



Award Review

ABC Proteins Protect the Human Body and Maintain Optimal Health

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Human *MDR1*, a multi-drug transporter gene, was isolated as the first of the eukaryote ATP Binding Cassette (ABC) proteins from a multidrug-resistant carcinoma cell line in 1986. To date, over 25 years, many ABC proteins have been found to play important physiological roles by transporting hydrophobic compounds. Defects in their functions cause various diseases, indicating that endogenous hydrophobic compounds, as well as water-soluble compounds, are properly transported by transmembrane proteins. *MDR1* transports a large number of structurally unrelated drugs and is involved in their pharmacokinetics, and thus is a key factor in drug interaction. *ABCA1*, an ABC protein, eliminates excess cholesterol in peripheral cells by generating HDL. Because *ABCA1* is a key molecule in cholesterol homeostasis, its function and expression are highly regulated. Eukaryote ABC proteins function on the body surface facing the outside and in organ pathways to adapt to the extracellular environment and protect the body to maintain optimal health.

Key words: ATP Binding Cassette (ABC) proteins; *MDR1*; *ABCA1*; cholesterol; xenobiotics

Because the lipid bilayer of cell membranes prevents the passage of hydrophilic molecules, cells have evolved ways of transferring water-soluble compounds across their membranes to ingest essential nutrients, such as glucose and amino acids, and ions. Transmembrane proteins, which transport small water-soluble organic molecules and ions across the plasma membrane, are called transporters. Transporters recognize their substrates specifically and with high affinity. In general, specificity and high affinity are considered to be the most important biological and physiological features, but the discovery of *MDR1* 25 years ago indicated that important biological reactions are not always specific or with high affinity, and that endogenous hydrophobic compounds might also be transported by membrane proteins in the body. Many transporters in the same protein family, ATP Binding Cassette (ABC) proteins, have been found to play important physiological roles transporting hydrophobic compounds, and defects in their functions are related to various diseases (Table 1). In this review, the physiological roles of ABC proteins and the mechanisms of their functions are summarized.

I. The *MDR1* Gene Was Discovered as the First Eukaryote ABC Protein

The emergence and outgrowth of a population of tumor cells resistant to multiple anticancer drugs is a major obstacle in cancer chemotherapy. One form of resistance is characterized by the energy-dependent removal of a variety of structurally unrelated cytotoxic agents by membrane transporter proteins. Human *MDR1* cDNA, now also called *ABCB1* by the HUGO (Human Gene Organization) Nomenclature Committee, was isolated from a multidrug-resistant KB carcinoma cell line, KB-C2.5, selected for its resistance to colchicines, in 1986,^{1,2)} and was found to code for P-glycoprotein,³⁾ a surface glycoprotein reported to be overexpressed in drug-resistant Chinese hamster ovary cell mutants.⁴⁾ Overexpression of human and mouse *MDR1* conferred resistance to many drugs, including *Vinca* alkaloids, anthracyclines, epipodophyllotoxins, and taxol.^{5–7)} A few years later, Kioka isolated human *MDR1* cDNA from the adrenal, and found that cDNA isolated from KB-C2.5^{1,2)} was associated with a Gly-to-Val substitution at position 185 in the predicted cytoplasmic loop between TM2 and TM3.⁸⁾ This mutation increased resistance to colchicine and decreased resistance to vinblastine.^{8,9)}

MDR1 is a 1,280 amino-acid protein with two symmetrical halves connected by a short linker region.¹⁾ Each half consists of six transmembrane α -helices (TM) followed by a nucleotide binding domain (NBD),¹⁰⁾ in which ATP is hydrolyzed to energize transport (Fig. 1). The NBDs of *MDR1* share homology with those of peripheral membrane components of bacterial active transporter systems, and those eukaryotic and bacterial transporters were collectively named ABC transporters or ABC proteins after the highly conserved ATP binding domains.¹¹⁾ *MDR1* was discovered as the first eukaryote ABC protein.^{1,12)} Now we know that 48 or 49 ABC protein genes exist on human chromosomes,¹³⁾ and that they can be classified into seven subgroups (A to G) based on the amino acid sequence of the ATP binding domain (Fig. 1).

II. *MDR1* Protects Human Body against Xenobiotics

In daily life, we are exposed to various hydrophobic compounds in food and the environment that pass

Table 1. Human ABC Proteins and Diseases

Gene (symbol)	Phenotype, disease/function
ABCA subfamily	
ABCA1	HDL deficiency/Cholesterol and phospholipid efflux
ABCA3	Pulmonary surfactant deficiency in newborn
ABCA4 (ABCR)	Stargardt disease 1
ABCA12	Harlequin ichthyosis
ABCA13	Schizophrenia, Bipolar Disorder, Depression
ABCB subfamily	
ABCB1 (MDR1)	Multidrug resistance in cancer/Export of xenobiotics
ABCB2 (TAP1)	Behçet's disease/Antigen peptide transport into ER lumen
ABCB3 (TAP2)	Behçet's disease/Antigen peptide transport into ER lumen
ABCB4 (MDR3)	Intrahepatic cholestasis/Secretion of phosphatidylcholine into bile
ABCB7	Sideroblastic anemia/Transport of iron-sulfate complexes in mitochondria
ABCB11 (BSEP, SPGP)	Intrahepatic cholestasis/Export of bile acid
ABCC subfamily	
ABCC1 (MRP1)	Multidrug resistance in cancer/Export of xenobiotics
ABCC2 (MRP2/cMOAT)	Dubin-Johnson syndrome/Export of bilirubin
ABCC6 (MRP6)	Pseudoxanthoma elasticum
ABCC7 (CFTR)	Cystic fibrosis/Cl ⁻ channel
ABCC8 (SUR1)	PHHI/ATP sensitive K ⁺ channel regulator in pancreatic β -cells
ABCD subfamily	
ABCD1 (ALDP)	Adrenoleukodystrophy/Peroxisomal transport of very long fatty acid
ABCD2 (ALDR)	Adrenoleukodystrophy/Peroxisomal transport of very long fatty acid
ABCG subfamily	
ABCG2 (BCRP)	Gout/Export of Uric acid
ABCG5	Sitosterolemia/Export of phytosterols
ABCG8	Sitosterolemia/Export of phytosterols

through the lipid bilayer freely and penetrate the body. Unfortunately, many of them have toxic effects. Therefore, the body has to deal with numerous hydrophobic compounds, but it is impossible to cover the intestine epithelia with cell walls, like bacteria, or to express the huge number of membrane transporters that excrete each hydrophobic toxic compound with high efficiency. Animals have in the main developed two strategies to cope with these hydrophobic toxic substances. One strategy is to conjugate them enzymatically with glutathione, glucuronate, or sulfate, whereby they become more hydrophilic and detoxified. At the same time, these markings make toxic compounds much easier to recognize by transporters. Several ABCC subfamily proteins (MRPs) are involved in transporting these marked compounds out of cells.¹⁴⁻¹⁸⁾ This pathway is very efficient but has an intrinsic defect, because toxic substances must be taken into cells to be marked.

The other strategy is to recognize hydrophobic compounds as they pass through the plasma membrane and to excrete them directly out of the cells. This is accomplished mainly by two ABC proteins, MDR1 and ABCG2,^{19,20)} which prevent the absorption of lipophilic toxic compounds of various structures from the intestine, and expel them into the bile and urine from the liver and kidney respectively, although their binding affinities are rather low, in the micromolar range. MDR1 in the capillary endothelial cells of the brain and testes limits their penetration. Saeki *et al.* found that cardiac glycoside digoxin, the calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK506), and steroid hormones are the transport substrates for MDR1 in transepithelial transport experiments,²¹⁻²³⁾ indicating that drugs other

than anticancer agents and even endogenous hydrophobic compounds are transported by MDR1. To date, it has been reported that MDR1 transports a huge number of structurally unrelated drugs and is involved in their pharmacokinetics, and thus MDR1 is a key factor in drug interaction.²⁴⁾

III. How Does MDR1 Recognize Multiple Drugs?

The most puzzling feature of MDR1 is its recognition and transport of a wide variety of substrates. It has been proposed that it possesses multiple drug-binding sites²⁵⁻²⁷⁾ located in the middle of the lipid bilayer.²⁸⁻³⁰⁾ Sometimes, drug binding to one site stimulates transport by another. Kimura reported that cholesterol can directly bind to or allosterically affect the drug-binding site adjusting its size to the drug.^{31,32)} Since the binding affinity of drugs with molecular weights of 800 to 900 (vinblastine, vincristine, and paclitaxel) is not affected by the presence of cholesterol, the drug-binding site of MDR1 might best fit drugs of these sizes. When small drugs, with molecular weights of 350 to 500, bind to MDR1, cholesterol (MW, 386.7) can fill the empty space tightening the drug-binding site and help in the recognition of smaller drugs.

ABCB4, also called MDR3 in humans and MDR2 in mice, has an amino acid sequence with 76% identity and 86% similarity to that of MDR1. But their physiological substrates and roles are different. ABCB4 does not confer drug resistance, but is essential for the secretion of phosphatidylcholine (PC) into the bile.³³⁾ Because *Abcb4* knockout mice do not excrete any phospholipid into the

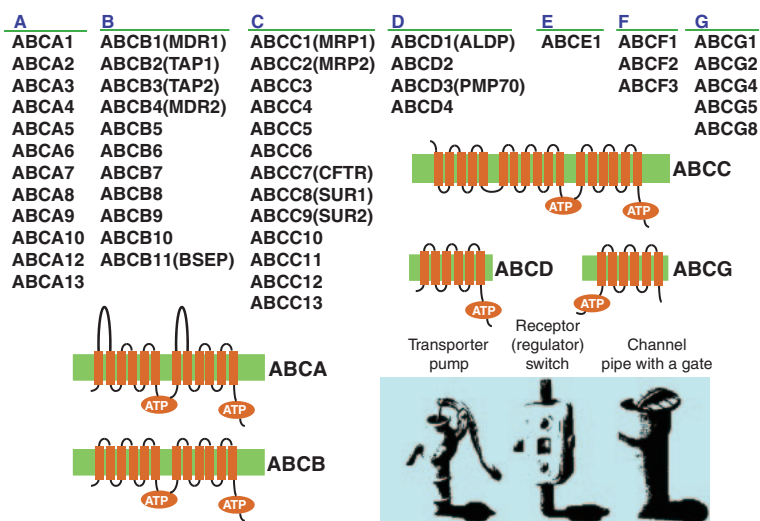


Fig. 1. Members of Subfamilies (A to G) of Human ABC Proteins, Their Typical Secondary Structures, and Drawings Representing the Three Types of ABC Proteins.

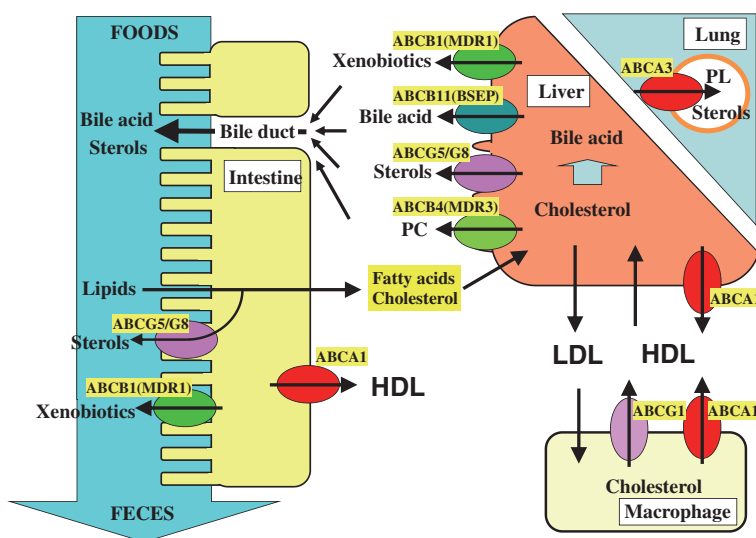


Fig. 2. Human ABC Proteins Transport Lipids and Xenobiotics in Various Tissues.

bile, despite the high expression of MDR1 on the canalicular membranes of hepatocytes, MDR1 has quite low if any ability to mediate phospholipid excretion. Indeed, HEK293 cells stably expressing human ABCB4 excrete phospholipids (preferentially PC) and cholesterol when bile salts are added to the medium, but MDR1 does not.³⁴⁾ Nevertheless, it is predicted that MDR1 and ABCB4 share conserved domains for substrate recognition and a conserved process transporting substrates.^{35–37)} To understand the precise mechanisms by which MDR1 recognizes multiple drugs and why ABCB4 transports PC but not drugs, 3D-structural analyses at high resolution are required.

IV. Eukaryotic ABC Proteins Can Be Classified into Three Major Groups of Membrane Proteins

ABC proteins have been generally recognized as drug efflux pumps. *MRP1/ABCC1*, isolated shortly after *MDR1*, also confers multi-drug resistance.³⁸⁾ The discovery of MDR1 and MRP1 had a strong impact on the

field of cancer chemotherapy and pharmacodynamics, and might be one of the reasons for the strong impression that ABC proteins function as drug transporters, but recent findings have suggested that their physiological role as self-defense mechanisms against xenobiotics is only one aspect of the importance of ABC proteins.

In 1992, mutations in the *ABCC7* gene were reported to cause cystic fibrosis, one of the most common serious diseases, which affects one in 2,000–2,500 people in northern Europe and the United States.³⁹⁾ *ABCC7* was originally called cystic fibrosis transmembrane conductance regulator (CFTR). It is a Cl⁻ channel, and is expressed in cells that line the lungs, digestive tract, and sweat glands, regulating Cl⁻ flux^{39–41)} maintaining a normal thin coating of fluid and mucus inside the lungs, pancreas, and other organs. Because mucus in the airway and lungs traps germs, which are then cleared out of the lungs, the thick, sticky mucus, due to defects in CFTR, causes infected lungs. In the pancreas, thick mucus blocks the digestion of foods, causing malabsorption of nutrients, especially fats.

The sulfonylurea receptor (SUR1) has been identified as a target protein for sulfonylureas, such as glibenclamide, which is most commonly used in the treatment of non-insulin-dependent diabetes mellitus.⁴²⁾ SUR1 (official gene name, *ABCC8*) is a subunit of the pancreatic β -cell K_{ATP} channel. The β -cell K_{ATP} channel is a heterooctamer composed of pore-forming Kir6.2 subunits and SUR1 that co-assemble with 4:4 stoichiometry.^{43–45)} It plays a key role in the regulation of glucose-induced insulin secretion. Matsuo *et al.* have suggested that SUR1 is not a channel or transporter itself, but a switch regulating the opening and closing of Kir6.2 channel subunits by monitoring the intracellular metabolic state, especially the ADP concentration.^{46–48)}

These findings suggest that eukaryote ABC proteins have evolved to serve various functions on the plasma membrane to protect against or cope with substances or germs in the environment and bodily fluid to maintain human health, and that eukaryote ABC proteins can be classified into three major groups based on function: transporters, regulators, and a channel, despite their similar assembly of functional domains (Fig. 1). The images in Fig. 1 show how these three groups of membrane proteins differ in function. MDR1 is an active transporter, in which ATP hydrolysis provides the free energy necessary to continue extruding chemicals from cells, like a water pump. CFTR, a chloride channel, is like a pipe with a gate, in which ATP hydrolysis allows the gate to open or close, but is not coupled to ion flow. SUR1 functions like a switch, which is controlled by intracellular ATP and ADP concentrations and regulates the K^+ channel.⁴⁶⁾

V. ABC Proteins Are Involved in Lipid Homeostasis

Membrane lipids, various phospholipids and cholesterol, move among organelles in cells and also move from the liver to various tissues and are excreted into the canalicular and intestinal lumen. Many ABC proteins have been found to be involved in these pathways (Fig. 2). ABCB4 and ABCB11 are involved in bile formation as transporters of PC and bile salts.^{33,49)} ABCA1 is required for HDL generation, which is the only pathway eliminating excess cholesterol from peripheral cells.^{50–52)} ABCA2 might be involved in lipid movement, generating the myelin sheath.^{53,54)} ABCA3 is expressed in lung alveolar type II cells and is involved in the biogenesis of lamellar body-like structures, in which pulmonary surfactant, consist of phospholipids, cholesterol, and surfactant proteins, is stored.^{55,56)} ABCA3 is thought to transport pulmonary surfactant lipids into vesicles.⁵⁷⁾ ABCG5 and ABCG8 excrete plant sterols into the canalicular lumen.⁵⁸⁾ ABCA12 is expressed predominantly in an epidermal keratinocyte, and defective ABCA12 results in loss of the skin lipid barrier, leading to a devastating skin disorder, harlequin ichthyosis.⁵⁹⁾ ABCA12 perhaps transports lipids, including glucosylceramide, into lamellar granules, which are secreted into the intercellular space forming the intercellular lipid layers,⁶⁰⁾ but in many cases it is hard to determine which lipids are directly transported due to the hydrophobicity and low binding affinity. In the second half of this review, the mechanisms of the

function and regulation of ABCA1, which have been most extensively studied among lipid-transporting ABC proteins, are summarized.

VI. ABCA1 Is a Key Molecule in Cholesterol Homeostasis

Cholesterol is a key component of the cell membrane and is required for cell proliferation, but excess accumulation of cholesterol is toxic to cells and excess deposition of it in peripheral tissues causes atherosclerosis. Excess cholesterol in peripheral tissues is reversely transported as high density lipoprotein (HDL) to the liver (Fig. 2). Because cholesterol is not catabolized in peripheral tissues, HDL formation is the only pathway by which excess cholesterol is removed from peripheral cells. The inverse relationship between plasma HDL levels and the risk of coronary artery disease (CAD) is well established⁶¹⁾ and HDL is often called “good cholesterol.” At least 70 mutations, including 23 missense, and 21 insertions or deletions, have been identified in the *ABCA1* gene, leading to Tangier disease and familial hypoalphalipoproteinemia, in which patients have a near absence of or decrease in circulating HDL.^{50–52,62,63)} ABCA1, expressed in cultured cells, mediates the secretion of PC and cholesterol when lipid-free apolipoprotein A-I (apoA-I), an extracellular lipid acceptor in the plasma, is added to the medium, and the more than 15 mutations examined show high correlations between PC and cholesterol efflux.^{64–66)}

VII. Where Does ABCA1 Mediate HDL Formation?

Several ABCA subfamily proteins function in intracellular vesicles. ABCA3 is expressed at the limiting membrane of the lamellar bodies in lung alveolar type II cells,^{55,56)} and Nagata *et al.* found that exogenous expression of ABCA3 in cultured cells promotes lipid uptake into intracellular vesicles that generate lamellar body-like vesicles.^{57,67,68)} ABCA12 is expressed at the limiting membrane of the lamellar granules⁶⁰⁾ as is ABCA3. ABCA4 is expressed in outer segment discs in rod and cone photoreceptor cells, and is associated with macular dystrophy Stargardt disease and age-related macular degeneration.⁶⁹⁾ ABCA4 perhaps transports retinal derivatives (Schiff base adduct of all-*trans*-retinal and PE) across disc membranes to eliminate it after photoexcitation.⁷⁰⁾

Differently from ABCA3, ABCA4, and ABCA12, ABCA1 localizes mainly on the plasma membrane, but sometimes in intracellular compartments. Two distinct mechanisms have been proposed for ABCA1-mediated HDL formation (Fig. 3). One is that ABCA1 mediates the complex formation of apoA-I with PC and cholesterol on the cell surface,^{71–73)} and the other, that apoA-I binds to ABCA1 on the cell surface and ABCA1/apoA-I complexes are subsequently internalized. ApoA-I/lipid complexes are formed (probably *via* ABCA1 activity) in late endosomes and are re-secreted by exocytosis.^{74–76)}

Azuma found that ABCA1 and apoA-I are endocytosed *via* a clathrin- and Rab5-mediated pathway and are recycled rapidly back to the cell surface, at least in

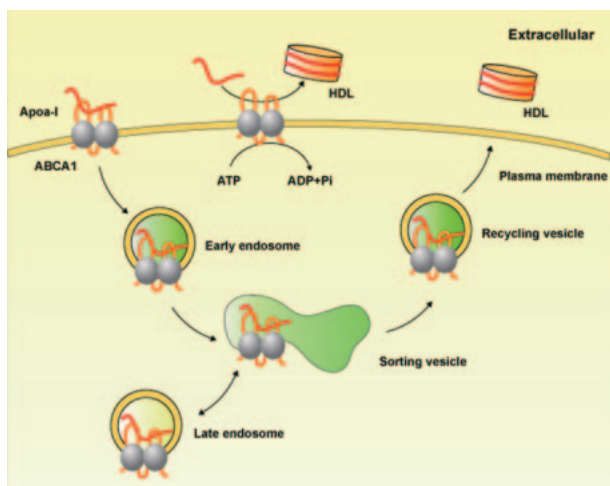


Fig. 3. Subcellular Localization of ABCA1 and HDL Formation.

ABCA1 mediates the complex formation of apoA-I with phospholipids and cholesterol on the cell surface. The retroendocytosis pathway of ABCA1/apoA-I also contributes to HDL formation when excess lipoprotein-derived cholesterol has accumulated in cells (modified from reference 117).

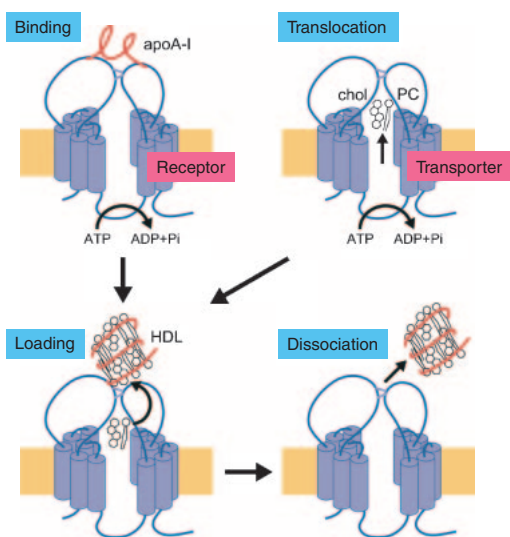


Fig. 4. Four-Step Model of HDL Formation Mediated by ABCA1.

At the apoA-I binding step, ATP binding and/or hydrolysis causes conformational changes within ECDs of ABCA1, to which apoA-I directly binds. At the translocation step, lipid translocation by ABCA1, which is apoA-I-independent, occurs. At the loading step, lipid loading of apoA-I bound to ABCA1 occurs. At the dissociation step, lipid-loaded apoA-I dissociates from ABCA1. In this model, the apoA-I binding step and lipid translocation step, both of which are mediated by ABCA1 in an ATP-dependent manner, are separable. The conformational transition of apoA-I caused by lipid loading probably facilitates the dissociation of apoA-I from ABCA1 (modified from reference 90).

part *via* a Rab4-mediated route; approximately 30% of endocytosed ABCA1 is recycled back to the cell surface.⁷⁷⁾ When clathrin-mediated endocytosis is inhibited, the level of ABCA1 at the cell surface increases and apoA-I internalization is blocked. Under these conditions, apoA-I-mediated cholesterol efflux from cells that have accumulated lipoprotein-derived cholesterol decreases, whereas efflux from cells without excess cholesterol increases.⁷⁷⁾ This suggests that the retroendocytosis pathway of ABCA1/apoA-I contrib-

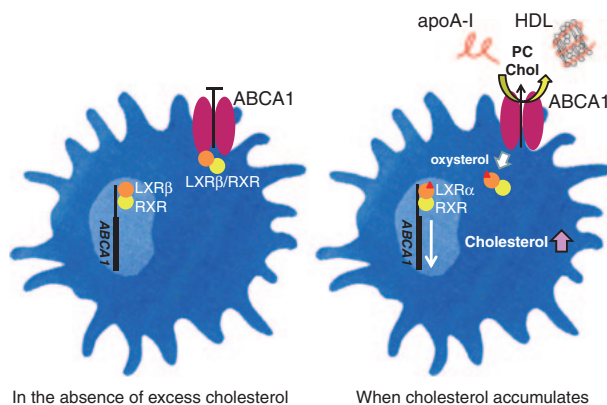


Fig. 5. LXR Regulates ABCA1 Not Only at the Transcriptional Level but Also at the Post-Translational Level by Direct Binding.

In addition to its well-defined role in transcription, LXR β directly binds the C-terminal region of ABCA1 mediating its post-translational regulation. In the absence of cholesterol accumulation, the ABCA1-LXR β /RXR complex stably localizes at the plasma membrane, but is inactive in HDL formation. When excess cholesterol accumulates, oxysterols bind to LXR β , leading to its dissociation from ABCA1. Because ABCA1 turns over rapidly, with a half-life of 1–2 h, and because the transcription, splicing, translation, and maturation of ABCA1, at more than 2,000 amino acid residues, take several h after transcriptional activation, cells are not able to cope with the acute accumulation of cholesterol for several h. This post-translational regulation allows ABCA1 to cause an immediate early response against acute cholesterol accumulation. LXR β has at least two distinct roles in controlling cholesterol homeostasis.

utes to HDL formation when excess lipoprotein-derived cholesterol has accumulated in the cells. This is also consistent with previous studies that blocking retroendocytosis of ABCA1 does not affect cholesterol efflux from cells in the absence of excess cholesterol.^{71–73)}

VIII. How Does ABCA1 Mediate HDL Formation?

ABCA1 and other members of the ABCA subclass are distinguished from other ABC transporter subclasses by the presence of large extracellular domains (ECDs) (Fig. 1).^{78,79)} Hozoji found that two intramolecular disulfide bonds are formed between ECD1 and ECD2 of ABCA1, and that these bonds are necessary for apoA-I binding and HDL formation.⁸⁰⁾ Direct interaction between ABCA1 and apoA-I has been shown *via* cross-linking experiments,^{64,81,82)} even with a cross-linker as small as 3 Å.⁸³⁾ These results indicate that the direct binding of apoA-I to ABCA1 is an essential step in HDL formation.

Kobayashi, Sano, and Morita found that ABCA1, and ABCB4 preferentially transport PC together with cholesterol, while ABCG1 preferentially transports sphingomyelin (SM) together with cholesterol.^{34,84–86)} Thus ABCA1 and ABCB4 mediate the transport of the same substrates, but their extracellular acceptors are different. ABCB4 mediates the secretion of PC and cholesterol to bile salts as acceptors, but cannot secrete lipids in the presence of apoA-I.^{34,87)} On the other hand, cholesterol secretion by ABCA1 is heavily dependent on the presence of lipid-free apoA-I.^{79,81,82)} To analyze the differences between ABCA1 and ABCB4, Nagao examined whether NaTC serves as an acceptor for lipids

secreted by ABCA1, and found that NaTC extracts both cholesterol and PC as efficiently as apoA-I from cells expressing ABCA1,⁸⁷⁾ suggesting that NaTC functions as a lipid acceptor for ABCA1, which mimics apoA-I. The NaTC-dependent efflux of cholesterol and PC is not physiologically relevant because it is unlikely that the plasma concentration of bile acids reaches 1 mM, but this system allowed us to analyze the role of apoA-I and to dissect the steps in HDL formation.

The Tangier mutation W590S, with one amino acid substitution in the first ECD, impairs HDL formation.^{64,66)} Nagao analyzed the function of this Tangier mutant in detail, and found that it greatly decreases NaTC-dependent cholesterol and PC efflux.⁸⁷⁾ The kinetics of apoA-I binding to cells expressing ABCA1-W590S were similar to those for cells expressing wild-type ABCA1, consistently with a previous report that the W590S mutation does not impair apoA-I binding,^{64,65,88,89)} but the W590S mutation delayed the dissociation of apoA-I from ABCA1.⁸⁷⁾ Based on these results, we proposed a four-step model for ABCA1-mediated HDL formation (Fig. 4),⁹⁰⁾ at the apoA-I binding step, ATP binding and/or hydrolysis causes conformational changes within the ECDs of ABCA1, to which apoA-I directly binds; at the translocation step, lipid translocation by ABCA1, which is apoA-I-independent occurs; at the loading step, lipid loading of apoA-I bound to ABCA1 occurs; and at the dissociation step, dissociation of lipid-loaded apoA-I from ABCA1 occurs. In this model, the apoA-I binding step and the lipid translocation step, both of which are mediated by ABCA1 in an ATP-dependent manner, are separable. The conformational transition of apoA-I caused by lipid loading probably facilitates the dissociation of apoA-I from ABCA1. ApoA-I undergoes a conformational transition in response to lipids,⁹¹⁾ and lipidated apoA-I does not interact with ABCA1.^{82,92)} Because the W590S mutation impairs the translocation step, apoA-I can remain in lipid-free conformation, which has high affinity for ABCA1. Thus ABCA1 is a unique membrane protein that functions both as a receptor and as a transporter. It has been reported that it also functions as a receptor for apoA-I to activate the JAK2/STAT3 pathway in an anti-inflammatory reaction, which is independent of the lipid transport activity of ABCA1.⁹³⁾

IX. ABCA1 Is Highly Regulated Both at the Transcriptional and the Post-Translational Level

ABCA1 is highly regulated at the transcriptional level. The response is mediated by nuclear receptors LXR α and LXR β , whose ligands are sterol metabolites such as 22-(*R*)-hydroxycholesterol, 24-(*S*)-hydroxycholesterol, 27-hydroxycholesterol, and 24-(*S*),25-epoxycholesterol.^{94,95)} LXR β is ubiquitously expressed, whereas LXR α is restricted to the liver, adipose tissue, adrenal glands, intestine, lungs, kidneys, and cells of myeloid origin. Human LXR α expression is highly regulated and can be autoregulated, whereas human LXR β is stably expressed even in the absence of excess cholesterol. In the basal state, the LXR β and retinoid X receptor (RXR) heterodimers are bound to liver X response elements (LXRE) in the promoters of the target

genes (Fig. 5).⁹⁶⁾ When cholesterol accumulates in the cells, intracellular concentrations of oxysterols increase; subsequently, LXR β , activated *via* the binding of oxysterols, stimulates the transcription of ABCA1.⁹⁷⁻⁹⁹⁾

ABCA1-mediated cholesterol efflux is also highly regulated at the post-translational level. Because cholesterol is an essential component of cells, excessive elimination of cholesterol can result in cell death. Consequently, ABCA1 protein turns over rapidly, with a half-life of 1–2 h, to prevent excessive elimination.¹⁰⁰⁻¹⁰⁴⁾ Several proteins, including apoA-I, α 1-syntrophin, and β 1-syntrophin, have been reported to interact with ABCA1 and to reduce the rate of ABCA1 protein degradation.¹⁰²⁻¹⁰⁵⁾ Syntrophins play critical roles in lipid homeostasis by suppressing ABCA1 degradation in the brain¹⁰³⁾ and liver.¹⁰⁴⁾

The degradation of ABCA1 is regulated and is carried out *via* several pathways: (i) Cell-surface ABCA1 is endocytosed and recycled back to the plasma membrane or is delivered to the lysosomes through early and late endosomes for degradation.^{77,106)} (ii) Calpain protease degrades ABCA1 on the plasma membrane^{101,102)} and intracellularly, especially when apoA-I does not bind to ABCA1.⁷³⁾ ABCA1 is also degraded through the ubiquitin-proteasome pathway.^{107,108)} The COP9 signalosome (CSN) complex, which plays an important role in the degradation of various proteins, such as I κ B α , associates with ABCA1 and controls the ubiquitinylation and deubiquitinylation of ABCA1.¹⁰⁹⁾

Recently, Hozoji and Munehira reported¹¹⁰⁾ that the LXR β /RXR complex binds to ABCA1 when the intracellular concentration of oxysterols is low, and that the ABCA1-LXR β /RXR complex is distributed on the plasma membrane but is inert in terms of cholesterol efflux (Fig. 5). When cholesterol accumulates and the intracellular concentration of oxysterols increases, oxysterols bind to LXR β and the LXR β /RXR complex dissociates from ABCA1. Once free from LXR β /RXR, ABCA1 is active in the formation of HDL, and decreases the local cholesterol concentration immediately. Upon binding to oxysterols, LXR β /RXR activates the transcription of ABCA1 and other genes. Consequently, LXR β can cause a post-translational response, as well as a transcriptional response, to maintain cholesterol homeostasis. This mechanism is an immediate early response to cope with rapid increases in intracellular cholesterol, such as when macrophages consume apoptotic cells.

X. ABC Proteins: A Means of Adaptation against the Environment

The plasma membrane is critical for the life of the cell, not only as the boundary maintaining the cytosolic environment differently from the extracellular environment, but also as a platform for protein assembly, which converts extracellular stimuli into intracellular signals. The plasma membrane contains hundreds species of lipids in two asymmetric leaflets, and their dynamic and transient assembly into specialized domains, such as lipid rafts, helps organize membrane protein assembly, as when they transduce signals.¹¹¹⁾ Cholesterol not only enhances the mechanical stability of the plasma membrane, but is also important for membrane domain

organization. Acquisition of cholesterol as a component of membranes might have been critical in the evolution of multi-cellular organisms, but because hyper-accumulation of it is toxic to cells, proper circulation of sterols and lipids in the body became important for animals. Some ABC proteins evolved as ATP-dependent lipid transporters that distribute lipids to peripheral tissues in need, and take them up from tissues in case of excess.

ABCA12 is involved in lipid barrier formation in the skin, which prevents water loss from the body surface. Very long-chain fatty acid secretion for cuticular wax formation, which prevents water loss from the plant surface, is also done by plant members of ABC proteins.¹¹²⁾ CFTR (ABCC7) promotes the movement of water across the membrane, which is necessary for the production of freely flowing mucus. Mucus coats the lining of the airways, digestive tract, reproductive system, and other organs, protecting them from infection and helping them to function. ABCA3 is involved in the secretion of pulmonary surfactant, consist of phospholipids, cholesterol, and surfactant proteins, into the alveolar space. Secreted surfactant coats the lumen of the alveoli, where it reduces the surface tension at the alveolar air/liquid interface, thus preventing alveoli from collapsing and lessening the effort of breathing. SUR1 (ABCC8) is a key molecule in the response to blood glucose, the most important energy source in food. A xenobiotics transporter, MDR1, is also adapted against environments rich in hydrophobic toxic compounds. This suggests that many ABC proteins function on the body surface facing the outside and in organ pathways to adapt to the extracellular environment and to protect the body to maintain optimal health.

XI. Perspectives

A quarter century has passed since the discovery of the *MDR1* gene. Because defects in the function and expression of ABC proteins are related to various diseases (Table 1), their functions have been extensively studied, but the physiological functions and endogenous substrates of many ABC proteins remain unclear. One reason is that it is still difficult to purify eukaryote ABC proteins and to analyze their functions after reconstitution. Only a few studies have reported the activities of purified eukaryote ABC proteins.^{113–116)} The mechanisms of substrate recognition and transport by eukaryote ABC proteins are still unclear due to a lack of 3D structure analysis at high resolution, but since studies are making steady progress in our laboratory and others, and new techniques, such as single molecular tracking, are being applied in functional analysis, hopefully the functions and mechanisms of human ABC proteins will be determined.

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