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Molecular Cloning of the Human ATP-Binding Cassette Transporter 1 (hABC1): Evidence for Sterol-Dependent Regulation in Macrophages

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We have cloned the full-length cDNA for the human ATP binding cassette transporter 1 (hABC1). The 6603-bp open reading frame encodes a polypeptide of 2201 amino acids resulting in a deduced molecular weight of 220 kDa. The hABC1 cDNA is highly homologous (62%) to the human rim ABC transporter (ABCR). hABC1 is expressed in a variety of human tissues with highest expression levels found in placenta, liver, lung, adrenal glands, and fetal tissues. We demonstrate that the hABC1 expression is induced during differentiation of human monocytes into macrophages *in vitro.* In macrophages, both the hABC1 mRNA and protein expression are upregulated in the presence of acetylated low-density lipoprotein (AcLDL). The AcLDL-induced increase in hABC1 expression is reversed by cholesterol depletion mediated by the addition of high-density lipoprotein (HDL_3). Our data, demonstrating sterol-dependent regulation of hABC1 in human monocytes/macrophages, suggest a novel role for this transporter molecule in membrane lipid transport.

Members of the ATP-binding cassette (ABC) transporters constitute a superfamily of highly conserved proteins involved in the membrane transport of a variety of substrates including ions, amino acids, peptides, sugars, vitamins and steroid hormones [1]. The full-size ABC proteins are characterized by two nucleotide binding folds (NBF) with conserved Walker A and B motifs and two transmembrane domains, each consisting of six membrane-spanning helices [2]. The specificity for the transported molecules appears to be determined by the transmembrane domains, whereas the energy required for the transport activity is provided by the degradation of ATP at the NBF [3, 4]. Several of the ABC transporters, which have as yet been identified in humans, have been implicated in the pathogenesis of diseases [5]. For example, cystic fibrosis is caused by mutations of the CFTR (cystic fibrosis transmembrane conductance regulator) gene [6] and multidrug resistance of tumor cells has been associated with MDR (multidrug resistance) proteins [7]. Luciani *et al.* recently have isolated a novel mouse ABC transporter molecule, termed ABC1, using a PCR-based cloning approach [2]. This protein contains all typical features of full-size ABC transporters including a symmetric structure with two transmembrane domains linked by a highly hydrophobic segment and two NBF. Functional studies in mice revealed that ABC1 is involved in the engulfment of apoptotic cells by macrophages during development [8] and in macrophage interleukin-1β secretion [9]. Furthermore, it has been shown that ABC1 is able to generate an anion flux across cell membranes [10]. In this article, we report the cloning of the full length coding region of human ABC1, its tissue-specific expression, and we demonstrate that the expression of hABC1 is regulated by sterol import and export.

MATERIALS AND METHODS

Cell culture. Peripheral blood monocytes were isolated by leukapheresis and counterflow elutriation [11]. To obtain fractions containing >90% CD14+ mononuclear phagocytes, cells were pooled and cultured on plastic Petri dishes in macrophage SFM medium (Gibco BRL, Karlsruhe) containing 25 U/ml recombinant human M-CSF (Genzyme, Alzenau) for various time periods in at 37°C (CO_2 content: 5%). The cells were incubated in the presence or absence (controls) of AcLDL (100 μg/ml) to induce sterol loading. Following

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Sequence data from this article have been deposited in the EMBL/NCBI-GenBank Data Libraries under Accession No. AJ012376.

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this incubation, the cells were cultured in fresh medium supplemented with or without (controls) HDL_3 (100 μg/ml) for an additional 12 h to induce cholesterol efflux to the acceptor complex HDL_3.

Isolation of lipoproteins and preparation of AcLDL. LDL (d = 1006 to 1063 g/ml) and HDL_3 (d = 1,125 to 1,21 g/ml) lipoproteins were prepared from human plasma obtained from healthy volunteers according to standard protocols. The preparation was performed in a Beckman L-70 ultracentrifuge (70 Ti rotor) at 4°C. All densities were adjusted with solid KBr. Lipoprotein fractions were dialyzed several times in phosphate-buffered saline (PBS) containing 5 mM EDTA. After the final dialysis step in 0.15 M NaCl, lipoproteins were sterilized using a 0.45-μm sterile filter (Sartorius, Göttingen). LDL was acetylated by repeated addition of acetic anhydride, dialyzed in PBS [12], and the acetylation was verified by an increase in electrophoretic mobility.

Preparation of RNA and Northern blot analysis. Total cellular RNA was isolated from the cells by guanidium isothiocyanate lysis and CsCl centrifugation [13]. The RNA was quantitated spectrophotometrically and 15 μg samples were separated on a 1.2% agarose-formaldehyde gel and transferred to a nylon membrane (Schleicher & Schüll, Dassel) followed by UV crosslinking (Stratalinker model 1800, Stratagene, La Jolla, CA). For the detection of hABC1 mRNA, the membranes were hybridized with a 1 kb DNA fragment derived from PCR amplification using primers mABC1 3622f and mABC1 4620r. Hybridization of the same filters with a human β-actin probe verified that comparable RNA amounts were separated on the gel (not shown). The tissue-specific expression of hABC1 was assessed utilizing a multiple tissue RNA master blot containing poly A^+ RNA from 50 human tissues (Clontech, Heidelberg). Probes were radiolabeled with [α-^{32}P]dCTP (Amersham, Freiburg) using an oligolabeling kit (Pharmacia, Freiburg). Hybridization and washing conditions were performed according to previously described protocols [14]. The blots were quantitated densitometrically, and the individual signal densities were translated into a linear integer scale ranging from 0 to 5 (0, no expression; 5, maximum expression).

cDNA cloning of human ABC1. For the amplification of human ABC1 cDNA, primers for RT-PCR analysis were designed based on sequence information of mouse ABC1 cDNA [2]. From mononuclear phagocytes that were allowed to differentiate for 5 days, 1 μg RNA was reverse transcribed in a 20-μl reaction using the RNA PCR Core Kit (Perkin–Elmer, Langen). An aliquot of the cDNA was amplified in a 100-μl reaction volume using AmpliTaq Gold (Perkin–Elmer) DNA polymerase. The following primer combinations were used (primer names indicate the position in the corresponding mouse cDNA sequence): mABC1-144f (5′-CAAACATGTCAGCTGTTACTGGA-3′) and mABC1-643r (5′-TAGCCTTGCAAA-AATACCTTCTG-3′), mABC1-1221f (5′-GTTGGAAAGATTCTCTATACACCTG-3′) and mABC1-1910r (5′-CGTCAGCACTCTGATGATGGCCTG-3′), mABC1-3622f (5′-TCTCTGCTATCTCCAACCTCA-3′) and mABC1-4620r (5′-ACGTCTTCACCAGGTAAT-CTGAA-3′), mABC1-5056f (5′-CTATCTGTGTCATCTTTGCGATG-3′) and mABC1-5857r (5′-CGCTTCCTCCTATAGATCTTGGT-3′), mABC1-6093f (5′-AAGAGAGCATGTGGA-GTTCTTTG-3′) and mABC1-7051r (5′-CCCTGTAATGGAATTGTGTTCTC-3′), hABC1-540f (5′-AACCTTCTCTGGGTTCCTGTATC-3′) and hABC1-1300r (5′-AGTTCCTGGAA-GGTCTTGTTCAC-3′), hABC1-1831f (5′-GCTGACCCCTTTGAGGACATGCG-3′) and hABC1-3701r (5′-ATAGGTCAGCTCATGCCCTATGT-3′), hABC1-4532f (5′-GCTGCC-TCCTCCACAAAGAAAAC-3′) and hABC1-5134r (5′-GCTTTGCTGACCCGCTCC-TGGATC-3′), hABC1-5800f (5′-GAGGCCAGAATGACATCTTAGAA-3′) and hABC1-6259r (5′-CTTGACAACACTTAGGGCACAAT-3. All PCR products were cloned into the pUC18 plasmid vector and the nucleotide sequences were determined on a Pharmacia ALFexpress sequencer using the dideoxy chain-termination method [15] and fluorescent dye-labeled primers.

Western analysis. Total protein was prepared from monocytes and macrophages after various incubation periods according to standard protocols. Aliquots of 75 μg total protein in Laemmli buffer (supplemented with 8 M urea) were heated for 2 min at 50°C, separated on a 6% SDS–PAGE [10], and electroblotted for 24 h onto a nitrocellulose membrane. hABC1 was detected using Ab16, a rabbit polyclonal antiserum (AB16 1:500), recognizing the highly conserved first NBF domain of murine ABC1 [8], in combination with a peroxidase conjugated secondary antibody (1:7000, Renaissance, NEN, Paris).

Computer sequence analyses. DNA and protein computer analyses were performed using the HUSAR GCG software package (DKFZ, Heidelberg). For sequence comparison and protein alignment studies, the FASTA and MOTIFS applications were used.

RESULTS AND DISCUSSION

hABC1 cDNA and primary protein structure. An RT-PCR approach was performed using primers derived from mouse ABC1 cDNA to amplify corresponding human sequences. Initially, five cDNA fragments were obtained that spanned different regions of the human cDNA. These fragments were cloned and the derived sequence information was used to generate human primers that were used in further RT-PCR experiments to fill the gaps between the initially identified cDNA fragments. Using this strategy, we cloned a 6880-bp cDNA containing the complete coding region of the human ABC1 gene (the detailed sequence has been deposited at the EMBL/NCBI GenBank under Acc. No. AJ012376). The open reading frame comprises 6603 bp and encodes a 2201-amino acid protein with a predicted molecular weight of 220 kDa (Fig. 1). The polypeptide structure of hABC1 contains two highly conserved ATP-binding cassettes including Walker A and B motifs and thus conforms to the model of a full-size transporter (Fig. 1). Homology comparison of the predicted hABC1 peptide sequence with the known mouse ABC1 revealed 94% amino acid identity (Fig. 1). The protein can therefore be considered as the human ortholog of the mouse ABC1 molecule. Interestingly, the lowest homology between the hABC1 and the murine ABC1 peptide sequences is located in a segment within the initial 220 N-terminal amino acids, which represents the first intracellular domain [10]. Among all as yet known human ABC transporters, the rim ABC transporter (ABCR) showed the highest homology with hABC1 (62% nucleotide identity, not shown).

hABC1 mRNA tissue distribution. The tissue-specific expression of hABC1 was assessed using a multiple tissue RNA master blot containing poly A^+ RNA from 50 human tissues. Northern Blot analysis demonstrated the presence of hABC1 specific mRNA in all tissues examined (Table 1). The highest expression levels were detected in placenta, liver, lung, adrenal glands and fetal tissues, the lowest expression of hABC1 was found in kidney, pancreas, the pituitary gland, mammary gland and bone marrow (Table 1). The high expression in placenta and fetal liver is in agreement with the expected role of ABC1 in pro-

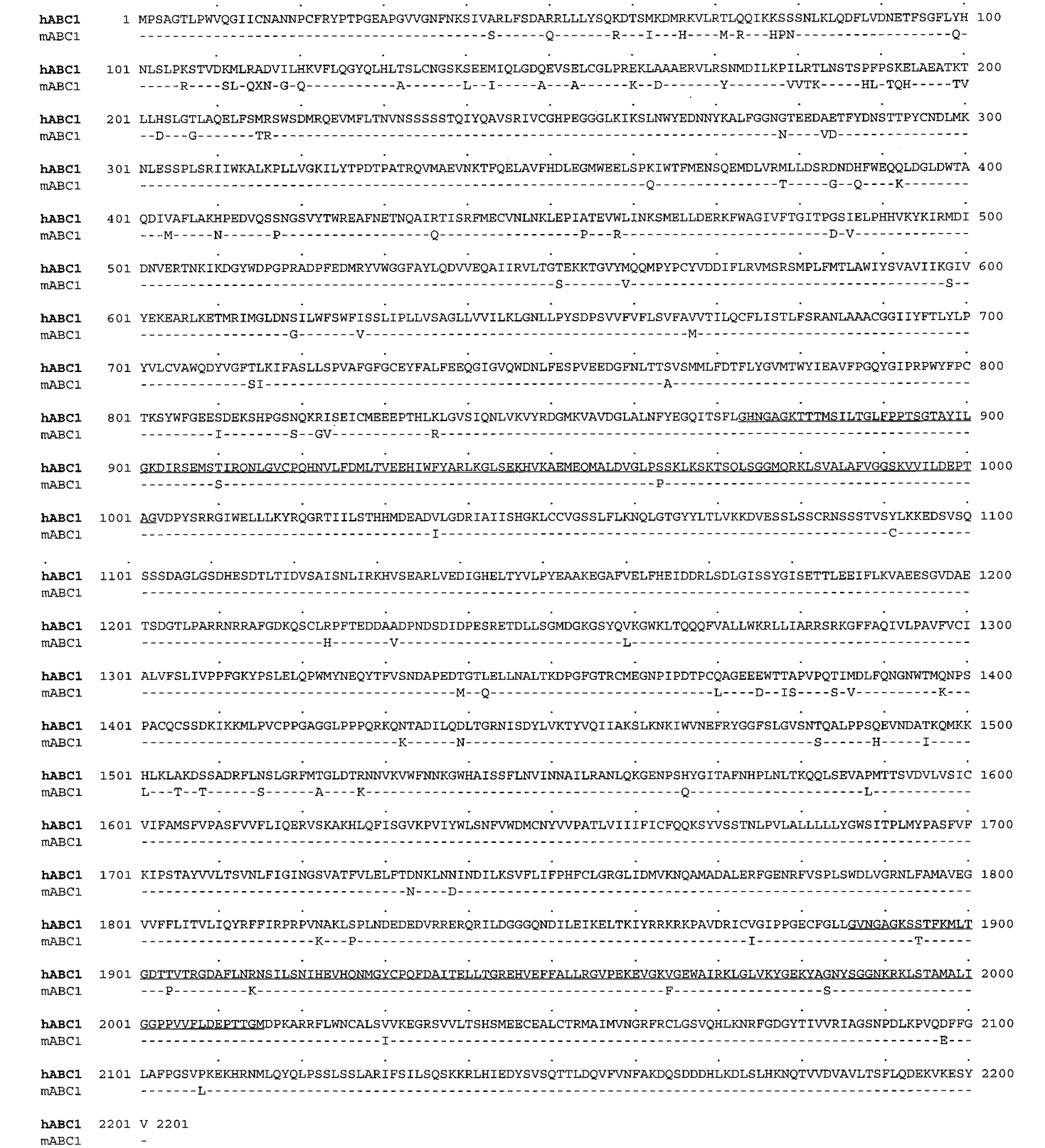

FIG. 1. Amino acid sequence of human ABC1 (hABC1) aligned with murine ABC1 (mABC1). Amino acids are indicated in the single letter code, the two ATP-cassette motifs are underlined. Identical amino acids in the mouse sequence are represented by dashes. The complete cDNA sequence for hABC1 can be obtained from the EMBL/NCBI-GenBank (Accession No. AJ012376).

TABLE 1

Expression of hABC1 mRNA in Various Human Tissues

Tissue	Relative ABC1 mRNA expression	Tissue	Relative ABC1 mRNA expression
Whole brain	●●	Pancreas	●
Amygdala	●●	Pituitary gland	●
Caudate nucleus	●●	Adrenal gland	●●●
Cerebellum	●	Thyroid gland	●●
Cerebral cortex	●	Salivary gland	●
Frontal lobe	●	Mammary gland	●
Hippocampus	●●	Kidney	●
Medulla oblongata	●	Liver	●●●
Occipital lobe	●●●	Small intestine	●●●
Putamen	●●●	Spleen	●●
Substantia nigra	●●	Thymus	●●
Temporal lobe	●	Peripheral leukocyte	●
Thalamus	●	Lymph node	●
Acumens	●	Bone marrow	●
Spinal cord	●●	Appendix	●●●
Heart	●●	Lung	●●●
Aorta	●●	Trachea	●●
Skeletal muscle	●	Placenta	●●●●●
Colon	●	Fetal brain	●●
Bladder	●	Fetal heart	●●●
Uterus	●●	Fetal kidney	●●●
Prostate	●	Fetal liver	●●●●
Stomach	●	Fetal spleen	●●●
Testis	●	Fetal thymus	●●
Ovary	●	Fetal lung	●●●

Note. mRNA expression levels for individual tissues were assessed by dot blot analysis and quantitated densitometrically (Materials and Methods). The signal densities were translated into a linear scale (0–5) and are indicated as dots.

grammed cell death [8], a scenario in which it is involved in the clearance of apoptotic cells by macrophages.

hABC1 in human macrophages is regulated by sterol import and export. In the present study, we established an *in vitro* model to study the influence of cholesterol import/export on the expression of hABC1 in monocytes/macrophages. Elutriated monocytes were cultured for different time periods in serum-free medium containing M-CSF as differentiation inducing agent. After an incubation time of four days, the medium was replaced and AcLDL was added to induce sterol uptake. Following further incubation for three days the medium was changed again and supplemented with the cholesterol acceptor HDL_3. The regulation of hABC1 mRNA expression in monocytes/macrophages during cholesterol loading/deloading was analyzed by Northern Blot using a 1 kb specific probe (Materials and Methods). *In vitro* differentiation of freshly isolated monocytes induced an upregulation of hABC1 mRNA levels within four days followed by a downregulation to baseline levels at 7 days (Fig. 2A, lanes 2–5) demonstrating that the hABC1 gene expression in monocytes is differentiation dependent. AcLDL loading of four days preincubated macrophages for 1, 2, and 3 days, respectively (Fig. 2A, lanes 6–8), induced a strong increase in mRNA expression compared to untreated controls (lanes 2–5). Subsequent deloading of macrophages with HDL_3 for 12 h (lane 9) reversed the AcLDL induced increase in hABC1 expression. In contrast, hABC1 expression in control macrophages remained at an elevated level in the absence of HDL_3 (lane 10).

The hABC1 mRNA regulation profile in macrophages during cell differentiation and sterol import/export was also detected at the protein level. Western blot analysis using an anti-mouse ABC1 specific antibody showed an increase in protein expression during monocyte differentiation, a significant upregulation of protein levels after sterol loading, and subsequent downregulation following the deloading of macrophages with HDL_3 (Fig. 2B).

Taken together, our results indicate that hABC1 is a sterol-sensitive gene which is inversely regulated by cholesterol import and export. The exact role of hABC1 in the lipid transport system is presently unknown. It is possible that hABC1 itself acts as a lipid transporter molecule in distinct cell membrane

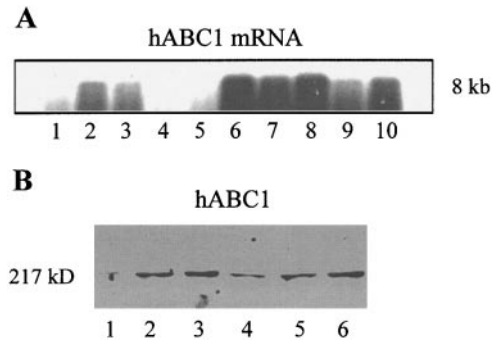

FIG. 2. Expression of hABC1 mRNA and protein in human macrophages is differentiation dependent and regulated by AcLDL and HDL_3. (A) Shown is the expression of hABC1 mRNA in human monocytes that were cultivated for various time periods in the presence or absence of AcLDL or HDL_3. Lane 1, freshly obtained human monocytes; lanes 2–5, monocytes maintained in culture in the presence of M-CSF for 4, 5, 6, and 7 days, respectively; lanes 6–8, 4-day-old M-CSF treated macrophages incubated with AcLDL for 1, 2, and 3 days, respectively; lanes 9 and 10, AcLDL laden macrophages as in lane 8 incubated for 12 h in the presence (lane 9) or absence (lane 10) of the cholesterol acceptor HDL_3. (B) Regulation of hABC1 protein expression by sterol loading and deloading. Lane 1, freshly obtained monocytes; lane 2, monocytes maintained in culture in the presence of M-CSF for 4 days; lane 3, 4-day-old old M-CSF treated macrophages incubated with AcLDL for an additional 2 days; lane 4, macrophages as in lane 3 without AcLDL loading (control); lane 5, 5-day-old macrophages loaded with AcLDL for an additional 2 days and subsequent deloading with HDL_3 for 12 h; lane 6, macrophages as in lane 5 but in the absence of HDL_3. A rabbit polyclonal antiserum (AB16 1:500), recognizing the highly conserved first NBF domain of ABC1, was used for the detection of hABC1.

domains. For example, it has been demonstrated that cholesterol and sphingolipid rich microdomains [20] are involved in the cholesterol efflux to the acceptor particle HDL [21]. Interestingly, an involvement in the translocation of phospholipids across the plasma membrane has been suggested for MDR1, MDR3, and MRP1, three other members of the ABC transporter family [16–19, 22]. Since the membrane cholesterol content is tightly associated with its phospholipid composition, it is conceivable that hABC1 bears phospholipid translocase activity. Experiments are currently underway to study in detail the role of ABC1 in cellular lipid trafficking.

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