP-like (TAPL). TAPL is a highly conserved gene with a sequence identity of 95% between human and rat or mouse. Homologs are present in phylogenetically more distant organisms such as sea lamprey or even *Ceanorhabditis elegans* [60,61]. The orthologous HAF-4 and HAF-9 proteins in *C. elegans* are localized in large, nonacidic gut granules belonging to LROs [60]. Interestingly, TAPL distribution shows a similar pattern in sea lamprey and mammalians [61]. Though a Coordinated Lysosomal Expression And Regulation element (CLEAR) is found in the promoter region of *abcb9*, its expression is not regulated by the major regulator of lysosome function and biogenesis the transcription factor EB [62]. At a transcriptional level, ABCB9 was detected in nearly every tissue with high expression in the central nervous system and testis [63,64]. Expression of the TAPL protein is high in Sertoli cells and the endothelial cells of the blood-brain barrier [65], and strong expression was detected in antigen presenting cells including dendritic cells and macrophages [66].

ABCB9 belongs to group of type IV ABC exporters [24] and forms a homodimer with a TMD composed of 2×6 transmembrane helices, and two cytosolic NBD. This dimer of core-transporters is capable of peptide transport, but is mislocalized to the plasma membrane [66]. ABCB9 contains an additional N-terminal TMD, TMD0. As determined by solution NMR, TMD0 is composed of four transmembrane helices (TMH) and a short luminal helical element connecting transmembrane helices 1 and 2 [67,68]. The TMD0 of TAP1 and TAP2 also contain four TMHs, whereas in other ABC transporters the TMD0 segment contains five transmembrane helices [69,70]. TMD0 is targeted to lysosomes, and can guide the core-ABCB9 to lysosomes through noncovalent interactions [66,71]. Additionally, TMD0 functions as an interaction hub for other membrane proteins. In lysosomes, TMD0 mediates interaction with the lysosomal associated

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membrane proteins (LAMP) LAMP-1 and LAMP-2B but not with the splice isoform LAMP-2A [72]. Interaction with LAMP-1 stabilizes ABCB9 significantly against proteolytic degradation, potentially by retaining the transporter in the limiting membrane of lysosomes. In the ER, TMD0 interacts with the transmembrane region of YIP1-interacting factor homolog B (YIF1B) [73]. The YIF1B is a factor involved in the anterograde shuttling of the 5-HT1A serotonin receptor to the cis-Golgi. In the case of ABCB9, YIF1B is important but not essential for the release from the ER.

After the discovery of ABCB9 in 1999, its subcellular localization was subject to controversies. Zhang *et al*. showed lysosomal localization of ABCB9 by fluorescence microscopy and by subcellular fractionation in stably transfected SK-OV-3 cells [64]. In contrast, in transiently transfected HEK-293 and COS-1 cells, a retention of ABCB9 in the ER was reported [74]. However, lysosomal localization of ABCB9 was confirmed in several subsequent studies [66,73].

Intracellular trafficking of ABCB9 was characterized by the retention using selective hooks (RUSH) assay [75]. In the RUSH assay, ABCB9 tagged with the streptavidin binding peptide is artificially retained in the ER by a hook protein containing streptavidin. With the addition of biotin, ABCB9 is released from the ER in a synchronized manner and the intracellular route can be followed by fluorescence confocal microscopy. The RUSH assay unanimously proved that ABCB9 is shuttled by the direct route from the Golgi *via* early endosomes to lysosomes (Fig. 6). Although ABCB9 was never observed in the plasma membrane, the drawback of this elegant method is that a short-lived intermediate step at the plasma membrane can be missed. However, plasma membrane expression of ABCB9 was excluded by applying the endocytosis inhibitor Dyngo4a, which also ruled out indirect lysosomal targeting [73]. Bioinformatics combined with mutational studies could not identify a sorting motif in the cytosolic loops of TMD0, the sorting domain of ABCB9. Sequence alignments of phylogenetically distant ABCB9 orthologs identified conserved charged residues in the first three transmembrane helices [73]. A salt bridge between D17 and R57 was found to be essential for the release of ABCB9 from the ER, whereas the network of D45, D49 and K100 is mandatory for the release from the Golgi. Following substitution of D17 with asparagine, ABCB9 is retained in the ER and its interaction with the sorting chaperone

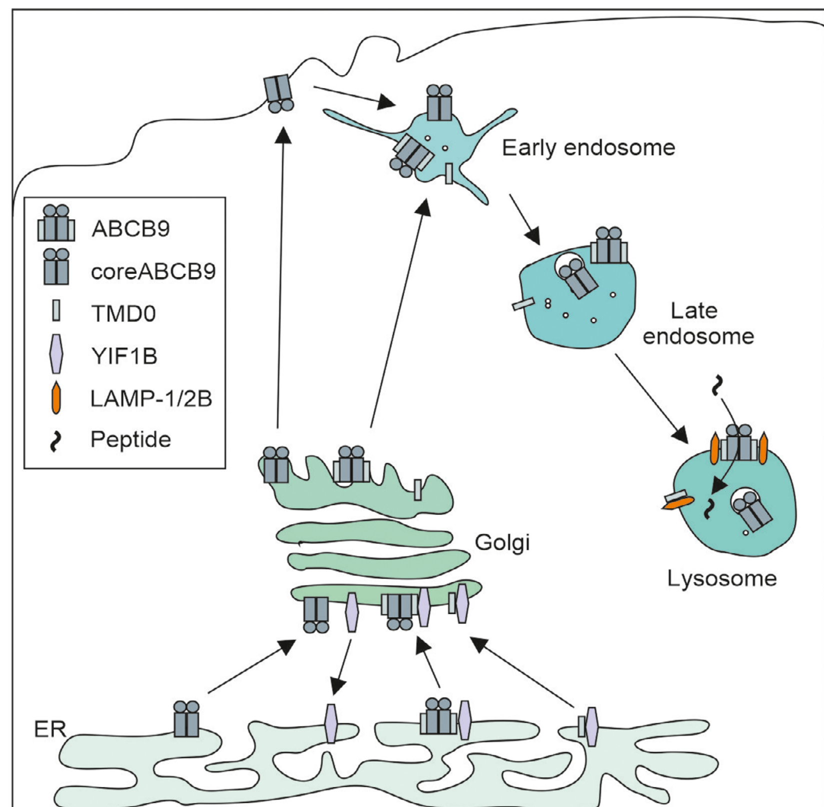

Fig. 6. Intracellular trafficking of ABCB9. The TMD0 of ABCB9 interacts in the ER with the shuttling factor YIF1B, which facilitates translocation from the ER to the Golgi. Subsequently, ABCB9 is trafficked *via* early and late endosomes to lysosomes where it interacts with LAMP-1/2B in the limiting membrane. Isolated TMD0, the trafficking domain of TAPL, follows the same route as full length ABCB9. In contrast, coreABCB9 is shuttled to the plasma membrane, internalized, packed in late endosomes and is ultimately degraded in lysosomes.

YIF1B is significantly weakened. Therefore, it can be speculated that the network of conserved, charged residues in the transmembrane helices of ABCB9 induces the correct conformation for the interaction with auxiliary proteins essential for lysosomal sorting.

Peptide transport by ABCB9 was first shown with crude membranes of insect cells expressing human ABCB9 [76]. Subsequently, ABCB9 dependent peptide transport into isolated lysosomes of stably transduced Raji cells or proteoliposomes containing reconstituted ABCB9 was demonstrated [77]. For peptide recognition, the N-terminal and C-terminal residue is important with a preference for positively charged and large hydrophobic residues. In contrast, negatively charged residues and residues like asparagine or methionine are disfavored at the termini. The sequence in between both ends can be highly promiscuous and of varying length, ranging from 6- to 59-mer peptides. Overall, positively charged peptides are transported more efficiently, suggesting that the negatively charged membrane enriches the positively charged peptides in spatial proximity to the transporter [77]. Although shorter and longer peptides are equally well recognized, the longer peptides are 20-fold slower transported due to significant higher activation energy corresponding to larger conformational changes. Moreover, ATP and GTP are hydrolyzed with the identical rate constants but ATP energizes the transport significantly more efficiently [78]. Peptide transport kinetics are determined most often in bulk, meaning that transport is averaged over thousands of transporters independent of their activity. By dual color fluorescence burst analysis, a method in which fluorescence bursts arising from single liposomes diffusing through the confocal volume of a microscope are quantified, single transporter kinetics were analyzed [79]. By this method, we established the transport rate of a 9-mer peptide as 8 peptides per minute, showing that ABCB9 is indeed a primary active transporter accumulating peptides against a 1000-fold gradient. Although peptide accumulation was not inhibited by an electrochemical gradient, luminal peptide concentrations never exceeded 1 mM, which is far below the theoretical accumulation that may be achieved from the free energy of ATP hydrolysis. Therefore, a trans-inhibition mechanism was postulated in which the luminal peptide inhibits further translocation similar to the product inhibition of enzymes [79].

Based on the broad tissue distribution and multiple GC-boxes in the promoter region for binding of the ubiquitous transcription factor Sp1 [80,61], ABCB9 seems to fulfill the function of a housekeeping factor preventing the accumulation of potentially cytotoxic or stressful peptides in the cytosol. ABCB9 translocates a similar spectrum of peptides as the transporter associated with antigen processing (TAP), which is an essential factor in the major histocompatibility class I (MHC I) mediated cellular immune response. TAP transports antigenic peptide into the ER lumen where they are loaded on MHC I for presentation on the cell surface to $CD8^+$ cytotoxic T cells. However, overexpression of ABCB9 in TAP1 or TAP2 deficient cells could not restore the MHC I surface presentation of antigenic peptides [66]. Moreover, ABCB9 was also not involved in the presentation of TAP-independent epitopes in tumor cells deficient in TAP [81]. Recently, a detailed study has characterized the immune system of ABCB9 knockout mouse in great detail. Lack of ABCB9 did not affect the number of $CD8^+$ thymocytes, $CD4^+$ or $CD8^+$ splenocytes, dendritic cells, B-cells, natural killer (NK) or NK-T cells or the expression of MHC I and MHC II molecules [82]. In further studies, a contribution of ABCB9 to cross-presenting exogenous antigens on MHC I or cytosolic antigens on MHC II could not be demonstrated. However, it was shown that ABCB9 modulates the maturation of phagosomes, which has an effect on the degradation of the antigenic peptides. In *C. elegans*, the orthologous heterodimeric proteins HAF-4 and HAF-9 are localized in large granules in the intestinal cells [60,83]. The granules are neither lipid droplets nor acidic granules instead, they are LROs associated with the endocytic pathway [84]. In the absence of either functional HAF-4 or HAF-9, this subset of granules disappears from the intestinal cells, and the animals show defects in brood size, growth rate and defecation rate [60]. Taken together, ABCB9 seems to have a function in the biogenesis of LROs in *C. elegans* and in the modulation of phagosome maturation.

The enigmatic ABCB6 transporter: a journey along the endolysosomal continuum

ABCB6 is widely expressed in many tissues, especially in the heart, liver, skeletal muscles [85], the red blood cells [86,87], and the skin [88]. ABCB6 is a homodimeric protein containing a unique N-terminal (TMD0) region with five transmembrane helices, followed by the ABC-core consisting of a TMD and a cytoplasmic NBD. ABCB6 was also named P-glycoprotein related protein (PRP) [89], and MTABC3, as it was believed to be an ortholog of Atm1p, a yeast mitochondrial protein localized to the inner mitochondrial membrane contributing to the biogenesis of cytosolic Fe/S proteins thorough mitochondrial export [85]. Thus, ABCB6 was initially classified as a

mitochondrial ABC transporter, despite the fact that, unlike ABCB7, ABCB8 and ABCB10 that reside in the inner mitochondrial membrane, ABCB6 does not contain a mitochondrial targeting sequence [90]. To date, the intracellular localization of ABCB6 is a matter of debate, with conflicting reports suggesting mitochondrial or endolysosomal expression. Current databases summarizing quantitative mass spectrometric data of subcellular fractions list ABCB6 as a mitochondrial [91] or a lysosomal protein [9]. Below we discuss some aspects of this controversy, arguing for the classification of ABCB6 as an endolysosomal protein. We refer the interested reader to a recent review by Boswell-Casteel and coworkers for an alternative view [92].

In 2006, Krishnamurthy and colleagues established ABCB6 as an outer mitochondrial membrane protein responsible for the mitochondrial import of porphyrin metabolites [93]. Synthesis of heme starts and ends in the mitochondrial matrix, including several enzymatic steps that take place in the cytosol. Following the formation of δ-aminolevulinic acid (ALA), the tetrapyrrole coproporphyrinogen III molecule (CPIII) enters the mitochondria, where protoporphyrin IX is formed by oxidation steps. Confocal microscopy analysis and fractionation of cells expressing Flag-tagged ABCB6 indicated that ABCB6 is localized to the mitochondria, and measurements using isolated mitochondria suggested that the ABCB6 ATPase is stimulated by CPIII. Based on these results the authors concluded that ABCB6 is located to the outer mitochondrial membrane, and its function is required for mitochondrial porphyrin uptake [93,94]. Since the first report, several studies from the same laboratory have been published in support of this model (reviewed in [92]). For example, the biological impact of ABCB6 was expanded to include protection against oxidative stress by means of increasing the availability of heme needed for the function of catalase [95].

Independent studies confirmed an (indirect) link between ABCB6 and multidrug resistance (MDR), based on the correlation of ABCB6 expression and *in vitro* resistance against chemotherapeutics such as camptothecin, cisplatin [96], paclitaxel/FEC [5-fluorouracil (5-FU), epirubicin, and cyclophosphamide] [97], paclitaxel [98] or 5-FU [99]. At the same time, ABCB6 was spotted in other organelles, and mounting evidence from several independent laboratories indicated that the suggested mitochondrial localization may be incorrect. In addition to an intracellular localization interpreted to correspond to the mitochondria, Paterson and coworkers identified ABCB6 in the plasma membrane [100]. Based on subcellular fractionation and immunofluorescence analyses of native and fluorescently tagged proteins, ABCB6 was localized to the Golgi system [101], the endolysosomal continuum [102] including the plasma membrane [86,87] and intracellular vesicles [102–106,88]. Quantitative mass spectrometric analysis coupled with subcellular fractionation identified ABCB6 as a lysosomal protein. The 'balanced sheet' analysis used in that study clearly shows that ABCB6 resides in lysosomes, which often contaminates preparations of 'purified' mitochondria [9]. In 2012, ABCB6 was identified as the molecular basis of a rare blood group antigen called Langereis (Lan), establishing ABCB6 as an erythrocytic protein (red blood cells are devoid of mitochondria) [86]. At present it is not clear how these contradictory reports may be reconciled. Subcellular expression can be influenced by experimental conditions such as the metabolic state of the cells, or artifacts linked to tagging, overexpression or fractionation. Our own laboratory has failed to detect ABCB6 in the mitochondria, regardless of the cellular or experimental models. As opposed to ABCB7/8/10, which showed the expected mitochondrial expression pattern, endogenous or cDNA-derived ABCB6 was consistently localized in extramitochondrial compartments by confocal or electron microscopy, and could be no longer found in pure mitochondrial fractions devoid of lysosomal contamination [107,105,108,87,109].

The overwhelming data in support of the endolysosomal localization called for a paradigm shift to remove ABCB6 from the list of mitochondrial proteins [105,90]. However, it may be argued that localization studies are relevant only in the context of complementary functional evidence, linking expression and function of a protein to a specific subcellular compartment. Although initial findings by Krishnamurthy *et al.* [93] suggested that loss of one Abcb6 allele in embryonic stem cells impairs porphyrin synthesis, knockout mice were phenotypically normal, exhibiting elevated sensitivity to phenylhydrazine-induced stress [110]. While ABCB6 was shown to be a genetic modifier of porphyria [111], loss of ABCB6 is dispensable for erythropoiesis in humans [86], questioning the requirement of ABCB6 in the mitochondrial import of porphyrins. At the same time, the presumed endolysosomal function of ABCB6 remains unknown. Unfortunately, the phenotypes associated with ABCB6 mutations do not provide an easy clue. Whereas deletion of ABCB6 in Lan-negative individuals or mice does not result in an overt phenotype, ABCB6 mutations cause various pathological conditions encompassing a wide range of symptoms such as coloboma [112], pseudohyperkalemia

[113], and dyschromatosis universalis hereditaria (DUH) [88]. Ocular coloboma is a developmental defect leading to the incomplete closure of the optic fissure, pseudohyperkalemia is characterized by increased serum potassium levels in whole blood stored at or below room temperature, whereas DUH is a pigmentary disorder characterized by hyperpigmented and hypopigmented macules distributed randomly over the body. Electron microscopy of DUH samples revealed an abnormal pattern of mature melanosomes and immature melanosomes in the basal layer of hypo- and hyperpigmented skin [114]. As LROs, melanosomes are derived from the endolysosomal continuum, and the association of ABCB6 with a pigmentary defect suggested a functional link between ABCB6 and melanin synthesis. Indeed, using confocal microscopy and ultrastructural analysis, we were able to demonstrate that ABCB6 localizes to the membrane of early melanosomes and lysosomes of the human melanocytic cell line MNT-1 (Fig. 7). It is important to emphasize that ABCB6 was detected in the limiting membrane of lysosomes, as opposed to cargo proteins destined for lysosomal degradation that are sorted into ILVs and remain in the endosomal lumen. Further, depletion of ABCB6 by siRNA induced an aberrant accumulation of multilamellar aggregates in pigmented melanosomes, which could be rescued by the overexpression of wild-type ABCB6 but not by variants containing DUH mutations [107]. Despite localization of ABCB6 to LROs, its functional contribution to the early steps of melanogenesis remains unknown. Since Abcb6−/− mice (developed on a C57/Bl6 background) and Lan-negative individuals do not show any overt sign of pigmentation defect, ABCB6 is clearly dispensable for melanogenesis. We speculate that ABCB6 is required for maintaining the intraluminal homeostasis of the maturing early melanosome, which is needed for efficient fibrillation and matrix formation [107].

Endogenous ABCB6 is glycosylated in multiple cell types, indicating trafficking through the endoplasmic reticulum (ER). Disruption of the atypical glycosylation site (NXC) at the amino terminus blocked ER exit and resulted in ABCB6 degradation [115]. Several disease-causing ABCB6 mutations were shown to influence intracellular trafficking, resulting in the trapping of mutant ABCB6 variants in the ER [87] or the Golgi [112,88]. A timely and targeted passage of ABCB6 through the endolysosomal continuum is probably also important for the regulation and function of ABCB6. To understand the dynamic process of ABCB6

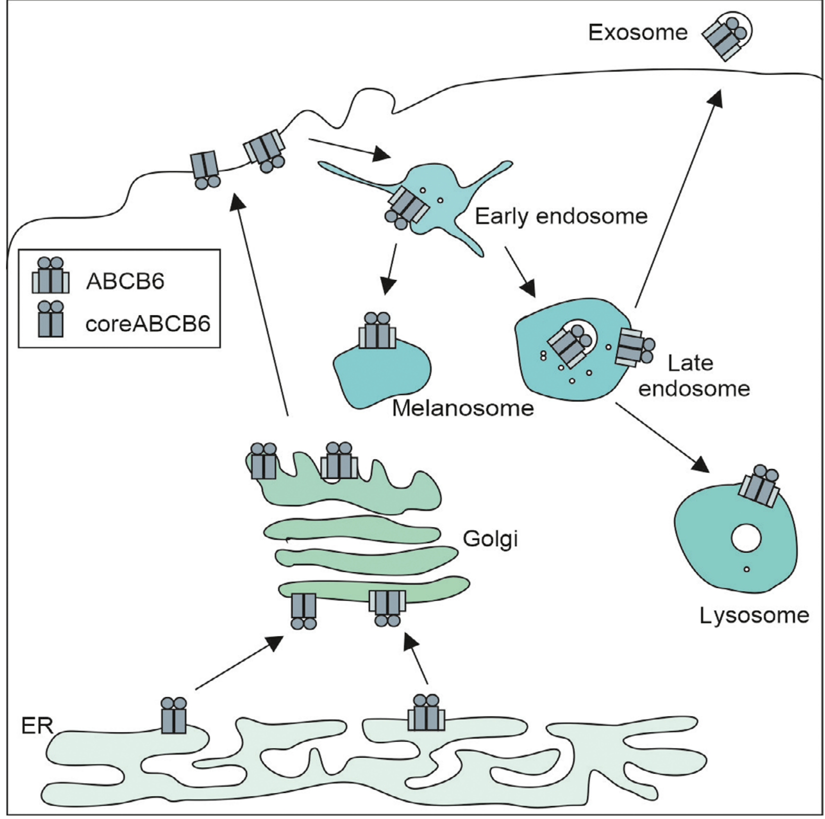

Fig. 7. Lysosomal trafficking of ABCB6. Following passage through the ER and Golgi, ABCB6 is targeted to the cell surface. ABCB6 is rapidly internalized from the plasma membrane through endocytosis, to be distributed to late endosomes and lysosomes. ABCB6 is present in the membrane of mature erythrocytes and in exosomes released from reticulocytes during the final steps of erythroid maturation. CoreABCB6 is also targeted to the plasma membrane but is not internalized. In pigment cells, ABCB6 is localized to early endosomes, which give rise to maturing stage II-IV melanosomes responsible for the synthesis, storage, and transport of melanin.

distribution beyond the snapshots of dynamically fixed end points offered by immunocytochemical analyses, we followed the trafficking of the endogenous ABCB6 protein in live cells. These results showed that ABCB6 is first targeted to the plasma membrane, and is rapidly internalized through endocytosis to be distributed to the limiting membrane of multivesicular bodies and lysosomes [108]. ABCB6 does not contain consensus signals associated with either lysosomal or mitochondrial targeting, and its N-terminal extension TMD0 bears no resemblance to any other proteins. Interestingly, the N terminally truncated core ABCB6 was retained in the plasma membrane, whereas a TMD0-GFP chimaera was targeted to the endolysosomal system. Taken together, these results indicate that TMD0 is dispensable for the folding, dimerization, membrane insertion and ATP binding/hydrolysis of the core–ABCB6 complex, but has a crucial role in the lysosomal targeting of ABCB6 [108]. Almost identical results were obtained with CeHMT-1 (*C. elegans* Heavy Metal Tolerance factor 1), which shares significant sequence and topological similarity with ABCB6 [116,117]. The evolutionarily conserved role of the HMT-1 proteins is to confer tolerance to heavy metals through the intracellular sequestration of metal complexes. Significantly, the hypersensitive phenotype of *Schizosaccharomyces pombe* and *Caenorhabditis elegans* strains defective for HMT-1 could be rescued by the human ABCB6 protein, based on the ABCB6-mediated vacuolar sequestration of cadmium. Modulation of ABCB6 levels by overexpression or gene silencing in human glioblastoma cells resulted in a parallel change in cadmium sensitivity, suggesting that endolysosomal ABCB6 may have an orthologous function in human cells. These results revealed that ABCB6 is a functional ortholog of the HMT-1 proteins, linking its function to the highly conserved mechanism of intracellular cadmium detoxification [109]. Interestingly, ABCB6 was implicated in the treatment susceptibility of *Leishmania panamensis,* which is an obligate intracellular parasite residing in the phagolysosomes of macrophages. ABCB6 was suggested to transport antimonial compounds into the Leishmania-containing phagosomes [118]. Given the conservation of HMT-1 proteins, we hypothesize that ABCB6 sequesters glutathione complexes. This model needs further experimental validation, especially considering that the effect of ABCB6 on heavy metal sensitivity was cell-dependent [109]. Similarly, the pathophysiological relevance of ABCB6 in heavy metal-related diseases, such as neurodegenerative conditions, dysfunction of the digestive tract and cancer will have to be confirmed by studies using relevant disease models.

Role of ABC transporters in lysosomal drug sequestration

Rescue of HMT-1 deficient organisms by ABCB6 clearly shows that the heterologously expressed ABCB6 sequesters heavy metals into the vacuoles of yeast and worm cells, but an orthologous function in mammalian cells has not been directly shown. Several other lysosomal ABC transporters have been implicated in the lysosomal sequestration of xenobiotics. A strong correlation between ABCA2 expression and *in vitro* drug resistance to cisplatin, mitoxantrone and estrogen derivatives was observed, and it is assumed that ABCA2 sequesters drugs or damaged lipids into lysosomes [119,120,50,121]. ABCA3 was also implicated in drug resistance through intracellular drug sequestration, and high levels of ABCA3 were significantly associated with the overall survival of acute myeloid leukemia patients [122]. The relevance of ABCA3 was also demonstrated in chronic myeloid leukemia, where it was shown that ABCA3 sequesters imatinib to the lysosomes [123]. Interestingly, ABCA5 was shown to be a urine diagnostic marker for high-grade prostatic intraepithelial neoplasia in biopsy-confirmed patients [124]. ABCA5 has been implicated in drug resistance, but its involvement in the transport of xenobiotics has not been shown [125]. The impact of ABCB9 in drug resistance was analyzed in the ovarian cancer cell line SK-OV-3. Stable expression of ABCB9 did not confer resistance to colchicine, vinblastine, methotrexate, daunorubicin, and cisplatin [64]. Recently, low expression of ABCB9 has been identified as a prognostic indicator of poor overall survival in ovarian cancer [126]. Paradoxically, overexpression of ABCB9 in the malignant pleural mesothelioma cell line NCI-H2452 resulted in a cisplatin sensitive phenotype, and treatment of the gastric cancer cell line HGC-27 with a combination of cisplatin, paclitaxel, and docetaxel generated drug-resistant cells which showed strongly reduced ABCB9 levels [127,128]. In contrast, overexpression of microRNA-24 increased the sensitivity to paclitaxel in drug-resistant breast carcinoma cell lines *via* targeting ABCB9 [129]. Clearly, further studies are needed to understand the contribution of ABCB9 lysosomal sequestration and drug resistance.

According to the textbook wisdom, the archetypal ABC transporter ABCB1 (MDR1/P-gp) is expressed in the plasma membrane to protect cells by keeping the concentrations of cytotoxic drugs below a cell-killing threshold. Interestingly, numerous studies have reported resistance mechanisms linked to the intracellular trapping of cytotoxic drugs, implying that P-gp localized in intracellular compartments may mediate

active sequestration of anticancer drugs [130–132]. For example, a recent study speculated that intracellular P-gp is responsible for the synergistic effect of hydroxychloroquine-azithromycin combination in COVID-19 therapy, by increasing the lysosomal concentration of azithromycin (since this report the WHO has discontinued research on hydroxychloroquine treatment for COVID-19) [133]. Lysosomal P-gp was also suggested to confer drug resistance against the antibody-drug conjugate (ADC) brentuximab vedotin and its cytotoxic payload [134]. ADCs specifically bind to tumor-associated antigens that are highly expressed in tumor cells. Once ADCs bind to surface antigens, they are internalized into endosomes and lysosomes, where further processing releases the toxic payloads. Whereas receptor-mediated uptake might overcome the first line of defense mounted by the plasma membrane, P-gp expressed in the endolysosomal compartment can still sequester the payloads from the intended targets.

In addition to the plasma membrane, P-gp has been localized to various intracellular compartments, such as the ER and Golgi, endosomes, lysosomes, and even mitochondria. While mitochondrial localization was ruled out [135], many of these sites indicate intermediate locations along the trafficking pathway (synthesis in ER/Golgi, trafficking/recycling in endosomes, and degradation in lysosomes). Tagging of P-gp with EGFP in HeLa cells revealed that P-gp is transported from the ER to the Golgi and finally to the plasma membrane within 12–48 h. The half-life of cell-surface-expressed P-gp is in the range of 25–27 h, which is regulated by constant trafficking/recycling between the endosomal pool and the plasma membrane [136]. Interestingly, significant daunorubicin accumulation occurred in transfected cells when P-gp-EGFP was localized predominantly within the ER, and accumulation remained high when P-gp-EGFP was mainly localized in the Golgi. However, these studies have also demonstrated that ER and Golgi localization of P-gp is transient, and that lysosomal localization is less common [137]. Other studies concluded that lysosomal sequestration is not a major mechanism in intracellular sequestration [138]. Thus, intracellularly trapped P-gp remains active, and therefore it can contribute to the vesicular sequestration of drugs. Whether P-gp is also able to function in late endosomes or lysosomes remains an open question, especially considering the lysosomal degradation of P-gp [139]. Based on the study of Pgp-expressing MDR cells, a recent study claimed that sequestration of doxorubicin and further ionizable P-gp substrates to LAMP2-stained lysosomes is mediated by P-gp. Doxorubicin accumulation could be prevented by incubation with the established P-gp inhibitors valspodar or elacridar or by silencing P-gp expression with siRNA [140]. The same authors suggested that the toxicity of Dp44mT is increased by the function of lysosomal P-gp [141], linking lysosomal P-gp to drug resistance and collateral sensitivity [142]. It has to be noted that weak-base cytotoxic compounds such as doxorubicin, daunomycin, sunitinib [143], nintedanib [144], or vinblastine are trapped in acidic organelles independently from the fact that they are transported by P-gp. Remarkably, tariquidar (and presumably further weak-base P-gp inhibitors) is also trapped by lysosomes, thus preventing the accumulation of protonated weak bases by mere competition, independently from P-gp [145]. Direct evidence demonstrating the functional expression of P-gp in the limiting membrane of lysosomes is lacking. As a cargo protein destined for lysosomal degradation, P-gp must be sorted into luminal ILVs, where its presumed sequestering function cannot reduce intracellular drug levels. Once sorted to ILVs, P-gp molecules are doomed to destruction by acid hydrolases present in the lysosomal lumen. Incorporation of P-gp into the membrane of cytoplasmic vesicles may be the consequence of the high-level expression of P-gp in MDR cells selected for extreme MDR [146]. Indeed, selected cell lines express very high P-gp levels, reaching 20% of the total plasma membrane proteins in Chinese hamster cells selected with doxorubicin [147]. Obviously, this degree of overexpression will likely overburden trafficking pathways, resulting in mislocalization of membrane proteins [138]. Since results claiming that drug resistance or collateral sensitivity is mediated by lysosomal P-gp [142] cannot be universally reproduced in a wide range of P-gp expressing cells [148,149], the relevance of the particular *in vitro* models has to be questioned, especially considering the moderate P-gp levels observed in preclinical models of acquired drug resistance [150,151]. Although lysosomal drug trapping was demonstrated in brain endothelial cells, which sequester P-gp substrates in LAMP-2 and LysoTracker positive organelles identified as lysosomes [152], the role of lysosomal P-gp in anticancer therapy resistance remains to be demonstrated in a clinically relevant study using patient samples or genetically engineered mouse models of cancer [153,154].

Outlook

The substrates of lysosomal ABC transporters are not known but, on the other hand, the identification of the transported substrate—as in the case of ABCB9—does

not immediately reveal the physiological function. Since the functions of lysosomal ABC transporters are not fully understood, knowing how they are sorted, trafficked, and distributed can provide important hints related to their function. Localization and functional studies should be complemented with the analysis of regulatory elements [62], compartment- and localization-specific interactomes, the identification of subcellular targeting sequences and intracellular trafficking route(s) [75,155]. Ultimately, this research will clarify the link between the localization and physiological function of endolysosomal ABC transporters.

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