ABC\textsubscript{A1}, the ATP-binding cassette protein mutated in Tangier disease, mediates the efflux of excess cellular sterol to apoA-I and thereby the formation of high density lipoprotein. The intracellular localization and trafficking of ABC\textsubscript{A1} was examined in stably and transiently transfected HeLa cells expressing a functional human ABC\textsubscript{A1}-green fluorescent protein (GFP) fusion protein. The fluorescent chimERIC ABC\textsubscript{A1} transporter was found to reside on the cell surface and on intracellular vesicles that include a novel subset of early endosomes, as well as late endosomes and lysosomes. Studies of the localization and trafficking of ABC\textsubscript{A1}-GFP in the presence of brefeldin A or monensin, agents known to block intracellular vesicular trafficking, as well as apoA-I-mediated cellular lipid efflux, showed that: (i) ABC\textsubscript{A1} functions in lipid efflux at the cell surface, and (ii) delivery of ABC\textsubscript{A1} to lysosomes for degradation may serve as a mechanism to modulate its surface expression. Time-lapse fluorescence microscopy revealed that ABC\textsubscript{A1}-GFP-containing early endosomes undergo fusion, fission, and tubulation and transiently interact with one another, late endocytic vesicles, and the cell surface. These studies establish a complex intracellular trafficking pathway for human ABC\textsubscript{A1} that may play important roles in modulating ABC\textsubscript{A1} transporter activity and cellular cholesterol homeostasis.

Cholesterol is a membrane constituent that maintains structural domains that are important in the regulation of vesicular trafficking and signal transduction (1). In most cells, cholesterol is not catabolized. Thus, the regulation of cellular sterol efflux plays a crucial role in cellular sterol homeostasis. Cellular sterol can efflux to extracellular sterol acceptors by both nonregulated, passive diffusion mechanisms (2) as well as by an active, regulated, energy-dependent process (3, 4) mediated by the ABC\textsubscript{A1} transporter (5).

The human ABC\textsubscript{A1} transporter is a polytopic membrane-spanning ATP-binding cassette protein (6) that is essential for the formation of HDL\textsuperscript{1} via apoA-I-mediated efflux of cholesterol and phospholipids from peripheral cells (3–5, 7, 8). Recent studies suggest that the ABC\textsubscript{A1} transporter may function to regulate cellular sterol efflux by modifying adjacent membrane lipid domains, thereby allowing apoA-I and other apolipoproteins to bind to the cell membrane and remove cholesterol and phospholipids from cells (9–12). ABC\textsubscript{A1}-mediated cellular sterol efflux constitutes the initial step in the pathway of reverse cholesterol transport that ultimately leads to elimination of cholesterol from the body. Macrophages, which scavenge serum lipoproteins in an unregulated manner, are particularly dependent on ABC\textsubscript{A1}-mediated sterol efflux to prevent pathogenic sterol accumulation. Mutations in the human ABC\textsubscript{A1} transporter cause Tangier disease (13–17), which is characterized by the accumulation of cholesterol ester in reticuloendothelial cells, hypercatabolism of poorly lipidated serum apoA-I, exceedingly low serum HDL, and increased risk of coronary heart disease (17).

To date, the cellular distribution of ABC\textsubscript{A1} and its possible site(s) of function are not fully understood. Immunocytochemical studies have suggested that endogenously expressed human ABC\textsubscript{A1} resides solely on the plasma membrane (5, 11, 12). Stably and transiently expressed chimERIC ABC\textsubscript{A1}-GFP has been reported to reside in intracellular compartments as well as on the plasma membrane (6, 9, 18). It is currently thought that ABC\textsubscript{A1} at the plasma membrane functions in cellular lipid efflux. Several investigators have provided evidence suggesting that ABC\textsubscript{A1}-mediated lipid efflux involves intracellular trafficking of substrate lipids (19, 20) or the apoA-I acceptor (21).

In the present study, a functional fluorescent chimERIC human ABC\textsubscript{A1}-GFP protein expressed in living cells has revealed that early endosomes containing the ABC\textsubscript{A1} transporter shuttle between the plasma membrane and other endocytic compartments. These studies suggest that the trafficking of ABC\textsubscript{A1} in endocytic compartments may play important roles in apoA-I-mediated efflux of cellular lipids.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—HeLa and Chinese hamster vary (CHO) cells were grown in DMEM (Life Technologies, Inc.), supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/ml of penicillin, 100 \( \mu \)g/ml streptomycin, and 100 \( \mu \)g/ml G418. CHO cells were transiently transfected with FuGene-6 (Roche Molecular Biochemicals, Indianapolis, IN), using the expression plasmid pTRE2 (CLONTECH, Palo Alto, CA), encoding a chimERIC ABC\textsubscript{A1}-GFP protein (pTRE2-ABC\textsubscript{A1}-GFP). Enhanced GFP along with a 5 amino acid glycine linker (Quantum Biologics, Vancouver, Canada) was fused in frame to the carboxyl terminus of human ABC\textsubscript{A1}, after first deleting the stop codon from the full-length ABC\textsubscript{A1} hamster ovary; DiI, 1,1'-dioctadeyl-3,3',3'-tetramethylindocarbocyanine perchlorate; LDL, low density lipoprotein; DMEM, Dulbecco’s modified Eagle’s medium; ER, endoplasmic reticulum; GFP, green fluorescent protein; LAMP2, lysosomal-associated membrane protein 2.
cDNA (9). HeLa cells were also transiently and stably transfected with a chimeric ABCA1-GFP protein, as previously described (9). Briefly, HeLa (Tet-off) cells (CLONTECH, Palo Alto, CA) were co-transfected with pTRE2-ABCA1-GFP and pTK-Hyg (CLONTECH, Palo Alto, CA) at a ratio of 1:20 and selected with 500 μg/ml of hygromycin. Hygromycin-resistant cells were screened for expression of the fusion protein by fluorescence microscopy and positive clones were further purified by limiting dilution. Control cells were co-transfected with pTRE2 and pTK-Hyg (CLONTECH, Palo Alto, CA) at a ratio of 1:20 and selected with 500 μg/ml of hygromycin.

Apolipoprotein Isolation—ApoloA-I purified from human plasma (22) was over 99% pure, as determined by SDS-PAGE and amino-terminal sequence analysis.

Immunocytochemical Analyses—Cells in glass chamber slides were washed in phosphate-buffered saline and fixed in 3% paraformaldehyde for 30 min. Cells were immunolabeled using an indirect procedure in which all incubations were performed either in blocker solution containing filipin (0.05%) and goat IgG (2.5 mg/ml) or 10% fetal bovine serum in phosphate-buffered saline containing saponin (0.2%). Primary antibodies used were raised against human LAMP2, transferrin receptor, and p58K protein. Secondary Alexa-568-labeled antibodies were used to detect the primary antibodies.
ABCA1-GFP Localization and Trafficking

**RESULTS**

**ApoA-I-mediated Efflux Is Enhanced by ABCA1-GFP Expression in Stably Transfected HeLa Cells**—ApoA-I-mediated efflux of cellular cholesterol and choline-containing phospholipids was enhanced 5-fold or more by ABCA1-GFP expression in stably transfected HeLa cells compared with control cells (Fig. 1). Thus, as previously shown, the fusion of enhanced green fluorescent protein to the C terminus of ABCA1 does not interfere with its function (9, 18).

**Cellular Distribution of ABCA1-GFP**—ABCA1-GFP expressed either transiently or stably in HeLa cells resides at the cell surface and in intracellular compartments (Fig. 2, A and B). Similar patterns of distribution were observed in both cell types (HeLa, CHO) and expression systems examined. To identify the endocytic compartments containing ABCA1, HeLa cells transiently expressing ABCA1-GFP were immunostained with antibodies to the human transferrin receptor (early endocytic compartments (23)) or LAMP2 protein (late endocytic compartments (24)). As shown in Fig. 2C, ABCA1-GFP does not appreciably localize in the punctate intracellular structures containing the transferrin receptor. As shown in Fig. 2D, ABCA1-GFP does co-localize with LAMP2, a marker for late endocytic compartments, which include late endosomes and lysosomes (24). Endocytosed DiI-LDL as well as tetramethylrhodamine-dextran when used as vital markers for late endocytic compartments (25, 26) also co-localized with a subset of intracellular vesicles containing ABCA1-GFP (data not shown).

**ABCA1-GFP Trafficking between the Plasma Membrane and Endocytic Compartments**—To further elucidate the intracellular trafficking pathways used by ABCA1, HeLa cells transiently expressing ABCA1-GFP were treated with cycloheximide, to prevent further delivery of newly synthesized protein to the cell surface, as well as with brefeldin A or monensin, agents that block both intracellular vesicular trafficking (27, 28) and apoA-I-mediated cellular lipid efflux (8, 29). The effect of these agents on the cellular distribution of ABCA1-GFP and their effects on cellular lipid efflux by ABCA1-GFP overexpression were monitored.

**Cycloheximide Reduces Cellular ABCA1-GFP Levels and ABCA1-GFP-induced Cellular Sterol Efflux**—Confocal microscopy reveals that blocking cellular protein synthesis with cycloheximide substantially reduced the total cellular levels of ABCA1-GFP including the amount residing at the cell surface (Fig. 3B). Cycloheximide treatment reduced ABCA1-GFP mediated cellular efflux of cholesterol and choline-containing phospholipids by 5-fold or more (Fig. 3C). However, treatment of cells with brefeldin A, which causes the accumulation of newly synthesized protein in the cis-Golgi (27), did not affect the cellular distribution of ABCA1-GFP (Fig. 3D). To further determine whether ABCA1-GFP was retained within intracellular vesicles, HeLa cells transiently expressing ABCA1-GFP were treated with brefeldin A and cultured for 4 h. Confocal microscopy revealed that ABCA1-GFP-containing vesicles were reduced upon treatment with brefeldin A (Fig. 3E).

**ABCA1-GFP Trafficking upon Inhibition of Protein Synthesis or Transport**—Inhibition of protein synthesis or transport inhibits apoA-I-mediated efflux in HeLa cells expressing ABCA1-GFP. The effect of treatment with 5 μg/ml BFA or 10 μM monensin in the absence or presence of 100 μg/ml cycloheximide (CHX) for 6 h on the ability of apoA-I to mediate cholesterol efflux (as described under “Experimental Procedures”) in stably transfected HeLa cells expressing the human ABCA1-GFP fusion protein was examined.

**FIG. 4. Monensin traps plasma membrane-derived ABCA1-GFP in late endocytic compartments.** Confocal microscopy reveals that compared with nontreated controls (A–C), treatment with 10 μM monensin for 6 h (D–F) altered the cellular distribution of ABCA1-GFP (A and D) and LAMP2 (B and E) in HeLa cells transiently expressing ABCA1-GFP. Compared with nontreated controls (A, arrow), monensin treatment reduced ABCA1-GFP (green) at the plasma membrane (D, arrow). Note that in monensin-treated cells, ABCA1-GFP resides in large intracellular vesicles (D, arrowheads) that also contain LAMP2 (E, arrowheads), a marker for late endocytic compartments. These intracellular vesicles containing both ABCA1-GFP and LAMP2 appear yellow in the merged image (F, arrowheads) of D and E. Note in the merged image of the monensin-treated cell (F), the shift in distribution of ABCA1-GFP from the plasma membrane (arrow in C versus arrow in F) to the LAMP2-containing late endocytic vesicles (F, arrowheads). Bar = 10 μm.

**FIG. 5. Inhibition of protein synthesis or transport inhibits apoA-I-mediated efflux in HeLa cells expressing ABCA1-GFP.** The effect of treatment with 5 μg/ml BFA or 10 μM monensin in the absence or presence of 100 μg/ml cycloheximide (CHX) for 6 h on the ability of apoA-I to mediate cholesterol efflux (as described under “Experimental Procedures”) in stably transfected HeLa cells expressing the human ABCA1-GFP fusion protein was examined.

used at a 1:100 dilution. Fluorescence was viewed with a Zeiss 410 or 510 laser scanning confocal microscope, using a krypton-argon-Ommichrome laser with excitation wavelengths of 488 and 568 nm for enhanced green fluorescent protein and Alexa-568, respectively.

**Time-lapse Video Fluorescence Microscopy**—Time-lapse images were taken with a Zeiss Axiosvert 35 microscope equipped with a charged-coupled device camera (TEA/CCD-1317K/1, Princeton Instruments, Trenton, NJ). For live cell imaging, cells were prepared on 40-mm coverslips, and temperature was maintained at 37 °C in Focht Chamber System 2 with an Objective Heater System (Bioptechs, Butler, PA). A total of 40 GFP images were acquired at the rate of 1/s (0.3-s exposure). Structures in QuickTime movies were pseudocolored using Adobe AfterEffects 4.1 and Adobe Photoshop 5.0 software.

**Experimental Procedures**—Experimental Procedures were monitored.
mediated cellular sterol efflux to 51.9 ± 7.0% of controls (Fig. 5).

These results are readily explained if in the absence of delivery of newly synthesized ABCA1-GFP to the cell surface the amount of ABCA1-GFP at the cell surface is reduced with time, as the existing cellular pool of protein is degraded, presumably in lysosomes.

Brefeldin A Treatment Reduces ABCA1-GFP at the Cell Surface and Blocks ABCA1-GFP-induced Cellular Sterol Efflux—BFA causes the Golgi to fuse with the endoplasmic reticulum and blocks vesicular transport to the cell surface along the secretory pathway (27). Thus, BFA would be expected to cause newly synthesized ABCA1-GFP to accumulate in the fused Golgi-ER. In addition, BFA would be expected to reduce the amount of ABCA1-GFP at the cell surface and in endocytic compartments as ABCA1-GFP at the cell surface traffics along the endocytic pathway. As expected, ABCA1-GFP is considerably reduced at the surface of BFA-treated HeLa cells expressing ABCA1-GFP (Fig. 3C). Instead, ABCA1-GFP is seen to distribute in a cytosolic reticular pattern (Fig. 3C), consistent with the trapping of newly synthesized ABCA1-GFP in the fused Golgi-ER. Treatment with BFA in the presence of cycloheximide resulted in a similar pattern of distribution of ABCA1-GFP in the Golgi-ER (Fig. 3D). However, blocking protein synthesis greatly reduced the amount of ABCA1-GFP trapped by BFA in the hybrid organelle (Fig. 3D).

BFA reduced ABCA1-GFP-induced cellular efflux of cellular sterol to 60.3 ± 6.5% of control values (Fig. 5). Thus, together with the progressive loss of ABCA1-GFP at the cell surface, there is a concomitant loss of ABCA1-stimulated cellular sterol efflux.

**Fig. 6.** Dynamic interactions of ABCA1-GFP-containing endosomes in living cells. CHO cells were transiently transfected with ABCA1-GFP and time-lapse image acquisition of GFP fluorescence was performed at 37 °C as described under “Experimental Procedures.” The large top panel shows a cell imaged in the first frame. The white arrowheads indicate ABCA1-GFP at the cell surface, and the yellow arrows highlight representative large, static, perinuclear ABCA1-GFP late endocytic vesicles (shown in white). Small, mobile ABCA1-GFP-containing endosomes were pseudocolored magenta, green, and blue. High magnification time-lapse images of a selected region of the cell (yellow box) are indicated by the image number.

**Fig. 7.** ABCA1-GFP-containing endosomes interact with the cell surface in living cells. CHO cells were transiently transfected with ABCA1-GFP, and time-lapse image acquisition of GFP fluorescence was performed at 37 °C as described under “Experimental Procedures.” The large top panel shows a cell imaged in the first frame. The white arrowheads indicate ABCA1-GFP at the cell surface. Small, mobile ABCA1-GFP-containing endosomes are pseudocolored magenta, green, and blue. High magnification time-lapse images of a selected region of the cell (yellow box) are indicated by the image number. The dynamic interaction of a small, rapidly moving ABCA1-GFP vesicle (green) with the plasma membrane (arrowhead) can be seen. Note that the ABCA1-GFP-containing endosome pseudocolored magenta and blue are identical to the corresponding vesicles seen in Fig. 6, whereas the ABCA1-GFP-containing endosome pseudocolored green represents a different vesicle than that in Fig. 6.
ABCA1-GFP Localization and Trafficking

This is a summary of the localization and potential trafficking pathways that ABCA1 may take in serving its role to lipidate apoA-I to form nascent HDL. The arrows represent fusion/fission events or physical contact that allows exchange of vesicular components. A, delivery to early endocytic compartments; B, recycling to plasma membrane; C, delivery to late endocytic compartments (late endosomes/lysosomes); D, retrieval from late endocytic compartments.

efflux. Co-treatment with cycloheximide did not further reduce cellular sterol efflux (49.7 ± 8.4% of control values; Fig. 5). This latter finding suggests that the ABCA1-GFP trapped in the hybrid Golgi-ER organelle by the action of BFA does not promote efflux of cellular sterol.

Monensin Reduces ABCA1-GFP at the Cell Surface and Traps ABCA1-GFP in Late Endocytic Compartments—Monensin (28), like brefeldin A (27), blocks delivery of newly synthesized protein to the cell surface and thus is expected to reduce surface expression of ABCA1-GFP as well as apoA-I-mediated efflux. In addition, because monensin blocks protein degradation and trafficking out of late endosomes and lysosomes (28), ABCA1-GFP would be expected to accumulate in LAMP2(+) late endosomes and lysosomes.

Monensin treatment reduced the level of ABCA1-GFP residing at the cell surface, as revealed by confocal microscopy (Figs. 3E and 4). Monensin treatment also induced the localization of ABCA1-GFP in intracellular vesicles containing LAMP2 (Fig. 4) as well as in late endocytic vesicles vitally labeled with endocytosed DiI-LDL (25) or endocytosed tetramethylrhodamine-dextran (26) (data not shown). As seen in Fig. 3F, co-treatment with monensin and cycloheximide resulted in the accumulation of ABCA1-GFP in punctate cytoplasmic structures identified to be late endosomes/lysosomes by their co-localization with endocytosed DiI-LDL or tetramethylrhodamine-dextran (data not shown). The Golgi-specific marker p58 protein (30) did not appreciably colocalize with LAMP2 (data not shown). Taken together, these results indicate that monensin causes ABCA1-GFP derived from the cell surface to become trapped in late endocytic compartments. As expected, based on the results of BFA treatment (Figs. 3 and 5), associated with the monensin-induced loss of surface expression a considerable decrease (47.3 ± 8.8%) in apoA-I-mediated sterol efflux in HeLa cells stably transfected with ABCA1-GFP was observed (Fig. 5). Blocking protein synthesis with cycloheximide did not further reduce the monensin-induced block in ABCA1-GFP-stimulated sterol efflux (43.3 ± 6.4%) (Fig. 5). Taken together, these results suggest that monensin causes ABCA1-GFP to the cell surface and traps plasma membrane-derived ABCA1-GFP in late endocytic compartments.

ABCA1-GFP Trafficking in Living Cells—Time-lapse digital video fluorescence microscopy of ABCA1-GFP expressed in living cells revealed a complex intracellular trafficking pathway for the ABCA1-GFP transporter (Figs. 6 and 7; Movies 1–3 in the Supplemental Material). The fluorescent chimeric protein was seen in traffic in at least two types of intracellular vesicles: (i) small rapidly moving vesicles and (ii) large, static perinuclear vesicles (Figs. 6 and 7 and Movie 1 in Supplemental Material). Treatment of cells expressing ABCA1-GFP with U18666A (31) selectively enlarged the static, large ABCA1-GFP-containing perinuclear vesicles (data not shown). U18666A enlarges late endosomes/lysosomes as a result of the accumulation of cholesterol in these compartments (Refs. 32 and 33; data not shown). Thus, the large, static ABCA1-GFP-containing perinuclear vesicles are likely to represent late endocytic compartments, whereas the small, fast-moving ABCA1-GFP-containing vesicles are likely to represent, for the most part, early endosomes.

Analysis of these images revealed the dynamic interactions of ABCA1-GFP-containing early endosomes with one another as well as with ABCA1-GFP-containing late endocytic vesicles and the ABCA1-GFP-containing plasma membrane. As described below, ABCA1-GFP-containing early endosomes appear to shuttle between late endosomes/lysosomes and the plasma membrane in living cells.

As seen in Fig. 6 (see also Movie 2 in Supplemental Material), a small ABCA1-GFP endosome (pseudocolored green) interacts with two other small ABCA1-GFP endosomes in frames 1–9 and 28–31 (the endosome pseudocolored magenta) and in frames 2 and 3 (the endosome pseudocolored blue). The small ABCA1-GFP endosome (magenta) remains relatively stationary during frames 1–37, moves rapidly in frame 38, and then appears to move out of the plane of focus in frames 39 and 40.

As shown in Fig. 6, the small green ABCA1-GFP endosome appears to shuttle between the small magenta ABCA1-GFP endosome and the ABCA1-GFP-containing late endosomes (shown in white). The green ABCA1-GFP endosome remains associated with the magenta ABCA1-GFP endosome during frames 1–8, then rapidly descends vertically while tubulating (frames 9–11), fuses with the ABCA1-GFP-containing late endosomes (shown in white) during frames 11–12, and then remains fused to the ABCA1-GFP-containing late endosomes during frames 13–25. The green ABCA1-GFP endosome subsequently re-emerges from the ABCA1-GFP-containing late endosome compartment in frame 26, rapidly ascends vertically during frames 27–28, interacts with the magenta ABCA1-GFP endosome again during frames 28–31, rapidly descends to the vicinity of the ABCA1-GFP-containing late endosomes during frames 31–34, and then appears to interact with the ABCA1-GFP-containing late endocytic vesicles (white) again during frames 34–40.

An example of a small mobile ABCA1-GFP endosome that interacts with other small mobile ABCA1-GFP endosomes, as well as with the cell surface, can be seen in Fig. 7 and Movie 3 (Supplemental Material). The small ABCA1-GFP endosome (pseudocolored green) can be seen to interact intimately with the cell surface during frames 36–40. Note that prior to docking at the cell surface, the green ABCA1-GFP endosome interacts first with another small ABCA1-GFP endosome (shown in blue, during frames 16–18) and then fuses with very small ABCA1-GFP endosomes (shown in white, frames 28–31, 34, and 35).

Image analysis further reveals the complex trafficking itinerary that a single ABCA1-GFP endosome can take. As seen in Fig. 6 (see also Movie 2 in Supplemental Material), the small mobile blue ABCA1-GFP endosome moves in a linear fashion across the field in frames 1–8, interacting with the small green...
ABCA1-GFP endosome in frames 2 and 3, then descends in a linear manner in frames 7–10, briefly interacts (frame 10) with late endosomes (shown in white, highlighted by the yellow arrow in frame 1), and then ascends vertically in a linear manner (frames 11–17) until it goes out of frame (frame 18). Note that the same small mobile blue ABCA1-GFP endosome seen in Fig. 6 can also be seen in Fig. 7 (frames 2–19) interacting subsequently with yet another small mobile ABCA1-GFP endosome (pseudocolored green in Fig. 7) during frames 16–18 (Fig. 7). The blue ABCA1-GFP endosome, which goes out of frame in Fig. 7 (frame 19), can be seen in Movie 1 (Supplemental Material) to ascend vertically and dock at the plasma membrane (during frames 19–28). Thus, the small mobile blue ABCA1-GFP endosome first interacts briefly with another small mobile ABCA1-GFP endosome (green, Fig. 6, frames 2 and 3), then interacts briefly with a large, immobile perinuclear late endosome (Fig. 6, frame 10), and finally interacts with another small, mobile ABCA1-GFP endosome for a longer period of time (pseudocolored green in Fig. 7, frames 16–18) prior to docking at the cell surface.

Consistent with the perturbations observed by confocal microscopy (Fig. 3), time-lapse video microscopy of living cells expressing ABCA1-GFP (Movie 4, Supplemental Material) revealed that BFA treatment resulted in a loss of ABCA1-GFP fluorescence at the cell surface and trapping of ABCA1-GFP in the cytosolic Golgi-ER compartment. Monensin treatment revealed that BFA treatment resulted in a loss of ABCA1-GFP (Movie 4, Supplemental Material) re-capture of ABCA1-GFP protein to gain insight into the cellular localization and trafficking of the human ABCA1 transporter and its possible sites of function. We have identified at least three major cellular sites of ABCA1 localization in living cells, including the plasma membrane, as well as in early and late endocytic compartments (Fig. 8).

The ABCA1-GFP chimeric protein functions to efflux both cholesterol and choline-containing phospholipids. The present studies suggest that ABCA1 at the cell surface functions in cellular lipid efflux. This is supported by the observation that a reduction in the amount of ABCA1-GFP residing at the cell surface correlates with a reduction in apoA-I-mediated cellular lipid efflux.

The present confocal microscopic immunolocalization studies have revealed that ABCA1-GFP is expressed not only on the plasma membrane but also on the surface of LAMP2 (+)/DiI-LDL (+) late endocytic vesicles. ABCA1-GFP residing at the plasma membrane appears to traffic along the endocytic pathway to late endocytic compartments, because monensin causes ABCA1-GFP to accumulate in late endosomes/lysosomes while reducing the amount of ABCA1-GFP at the plasma membrane.

Time-lapse microscopy revealed that ABCA1-GFP resides on the surface of both small, mobile vesicles as well as on large, relatively immobile perinuclear vesicles. The small, fast-moving ABCA1-GFP-containing vesicles are likely to represent early endosomes, whereas the ABCA1-GFP-containing large, static, perinuclear vesicles are likely to represent late endocytic compartments. The ABCA1-GFP-containing early endosomes undergo fusion and fission events. The ABCA1-GFP-containing early endosomes interact transiently with one another, with the ABCA1-GFP-containing late endocytic vesicles, and with the cell surface. As illustrated in Fig. 8, the ABCA1-GFP early endosomes appear to shuttle between the ABCA1-GFP late endocytic vesicles and the cell surface. The ABCA1-GFP-containing early endosomes tubulate while moving vortically, consistent with directed movement along cytoskeletal elements such as microtubules or actin filaments.

In addition to ABCA1, the membrane components that may be transferred from the ABCA1-GFP early endosomes to other cellular compartments remain to be determined but may include substrate lipids for ABCA1 and/or acceptor apolipoproteins. Indeed, recent studies have provided evidence that extracellular apolipoproteins are endocytosed and trafficked via a recycling compartment back to the cell surface prior to their release from the cell (21, 34). Interestingly, the compartment that appears to mediate the recycling of apolipoproteins is distinct from the transferrin receptor recycling compartment (34). Thus, the possibility exists that apoA-I and other apolipoproteins that are lipidated in an ABCA1-dependent manner may share an endocytic trafficking itinerary with ABCA1 in a novel endocytic recycling compartment.

We have shown that delivery of the ABCA1 transporter to the cell surface is necessary for cellular lipid efflux. It remains to be determined, however, whether residence of the ABCA1 transporter at the surface is sufficient for its function or whether trafficking along the endocytic pathway plays a critical role in ABCA1 transporter function. Support for a potential functional role for ABCA1 in endocytic compartments is provided by the recent reports that the ABCA2 (35) and ABCB9 (36) transporters predominantly reside in and may function in endocytic compartments. Finally, delivery of ABCA1 to lysosomes for degradation may serve as a mechanism to modulate the surface expression of ABCA1 and hence cellular lipid efflux. Thus, the sorting of endocytosed ABCA1 into either the recycling or the lysosomal degradation pathways may serve to modulate cellular sterol efflux in a manner that may be amenable to pharmacological intervention.

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Cellular Localization and Trafficking of the Human ABCA1 Transporter

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