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REVIEW

Kei Takahashi · Yasuhisa Kimura · Koh Nagata
Akitsugu Yamamoto · Michinori Matsuo
Kazumitsu Ueda

ABC proteins: key molecules for lipid homeostasis

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Abstract Forty-nine ABC protein genes exist on human chromosomes. Eukaryotic ABC proteins were originally recognized as drug efflux pumps involved in the multidrug resistance of cancer cells. However, it is now realized that one of their major physiological roles is cellular lipid transport and homeostasis, and their dysfunction is often associated with human diseases. ABCA1 and ABCA7 mediate the apolipoprotein-dependent formation of a high-density lipoprotein–cholesterol complex. ABCA3 is indispensable for pulmonary surfactant secretion. ABCG5 and ABCG8 are involved in the secretion of plant sterols and cholesterol into bile. However, the primary substrates and mechanism of action of these ABC proteins have not been precisely defined. In this review article, we first describe the general structure and functions of eukaryotic ABC proteins. The current model of ABCA1 functionality is then explained based on studies on a topological model, subcellular localization, apoA-I dependence of HDL formation, functional defects of Tangier disease mutants, and ATP hydrolysis of purified ABCA1. ABCA1 is supposed to function as a transporter of lipids as well as a receptor for apoA-I. ABCA3 is likely involved in accumulating phospholipids and cholesterol in lamellar bodies and in generating multivesicular structures.

Key words Cholesterol · Transporter · ABCA1 · ABCA3 · ABCA7

Introduction

Eukaryotic ATP-binding cassette (ABC) proteins are generally recognized as drug efflux pumps that protect the body from various toxic substances. It is partly for historical reasons that *ABCB1* (*MDR1*), encoding P-glycoprotein, was the first of the ABC proteins to be identified in eukaryotes,[1–4] and another ABC protein gene *ABCC1* (*MRP1*) was isolated from multidrug resistant cancer cells[5] shortly after ABCB1. Both ABC proteins function as drug efflux pumps that protect the body from toxic substances. MDR1 extrudes a very wide array of structurally dissimilar compounds, all lipophilic and ranging in mass from approximately 300 to 2000 Da, including cytotoxic drugs that act on different intracellular targets. On the other hand, MRP1 transports various organic anionic conjugates, including glutathione, glucuronide, and sulfate conjugates. The discovery of MDR1 and MRP1 had a strong impact on the field of cancer chemotherapy and pharmacodynamics. This may be another reason for the strong impression that ABC proteins function as drug transporters. However, recent findings have suggested that the physiological role as self-defense machinery against xenobiotics is only one aspect of the importance of ABC proteins. The physiological importance of ABC proteins in lipid homeostasis has become obvious recently.

The importance of ABC proteins in lipid homeostasis was first suggested in 1993 with the discovery that ABCB4 (MDR2, also called MDR3) is a phosphatidylcholine translocator, indispensable for bile formation.[6] Two closely linked genes (*MDR1* and *MDR2*, also called *MDR3*) exist on chromosome 7 in humans, and three genes in mice (*mdr1, mdr2*, and *mdr3*, or *mdr1b, mdr2*, and *mdr1a*, respectively). Human *MDR1* and mouse *mdr1* and *mdr3* encode multidrug transporters whereas human MDR2 (MDR3) and mouse mdr2 are involved in phosphatidylcholine secretion into bile. The *ABCB4* gene is highly homologous to *ABCB1*, and exists next to *ABCB1* on chromosome 7q. The importance of ABC proteins in lipid homeostasis was firmly established by the discovery in 1999 that mutations in ABCA1 cause Tangier disease, a rare inherited disorder characterized by an absence of circulating high-density lipoprotein (HDL).[7,8]

Among 49 human ABC proteins, there still exist many whose physiological functions or endogenous substrates

K. Takahashi · Y. Kimura · K. Nagata · A. Yamamoto · M. Matsuo · K. Ueda (✉)
Laboratory of Cellular Biochemistry, Division of Applied Life Sciences, Faculty of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan
Tel. +81-75-753-6124; Fax +81-75-753-6104
e-mail: uedak@kais.kyoto-u.ac.jp

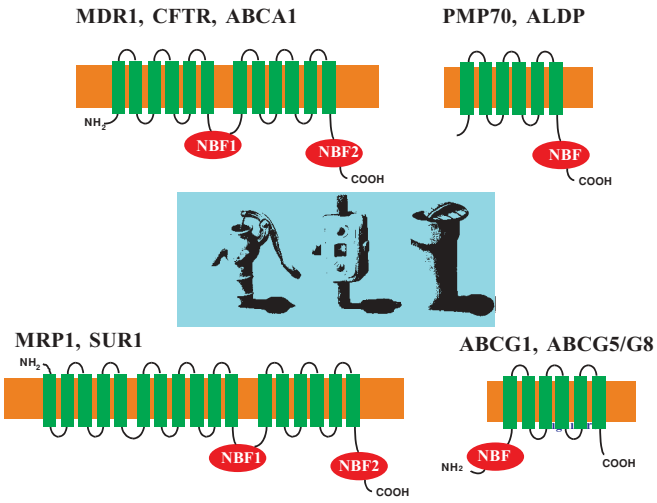

Fig. 1. Domain organization of eukaryotic ATP-binding cassette (ABC) proteins and drawings representing the three types of ABC proteins

are unclear. Also, awareness of the physiological importance of ABC proteins in lipid homeostasis is increasing. In this review article, we first describe the general structure and functions of ABC proteins and, in the second half, functions of ABCA subfamily proteins involved in lipid homeostasis.

Three major groups of eukaryotic ABC proteins

In eukaryotes, ABC proteins can be classified into three major groups based on function: transporters, regulators, and a channel (Fig. 1). Cystic fibrosis transmembrane conductance regulator (CFTR) is the only ABC protein clearly proven to function as a channel.[9–11] CFTR is a voltage-independent Cl^- channel found in the epithelial cells of many tissues and plays a major role in regulating Cl^- flux. Mutations in *CFTR* cause cystic fibrosis, one of the most common serious diseases, which affects 1 in 2000–2500 people in northern Europe and the United States. In addition to its Cl^- channel activity, CFTR is also suggested to act as a regulator of both an outwardly rectifying Cl^- channel (ORCC) and an epithelial Na^+ channel. Numerous abnormalities of cystic fibrosis are believed to be related to this multifunctionality of CFTR.[12]

The sulfonylurea receptor (SUR1) was identified as a target protein for sulfonylureas, such as glibenclamide, which is most commonly used in the treatment of noninsulin-dependent diabetes mellitus.[13] SUR1 is a subunit of the pancreatic β-cell K_{ATP} channel. The β-cell K_{ATP} channel is a hetero-octamer composed of pore-forming Kir6.2 subunits and SUR1 that coassemble with a 4:4 stoichiometry (5–8) and plays a key role in the regulation of glucose-induced insulin secretion. SUR1 is suggested to be not a channel or transporter itself but a switch that regulates the opening and closing of Kir6.2 channel subunits by monitoring the intracellular metabolic state, especially the ADP concentration.[14,15]

Most of the other eukaryotic ABC proteins seem to be transporters, although there are still many members with unknown functions. All the eukaryotic transporter-type ABC proteins studied to date transport substrates outwardly from cells. One exception may be a plant MDR-type ABC protein CjMDR1, which is involved in alkaloid transport in *Coptis japonica*, a perennial medicinal plant.[16] It is proposed that CjMDR1 is involved in the translocation of berberine from the root to the rhizome and functions as an influx pump for berberine.

Structure of ABC proteins

Domain structure

Most eukaryotic ABC proteins consist of four distinct domains (see Fig. 1). Two of these are highly hydrophobic, integral transmembrane domains (TMDs), each of which spans the membrane six times via α-helices. The other two are hydrophilic nucleotide-binding folds (NBFs), which share homology with potential nucleotide-binding sites of peripheral membrane components of bacterial active transporter systems. The individual domains are frequently expressed as separate polypeptides in prokaryotic ABC proteins,[17] whereas many eukaryotic ABC proteins have all four domains fused into a single polypeptide, as illustrated in Fig. 1.

Conserved sequence motifs

All the ABC proteins contain within each NBF at least three highly conserved sequence motifs, Walker A, Walker B, and the ABC signature sequence, known also as the C motif or a linker sequence. Walker A and B motifs are wildly conserved also among many nucleotide-binding proteins, such as ras P21 (Ras), RecA, adenylate kinase, myosin, and F_1-ATPase. The Walker A motif (G-X-X-G-X-G-K-S/T-S/T) is also known as a phosphate-binding loop (P-loop) or a glycine-rich loop. Residues within this motif interact with the phosphate groups and the magnesium ion of the bound Mg^{2+}–nucleotide complex. The Walker B motif is h-h-h-h-D, where h is a hydrophobic residue. In ATP- or GTP-binding proteins, this sequence constitutes a buried β-strand within the core of the NBF.[18] The highly conserved aspartate residue is involved in the coordination of the catalytic Mg^{2+} ion. An amino acid substitution of the conserved lysine residue in the Walker A motif or the conserved aspartate residue in the Walker B motif in either NBF results in a loss of the ATP hydrolysis activity of MDR1/P-glycoprotein.[19–21] The glutamate residue next to the aspartate residue of the Walker B motif is conserved among the ABC proteins except TAP1, NBF1s of MRPs, CFTR, or SURs. An amino acid substitution of this glutamate residue in the h-h-h-h-D-E sequence in either of the NBFs also abrogates steady-state ATP hydrolysis and

drug transport activities of MDR1 but does not impair vanadate-dependent nucleotide trapping.[22] An aspartate to glutamate substitution in NBF1 of MRP1 increases affinity for ADP and enhances ATP hydrolysis, whereas a glutamate to aspartate substitution in NBF2 of MRP1 decreases affinity for ATP and decreases ATP hydrolysis.[23] These results suggest that the acidic residue adjacent to the Walker B motif is involved in nucleotide binding, hydrolysis, and nucleotide release from NBF.

The C motif (L-S-G-G-Q-Q/R/K-Q-R) exists within each NBF of all the ABC proteins, but not of other nucleotide-binding protein families, such as GTP-binding proteins and the AAA superfamily. Therefore, it is also called the ABC signature motif. This motif is located immediately N-terminal to the Walker B motif. The C motif is suggested to be involved in the transduction of the energy of ATP hydrolysis to the conformational changes in the membrane integral domains required for translocation of the substrate.[17] According to the crystal structure of the *Escherichia coli* BtuCD protein, an ABC transporter mediating vitamin B_{12} uptake,[24] the two ATP-binding subunits (BtuD) are in close contact with each other in the crystal structure as predicted,[25] and the ATP molecules are proposed to be sandwiched between the Walker A sequence and the ABC signature motif of opposing BtuD subunits. This model is also supported by the structure of other prokaryotic ABC proteins such as MJ0796 and MalK.[26,27]

Other than the three conserved motifs mentioned above, glutamine located between the Walker A motif and the signature motif is highly conserved among the ABC proteins, and the loop containing this glutamine is called the Q-loop. A comparable amino acid substitution, Q1291R, in CFTR was observed in patients with cystic fibrosis, and the Q1291R CFTR shows no chloride channel function although it reaches the plasma membrane as a fully glycosylated mature protein.[28] A missense mutation of the corresponding glutamine in the second NBF of multidrug resistance protein 2 (MRP2, ABCC2) was detected in a patient with Dubin–Johnson syndrome (DJS), a hereditary disease characterized by hyperbilirubinemia. This mutation caused a lack of substrate-induced vanadate trapping, which may suggest that the glutamine is involved in the conformational change after ATP hydrolysis.[29]

Three-dimensional structure

An approximately 10-angstrom resolution structure of ABCB1 (MDR1/P-glycoprotein) was determined by electron cryomicroscopy of two-dimensional crystals.[30] The three-dimensional structure in the presence and absence of nucleotide was reported at a resolution of approximately 20 angstrom.[31] The TMDs form a chamber within the membrane that appears to be open to the extracellular milieu and may also be accessible from the lipid phase at the interfaces between the two TMDs. This structure may be consistent with a predicted model for the function of MDR1 and the general architecture of ABC proteins. MDR1 is predicted to act as a "vacuum cleaner" or a "flippase," with drug substrates gaining access to their binding site(s) from the inner leaflet of the lipid bilayer.[32] A gap present in the protein ring could allow substrates to access the central pore from the lipid phase. The substrates transported by different ABC proteins can vary widely, from small ions to large polypeptides and polysaccharides. A large pore could readily be adapted to accommodate different-sized substrates. The projection structures of MDR1 trapped at different steps of the transport cycle were also determined.[30] ATP binding, not hydrolysis, was proposed to drive the major conformational change associated with solute translocation. The crystal structure of the *E. coli* BtuCD protein, an ABC transporter mediating vitamin B_{12} uptake, was reported at a resolution of 3.2 angstroms.[24] The two ATP-binding subunits (BtuD) are in close contact with each other, as are the two membrane-spanning subunits (BtuC). However, because the BtuC subunits provide 20 transmembrane helices, we cannot predict the structure of eukaryotic ABC proteins directly from this conformation. Crystal structures at a higher resolution are prerequisite to understand the mechanism of substrate recognition and transport.

ABC proteins and lipid homeostasis

It is now realized that one of the major physiological roles of ABC transporters is cellular lipid transport and homeostasis, and their dysfunction is often associated with human disease phenotypes. The first break came with the finding that ABCB4 mediates the transport of phosphatidylcholine across the canalicular membrane during bile formation, and mutations in the *ABCB4* gene are a cause of progressive familial intrahepatic cholestasis.[33–35] Second, ABCA4 (ABCR) was suggested to transport a complex of retinaldehyde and phosphatidylethanolamine in the retina of the eye, and malfunctioning of this transporter results in Stargardt's macular dystrophy[36] and is related to age-related macular degeneration.[37] Third, half-size peroxisomal ABC proteins (ALDP, ALDR, PMP70, P70R) are suggested to be involved in the transport of long chain and very long chain fatty acids into peroxisomes.[38,39] Fourth, ABCB11 was proved to function as an ATP-dependent transporter of bile salt, which is converted from cholesterol in liver.[40] Finally, mutations in the *ABCA1* gene were causatively linked to familial high-density lipoprotein deficiency and Tangier disease.[41–45] ABCA1 plays a key role in the formation of HDL particles.[46–48] ABCG5 and ABCG8 are involved in the secretion of plant sterols and cholesterol into bile, and mutations in either of these genes are a cause of sitosterolemia.[49] ABCA7 also mediates apoA-I-dependent release of cholesterol and phospholipids.[50,51]

ABCA1 and HDL formation

Dietary cholesterol is absorbed from the small intestine and transported as chylomicron to the liver, from where it is

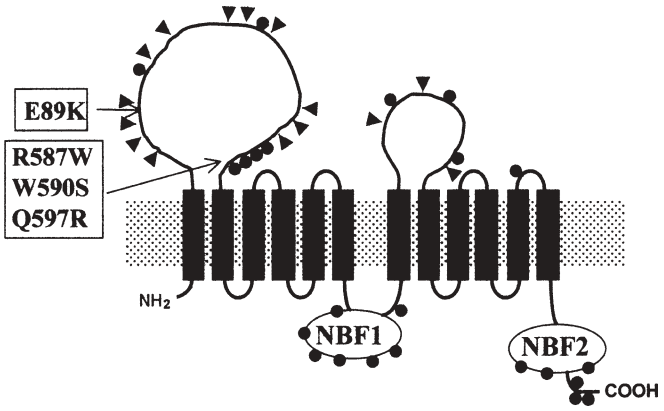

Fig. 2. Putative secondary structure of human ABCA1 and mutations that cause familial hypoalphalipoproteinemia and Tangier disease. ●, mutation site; ▲, putative glycosylation site

transported as low-density lipoprotein to peripheral cells. Cholesterol is not catabolized in the peripheral cells and therefore is mostly released and transported to the liver for conversion to bile acids to maintain cholesterol homeostasis. The assembly of HDL particles by helical apolipoproteins with cellular lipid is the major mechanism of cellular cholesterol release.[52,53] The importance of this active cholesterol-releasing pathway became apparent with the finding that it is impaired in cells from patients with Tangier disease.[7,8]

Tangier Island is located in Chesapeake Bay in Virginia, USA. Dr. Frederickson of the NIH discovered that a 5-year-boy living on the island had unusually large orange tonsils and virtually no HDL in the plasma in 1964.[54] More than 30 years after the discovery, mutations were identified in *ABCA1* of Tangier disease (TD) patients.[55–57] Mutations mapped to the *ABCA1* gene in patients with familial hypoalphalipoproteinemia (FHA) and TD seem to be clustered in several regions of the ABCA1 protein, such as the first extracellular domain (ECD1), the first nucleotide-binding fold (NBF1), the linker region, the second extracellular domain, and the C-terminal region (Fig. 2).

The primary substrates and mechanism of action of ABCA1 have not been precisely defined. The current model of ABCA1 functionality is based on the following.

1. Topological models. The current consensus topological model predicts two large extracellular loops between TM1 (amino acids 23–44) and TM2 (amino acids 642–660) and TM7 (amino acids 1346–1368) and TM8 (amino acids 1655–1677) (Fig. 2). It is supported by computer modeling,[58] tag insertion,[58] antibody reactivity,[58] and glycosylation analysis.[58–60] These large extracellular domains are unique to the ABCA subfamily, and other ABC proteins, such as MDR1, do not have them. We also predicted that the first hydrophobic segment serves as a signal peptide.[58] This idea is based on an estimation with the algorithm SignalP[61] and on the inability to detect an HA epitope fused to the N-terminus of ABCA1 by Western blotting.[58] However, the EGFP–ABCA1 fusion construct functioned without being processed by signal peptidase.[59] It is not clear yet if the segment serves as a signal peptide or a signal anchor sequence.
2. Subcellular localization of ABCA1–GFP. The subcellular distribution of ABCA1 was studied using a GFP-fused protein.[48,62] ABCA1–GFP was localized not only to the plasma membrane but also to the intracellular compartments. It was colocalized partly with a marker for the Golgi apparatus, with a marker for early endosomes, and with lysotracker, a marker for acidic compartments. When the cells were treated with monensin, which prevents delivery of protein from endosomes to the cell surface, after the blocking of new protein synthesis, the ABCA1–GFP on the cell surface decreased and the vesicular distribution increased. These results suggested that newly synthesized ABCA1–GFP was first delivered to the plasma membrane through the endoplasmic reticulum (ER) and Golgi and then shuttled rapidly between the plasma membrane and intracellular vesicles. Fluorescent apoA-I was accumulated into ABCA1–GFP-containing endosomes[63] that shuttled between late endosomes and the cell surface.[64] In late endocytic vesicles of cells from TD patients, both cholesterol and sphingomyelin were accumulated and massive amounts of NPC1 were retained. These late endocytic trafficking defects were corrected by adenovirally mediated ABCA1–GFP expression.[64]

   In polarized intestine[65] and liver cells,[66] ABCA1 is mainly expressed on the basolateral surface, suggesting an important role for ABCA1 in the absorption of dietary cholesterol from intestine[67] and for hepatocyte ABCA1 in the regulation of the levels of plasma HDL.[66]
3. ApoA-I dependence of ABCA1-mediated HDL formation. HEK293 cells stably expressing human ABCA1 show apoA-I-mediated release of cholesterol and choline phospholipids.[58,59,62] Without the addition of apoA-I, virtually no release of lipids is observed compared to the nontransfected HEK293 cells. ApoA-I is supposed to interact with ABCA1 directly, because ApoA-I retards calpain-mediated degradation of ABCA1 at the cell surface[68,69] and because apoA-I can be cross-linked with ABCA1.[46,47,70] Interaction with apoA-I could be required to allow ABCA1 to internalize from the plasma membrane to endocytic compartments. Takahashi and Smith[71] first reported that cellular cholesterol efflux involves endocytosis and resecretion of apoA-I. However, it is not yet clear that ABCA1 and apoA-I are internalized obligatorily together to early and late endocytic compartments after interaction on the cell surface.
4. Functional analysis of TD mutants. Many mutations in patients with TD and FHA have been identified in ECD1 of ABCA1, and three mutations (R587W, W590S, Q597R) cluster amino acids 587 to 597[46,48,72] (see Fig. 2). When these three TD mutations were introduced into ECD1 of ABCA1-GFP and the mutants were transiently or stably expressed in HEK293, R587W and Q597R appeared to be distributed mainly in the ER and not the plasma membrane. In contrast, the W590S mu-

tant was localized to the plasma membrane to the same extent as the wild type. Immunostaining with the antibody against ECD1 confirmed the proper orientation of W590S. However, the apoA-I-mediated release of cholesterol and choline phospholipids from cells expressing W590S was as low as that of the other two mutants and less than 10% of that in cells expressing the wild-type ABCA1. The W590S mutation does not have any apparent effect on ATP hydrolysis or the interaction between apoA-I and ABCA1. Analyses of W590S should facilitate our understanding of the mechanism of HDL formation.

5. ATP hydrolysis by ABCA1. We previously showed that ABCA1 is able to bind ATP and hydrolyze it using crude membrane of HEK293 cells stably expressing human ABCA1.[48] Recently, we expressed human ABCA1 in insect Sf9 cells and succeeded in purifying it (Takahashi et al., in preparation). The purified detergent-soluble ABCA1 showed significant ATPase activity, and the activity was stimulated by the addition of phospholipids. The Walker A lysine mutation K939M in the first nucleotide-binding fold, resulting in a defect in HDL formation, impaired ATPase activity. ATP hydrolysis is suggested to be involved in the apoA-I-dependent secretion of phospholipids and cholesterol.

Model for ABCA1-mediated HDL formation

Figure 3 is our working model of the action of ABCA1. The lipid-free apoA-I interacts with the extracellular domain of ABCA1. ABCA1–apoA-I complexes are internalized into early endosomes and can either recycle back to the plasma membrane or move to late endocytic compartments. ABCA1 likely loads phospholipid and cholesterol onto apoA-I in a manner dependent on ATP hydrolysis. However, it is not clear yet where this "lipidation of apoA-I" occurs. As described later, when human ABCA3, predominantly expressed in alveolar type II cells, was expressed in HEK293 cells, it localized to the intracellular vesicle membrane, and ABCA3 is involved in accumulating phospholipids and cholesterol in the vesicles.[73] ABCA1 may function as ABCA3 to accumulate phospholipids and cholesterol in intracellular compartments, in which apoA-I–lipid complexes are generated. A specific environment could be also required for the generation of the nascent HDL. ABCA1 in late endosomes may make it possible to remove the excess endocytosed LDL-derived cholesterol from the cell and likely plays a critical role in the macrophage, where unregulated uptake of oxidized LDL triggers foam cell formation. The "lipidated" apoA-I in intracellular compartments moves to the cell surface and is released as the nascent HDL particle. Alternatively, the nascent HDL particles may be generated either on the cell surface or in early or late endocytic compartments. Each nascent HDL particle could be generated from different pools of lipids. In any case, we speculate that ABCA1 functions as a transporter of lipids as well as a receptor for apoA-I.

Transcriptional and posttranscriptional regulation of ABCA1

The hyperaccumulation of cholesterol is harmful to cells, but at the same time cholesterol is indispensable for cells. Therefore, the expression of ABCA1 is highly regulated at both the transcriptional and posttranscriptional level. The transcription of ABCA1 is regulated by the intracellular oxysterol concentration via the LXR/RXR nuclear receptor,[74] and the turnover of ABCA1 protein occurs rapidly with a half-life of 1–2 h[68,69,75] to avoid depletion of cholesterol.

Some TD mutations are clustered at the C-terminal region of ABCA1,[76] and one mutation, which was identified in a TD patient and was predicted to delete the last 46 amino acids, impaired the function of ABCA1.[77] Furthermore, the C-terminal region contains amino acid sequences well conserved among ABCA subfamily proteins. Therefore, this region was expected to be functionally important. Proteins that interact with this region were screened using the yeast two-hybrid method and three PDZ-binding proteins, α_1- and β_2-syntrophin and Lin7, were isolated as candidate proteins interacting with ABCA1.[78,79] α_1-Syntrophin is mainly expressed in brain, skeletal muscle, and heart in mice, and indeed mouse ABCA1 was coimmunoprecipitated with α_1-syntrophin from mouse brain.[79] This interaction was confirmed to be specific, because ABCA1 was not precipitated from the brain of α_1-*Syn*$^{-/-}$ mice.

Coexpression of α_1-syntrophin in HEK293 cells retarded degradation of ABCA1 and made the half-life of ABCA1 five times longer than in the cells not expressing α_1-syntrophin. This effect is not common among PDZ-containing proteins interacting with ABCA1, because Lin7 did not have a significant effect on the half-life of ABCA1. Coexpression of α_1-syntrophin significantly increased the apoA-I-mediated release of cholesterol.[79] These results suggest that cellular proteins, including α_1-syntrophin, may be involved in intracellular signaling, which determines the stability of ABCA1 and modulates cellular cholesterol release.

There may be other motifs in the C-terminal region of ABCA1 that are involved in the regulation. A novel, highly conserved motif (VFVNFA) was reported to be required for lipid efflux.[80] Alteration of this motif, which is present in some but not all members of the ABCA family, did not prevent trafficking of the transporter to the plasma membrane but did eliminate its binding of apoA-I. When a peptide containing the VFVNFA sequence was introduced into ABCA1-expressing cells, ABCA1-mediated lipid efflux was markedly inhibited. It is not clear yet that this motif participates in novel protein–protein interactions.

Phosphorylation seems to play a role in controlling ABCA1 protein levels and activity. Interaction with lipid-free apoA-I stabilizes ABCA1 against degradation by calpain and increases its protein level in cells. Two protein kinases, protein kinase C (PKC) and protein kinase A (PKA), are reported to play an important role in the apoA-I-induced stabilization of ABCA1. It is suggested that the removal of sphingomyelin by lipid-free apoA-I in generat-

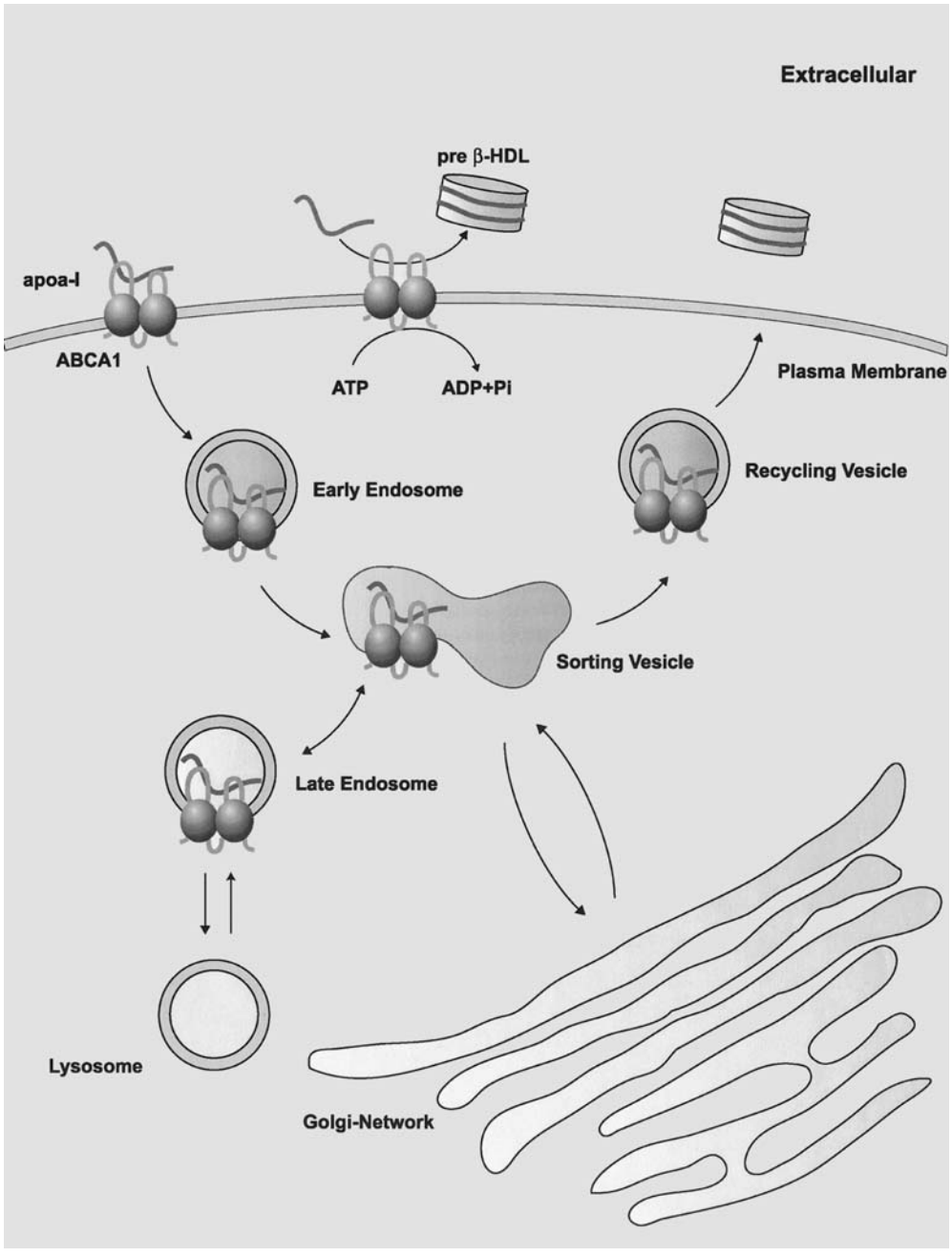

Fig. 3. Model for ABCA1-mediated high-density lipoprotein (HDL) formation

ing new HDL generates diacylglycerol by a replenishment reaction, and diacylglycerol activates PKC, which in turn phosphorylates and stabilizes ABCA1.[81] Peptides with an amphipathic helical structure that are competent in promoting lipid efflux from cells also promote the phosphorylation and stabilization of ABCA1.[82] ABCA1 contains a cytosolic PEST sequence that, when phosphorylated, directs calpain-mediated proteolysis of ABCA1.[83] The interaction of apolipoproteins with cells prevents this phosphorylation of PEST and thus stabilizes ABCA1. ApoA-I binding is also suggested to induce intracellular cAMP release, which leads to the phosphorylation of ABCA1 via a PKA-dependent mechanism,[84] and PKA-mediated phosphorylation of ABCA1 may be essential for optimal lipid transport activity.[85] It is also reported that apoA-I stimulates the autophosphorylation of protein-tyrosine kinase Janus kinase 2 (JAK2), which in turn activates a process that enhances apolipoprotein interactions with ABCA1 and lipid removal from cells.[86] However, it is not clear if JAK2 phosphorylates ABCA1 directly.

ABCA7 is also involved in HDL formation

ABCA7 is highly homologous to ABCA1. This gene product was first reported as a human sterol-sensitive ABC transporter.[87] While investigating the functional importance of ECD1 of ABCA1, we discovered that the amino acid sequence of autoantigen SS-N, an epitope of Sjögren's syndrome, is highly homologous to a part of the ECD1 of ABCA1, and isolated a ABC protein gene coding for autoantigen SS-N,[58] which was *ABCA7*.

ABCA7 induces an apolipoprotein-mediated assembly of cholesterol-containing HDL similar to that induced by ABCA1.[51,88] ABCA7 protein was detected with a polyclonal antiserum when macrophages were incubated with AcLDL[87] as ABCA1. However, ABCA7 is expressed in a rather tissue-specific manner compared to ABCA1, and mRNA of ABCA7 is predominantly found in myelolymphatic tissues (bone marrow, lymph node, spleen, thymus, and peripheral blood cells) and in brain and trachea.[87,88] Alternative splicing could be involved in the posttranscriptional regulation of the expression and function of human ABCA7.[88] The human *ABCA7* gene produces at least two types of splicing variants in a tissue-specific manner, and type II mRNA, which does not produce a functional ABCA7, was detected rather abundantly in many tissues.[88] We have failed to detect ABCA7 protein in human peripheral blood cells, although poly A^+ RNA was detected by Northern blot hybridization. There was a report that human ABCA7 is highly expressed in keratinocytes and upregulated during differentiation.[89] Mouse ABCA7 protein is expressed in spleen, lung, adrenal, and brain[87] whereas rat ABCA7 protein is expressed specifically in platelets and red blood cells.[90] Mouse ABCA7 was reported to promote the apoA-I-dependent release of phospholipids, but not cholesterol,[91] although human ABCA7 mediates the apoA-I-dependent release of cholesterol and phospholipids.[51,88] It is worth examining the difference in physiological function between human and mouse ABCA7.

ABCA3 and pulmonary surfactant secretion

ABCA3 is predominantly expressed in lung[92,93] and localized to the limiting membrane of lamellar bodies in alveolar type II cells in humans and rats.[94,95] Recently it was revealed that *ABCA3* gene mutations cause a fatal deficiency of surfactant in newborns.[96] The lamellar body is a member of lysosome-related organelles, in which pulmonary surfactant is stored. Lamellar bodies are secreted into the alveolar space by exocytosis, and secreted pulmonary surfactant coats the lumen of alveoli, where it reduces the surface tension at the alveolar air–liquid interface, thus preventing alveoli from collapsing and reducing the workload of breathing. Pulmonary surfactant is composed of lipids (90%) and surfactant proteins (SP-A, SP-B, SP-C, and SP-D), which are densely packed into multilamellar structures. The most abundant lipid in pulmonary surfactant is phosphatidylcholine, especially dipalmitolyphosphatidylcholine. The mechanism by which lipids are packed into lamellar bodies is unknown.

When expressed in HEK293 cells, human ABCA3 localized to the intracellular vesicle membrane.[73] The diameters of the vesicles observed in HEK293/hABCA3, where ABCA3 was expressed, were about 1 μm, corresponding to those of lamellar bodies. ABCA3 is efficiently labeled by 8-azido-[α-^{32}P]ATP, but not by 8-azido-[γ-^{32}P]ATP, when the membrane fraction is incubated in the presence of orthovanadate, indicating that ABCA3 shows strong ATPase activity in the isolated membrane. Photoaffinity labeling was largely reduced by membrane pretreatment with 5% methyl-β-cyclodextrin (MβCD), which depletes cholesterol. Therefore, cholesterol would be the first candidate of a transport substrate for ABCA3. Indeed, a filipin–

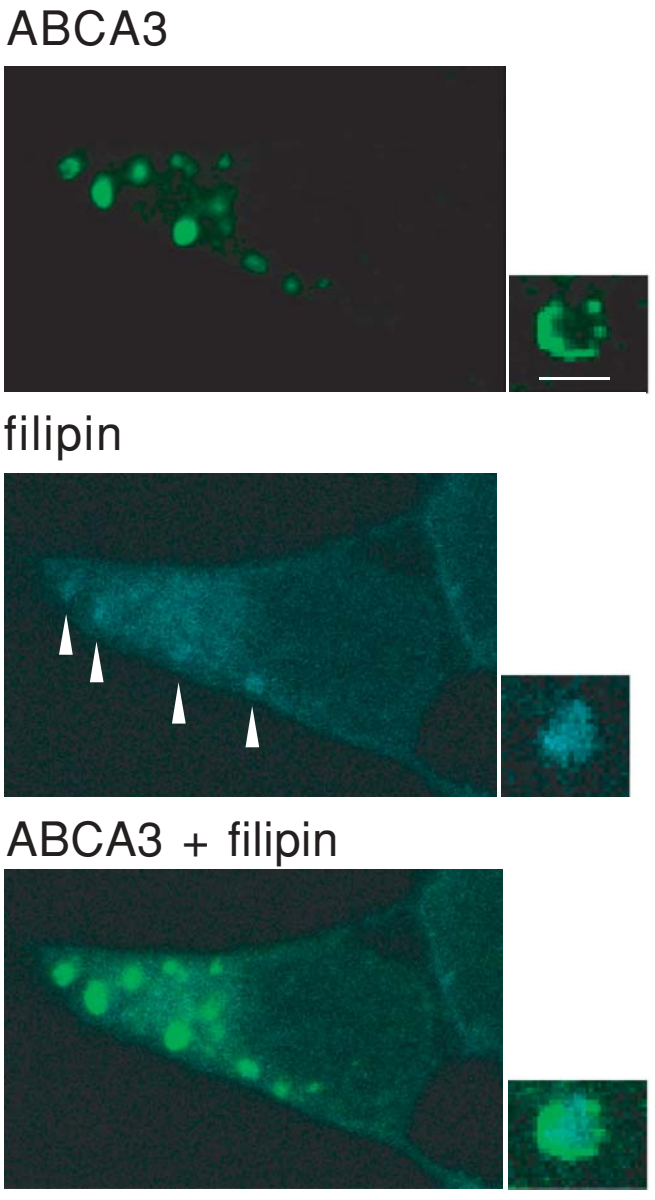

Fig. 4. *Filipin* staining of HEK293/hABCA3 cells. *A3 + filipin*, overlaid image of *ABCA3* fluorescence and *filipin* staining. Bar 1 μm

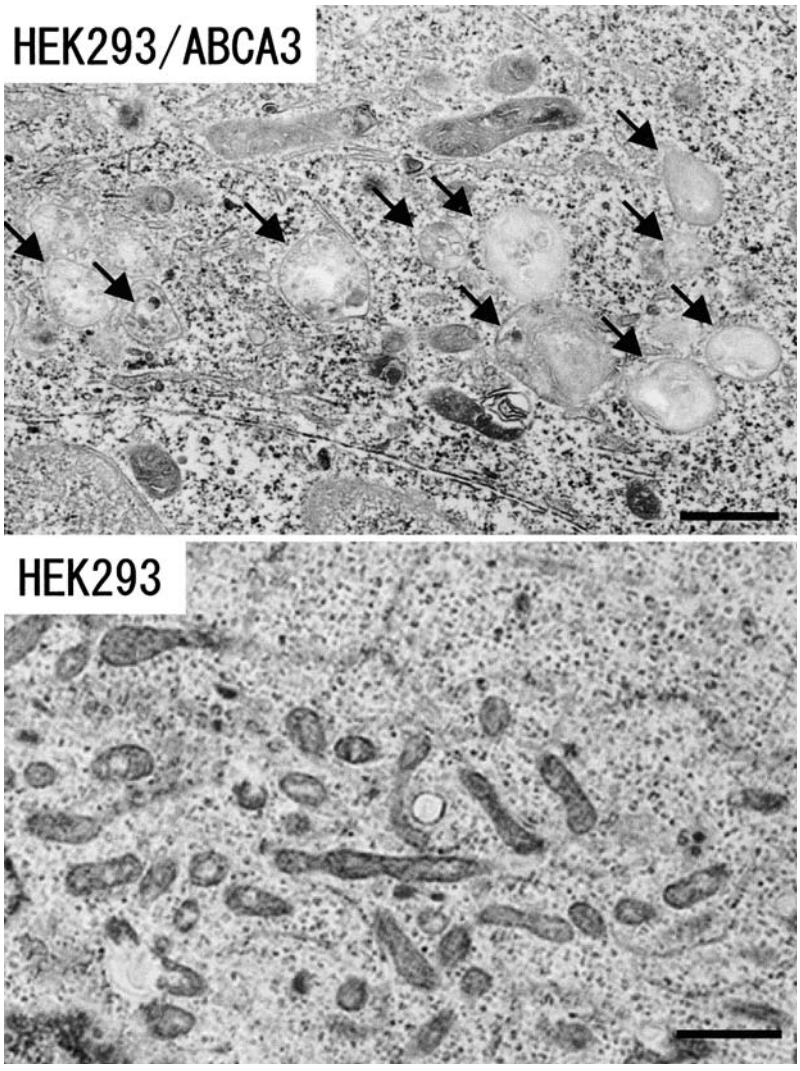

Fig. 5. Electron micrograph of *HEK293/hABCA3* cells (*top*) and *HEK293* host cells (*bottom*). Lamellar body-like structures are indicated by *arrows*. Modified from Nagata et al.[73] Bar 1 μm

cholesterol complex was preferentially detected in the vesicular structures, in which ABCA3 is located, when HEK293/hABCA3 cells were stained with filipin (Fig. 4). However, MβCD might deplete phospholipids together with cholesterol. Because dipalmitolyphosphatidylcholine, the most abundant lipid in pulmonary surfactant, has high affinity for cholesterol, it is possible that phospholipids with saturated fatty acid chains are depleted from the HEK293 cell membrane together with cholesterol. This might cause the suppression of vanadate-induced nucleotide trapping in ABCA3.

Electron micrographs show that HEK293/hABCA3 cells contain multivesicular, lamellar body-like structures, which do not exist in HEK293 host cells (Fig. 5). Some fuzzy components such as lipids accumulate in the vesicles.[73] These results suggest that ABCA3 shows ATPase activity, which is induced by lipids, and may be involved in accumulating phospholipids and cholesterol in lamellar bodies and in generating multivesicular structures.

Conclusion

Among 49 human ABC proteins, there still exist many whose physiological functions or endogenous substrates are unclear. Even though we know the human disease phenotype with which the dysfunction of ABC proteins is associated, it is difficult to define the primary substrates and mechanism of action of ABC proteins, because most of their substrates are lipophilic and their binding affinity for ABC proteins is quite low, and because the substrate specificity is often quite low also. The regulation of their expression and functions is sophisticated and thus very complicated. However, ABC proteins play critical roles in the homeostasis of lipids and other metabolites, making them important therapeutic targets. Basic biochemical studies as well as cellular level and animal model studies will improve our understanding of the physiological roles of ABC proteins.

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