Rme-1 regulates the recycling of the cystic fibrosis transmembrane conductance regulator

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Picciano, John A., Nadia Ameen, Barth D. Grant, and Neil A. Bradbury. Rme-1 regulates the recycling of the cystic fibrosis transmembrane conductance regulator. Am J Physiol Cell Physiol 285: C1009–C1018, 2003. First published July 2, 2003; 10.1152/ajpcell.00140.2003.—Endocytic motifs in the carboxyl terminus of cystic fibrosis transmembrane conductance regulator (CFTR) direct internalization from the plasma membrane by clathrin-mediated endocytosis. However, the fate of such internalized CFTR has remained unknown. Internalized membrane proteins can be either targeted for degradation or recycled back to the plasma membrane. Using cell surface biotinylation and antibody uptake studies, we show that CFTR undergoes constitutive endocytosis and recycling back to the plasma membrane. Expression of dominant negative Rme-1 (a protein that regulates exit from the endosomal recycling compartment in CFTR-expressing cells results in the expansion of recycling compartments. Transferin, a marker for the endosomal recycling compartment, and CFTR accumulate in these enlarged recycling endosomes. Such accumulation leads to a loss of cell surface CFTR because it is prevented from being recycled back to the cell surface. In contrast, traffic of the low-density lipoprotein (LDL) is unaffected by the expression of dominant negative Rme-1. In addition, chimeras containing the extracellular domain of the transferrin receptor and the carboxyl terminal tail of CFTR also enter Rme-1-regulated recycling compartments and accumulate in these compartments containing dominant negative Rme-1, suggesting that in addition to endocytic signals, the carboxyl terminal tail of CFTR also contains intracellular traffic information.

Cystic fibrosis transmembrane conductance regulator; Rme-1; endocytosis; recycling; channel

Cystic Fibrosis (CF), a common lethal genetic disease of Caucasians, results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ABC superfamily of transport proteins (28). CFTR is a cAMP-activated anion channel and channel regulator that functions at the apical membrane of polarized epithelia to regulate secretion in the respiratory and gastrointestinal tracts (37). Previous studies have shown that CFTR is not a stable component of the plasma membrane but is rather efficiently endocytosed from the cell surface (18, 27, 35) through clathrin-coated pits (4, 5). Furthermore, such entry of CFTR into the clathrin-dependent endocytic pathway is mediated by binding of a tyrosine-based endocytic motif in the carboxyl terminus of CFTR (27, 35) to the medium subunit of the AP2 clathrin adaptor complex (36). In addition to endocytic motifs, CFTR also interacts with PDZ-domain containing proteins (29, 32) and AMP kinase (10) at its carboxyl terminus and syntaxin at its amino terminus (24, 25). How all these interactions modulate cell surface expression of CFTR is not clear (2, 22), although morphological, biochemical, and functional data indicate that CFTR is present in cell surface and intracellular compartments in both heterologous expression systems and epithelial cells that natively express CFTR (1, 3, 33). Although much is now known about the mechanisms of CFTR internalization, the fate of internalized CFTR is less well documented. Previous studies have suggested that internalized CFTR is recycled (31), but the pathway and compartments through which CFTR traverses undergo recycling are not known.

Endocytosed material is directed initially to a sorting endosomal compartment from which it is directed to one of two destinations. Some molecules such as the low-density lipoprotein (LDL) are retained in the sorting endosome, which matures into and/or fuses with late endosomes (6). Molecules that are destined to be recycled back to the cell surface are initially targeted to the endosomal recycling compartment (ERC) (9, 23). While the ERC has been extensively characterized on the basis of studies of transferrin receptor recycling (39) and insulin-regulated recycling of GLUT-4 (14), there are no data concerning the entry of ion channels, such as CFTR, into this compartment for recycling back to the cell surface. Rme-1 is an epsin homology (EH) domain containing protein (8) recently identified as a key element in controlling exit from the ERC (15). A dominant negative Rme-1 (Rme-1 G429R), when expressed in mammalian cells, has no effect on the endocytosis of integral membrane proteins such as the transferrin receptor but dramatically inhibits the recycling of the transferrin receptor back to the cell surface.
surface, trapping the receptor in the ERC (15). Endocytosed proteins such as LDL, which are targeted to lysosomes and do not enter the ERC, are unaffected. We examined the fate of endocytosed CFTR by using dominant negative Rme-1 as a tool for probing transport of CFTR through the ERC.

METHODS AND MATERIALS

Plasmids. The transferrin receptor/CFTR chimera, in which the entire cytoplasmic domain of the transferrin receptor was replaced with amino acids 1,391–1,476 of CFTR, was a generous gift from Dr. Jim Collawn (University of Alabama at Birmingham) (27). TR/CFTR was subcloned into the mammalian expression vector pCMV4 (Stratagene) using ClaI, and orientation was confirmed by sequence analysis.

Cell culture. Isogenic 293 cells stably expressing wild-type CFTR were generated as previously described (30) and maintained in DMEM supplemented with 10% FBS and hygromycin (150 μg/ml). Two-hundred ninety-three cells expressing CFTR were transfected transiently by LipofectAMINE (GIBCO BRL) at 60% confluence and analyzed after 48–72 h. TRvb1, a Chinese hamster ovarian (CHO) cell line that fails to express transferrin receptors, was generously provided by Dr. Tim McGraw (Cornell University, Ithaca, NY) (20). TRvb1 cells were transiently transfected with a transferrin receptor/CFTR chimera using LipofectAMINE (GIBCO BRL) and 24 h later with G429R green fluorescent protein (GFP)-tagged Rme-1. Experiments were performed 48 h later.

CFTR recycling assay. To monitor recycling of CFTR, we used a modified cell surface biotinylation assay (7, 31, 34). Briefly, plasma membranes were biotinylated at 4°C using a cleavable reagent (Sulpho-NHS-SS-biotin; Pierce, Rockford, IL) and then warmed up to 37°C to load endocytic vesicles with biotinylated proteins (including CFTR). Remaining cell surface biotin was removed using MESNA as previously described (34). Subsequently, the cells were either lyzed or rewarmed to 37°C to allow internalized biotinylated CFTR to recycle to the cell surface. The cells were again cooled to 4°C, the disulfide bonds on recycled cell surface proteins were reduced with MESNA, and the cells were lyzed. Biotinylated proteins were isolated by streptavidin-agarose beads, eluted into SDS sample buffer, and resolved on a 7.5% SDS-PAGE gel. Biotinylated CFTR was detected by Western blot analysis as described above.

Fluorescent labeling and indirect immunofluorescence. CFTR-expressing 293 cells were grown on polylysine-coated glass coverslips. Two to three days posttransfection with or without GFP-tagged dominant negative Rme-1, cells were rinsed in ice-cold PBS supplemented with 2 mM CaCl2 and 1 mM MgCl2 (PBS-CM). Cells were fixed in 2% paraformaldehyde/0.1% Triton X-100 and exposed to antibodies against CFTR (M3A7 and L12B4; Upstate Waltham). Antibody binding was visualized by using Cy3-conjugated goat anti-mouse secondary antibodies (Molecular Probes, Eugene, OR). Antibodies against Rme were a generous gift from Dr. M. A. Robinson (University of Cambridge, Cambridge, UK). To label cells with transferrin, cells were incubated with 10 μg/ml Cy3-transferrin. DiLDL (LDL labeled with 3′,3′-diodoacetyldichlorocyanine) was from Molecular Probes. Confocal images were obtained on a Zeiss 510 META confocal scanning microscope.

Cryoimmunoelectron microscopy. Staining for CFTR was performed essentially as described (1). Briefly, 293 cells were fixed in 2% paraformaldehyde in PBS for 2 h at room temperature, pelleted, and then embedded in 3% gelatin and placed on ice for 10 min. The pellet was infused with 2.3 M sucrose in 0.1 M PBS overnight at 4°C. The pellet was frozen onto ultracyotome stubs under liquid nitrogen and stored in liquid nitrogen until use. Ultra thin sections (70–100 nm) were cut by using a Reichert Ultracut U ultramicrotome with a FC4S cryoattachment. Sections were picked up onto a drop of 2.3 M sucrose and mounted on Formvar-coated copper grids. Briefly, sections were washed with PBS and then with nonspecific binding blocked with PBS containing 0.5% bovine serum albumin (BSA) and 0.15% glycine (in PBS containing 0.5% BSA), followed by a 30-min incubation with 5% normal goat serum. Sections were incubated in primary antibodies (1:50) for 1 h and labeled with colloidal gold (1:25) at room temperature for 1 h (Amersham, Piscataway, NJ). Sections were washed in PBS containing 0.5% BSA and PBS and fixed with 2.5% glutaraldehyde. Sections were poststained in 4% uranyl acetate and embedded in 1.25% methyl cellulose. Labeling was observed on a JEOL JEM 1210 electron microscope (Peabody) at 80 kV.

Electrophoresis and immunoblotting. Cells were washed with ice-cold PBS and lysed in TGH buffer (1% vol/vol Triton X-100, 10% vol/vol glycerol, 25 mM HEPES-Na [pH 7.4]) containing protease inhibitors (Complete EDTA-free protease inhibitors; Roche) for 20 min on ice. Nuclei and unbroken cells were removed by centrifugation (at 15,000 g for 5 min at 4°C). Soluble proteins were resolved on a 7.5% SDS-PAGE gel and transferred to a nitrocellulose membrane. After block, membranes were probed with antibodies (CFTR; M3A7 Upstate, transferrin receptor; BD Pharmingen) and visualized by enhanced chemiluminescence (Pico West) as described (36).

 Immunomagnetic isolation of CFTR containing vesicles. CFTR-expressing 293 cells transfected with G429R Rme-1 were washed in PBS and homogenized by passing the cells 20 times through a 25-gauge needle in buffer (250 mM sucrose containing 50 mM Na-HEPES [pH 7.4] and protease inhibitors). Postnuclear supernatants were obtained by centrifugation and incubated overnight at 4°C with 1 μg/ml each of M3A7 and LB12B4 anti-CFTR antibodies. Washed paramagnetic beads (25 μl) coated with sheep anti-mouse antibodies (Dynal Biotech) were added for a further 90-min incubation at 4°C. Vesicles were washed and isolated using standard magnetic separation protocols and subjected to immunoblot analysis by using antibodies described in the text.

RESULTS

CFTR undergoes constitutive recycling. To investigate endocytic recycling of CFTR, we initially confirmed that CFTR undergoes constitutive internalization. As shown in Fig. 1A, we observed a linear increase in the endocytic internalization of CFTR between 0 and 8 min. After 6–8 min, the amount of apparent CFTR internalization did not increase because CFTR is rapidly recycled back to the cell surface (Fig. 1B). We observed a linear increase in endocytic recycling of CFTR back to the cell surface between 0 and 5 min. Thereafter, the amount of apparent CFTR recycling back to the cell surface failed to increase, presumably due to the rapid reinternalization of CFTR from the plasma membrane (Fig. 1A).

Rme-1 identifies a recycling compartment in 293 cells. To characterize further the endocytic recycling of CFTR, we investigated whether internalized CFTR entered compartments whose turnover was regulated by Rme-1. Anti-Rme-1 antibodies labeled three struc-
AND METHODS. Results are expressed as means and recycling (B) under- goes constitutive endocytosis and recycling. Endocytosis (A) and recycling (B) of CFTR were monitored as described in MATERIALS AND METHODS. Results are expressed as means ± SE of at least 3 separate experiments.

Fig. 1. Cystic fibrosis transmembrane conductance regulator (CFTR) undergoes constitutive endocytosis and recycling. Endocytosis (A) and recycling (B) of CFTR were monitored as described in MATERIALS AND METHODS. Results are expressed as means ± SE of at least 3 separate experiments.


cells for 60 min at 37°C in the continued presence of Cy3-labeled transferrin. In untransfected cells (Fig. 3a), transferrin-positive compartments were seen as discrete perinuclear vesicles throughout the cytoplasm of the cell. When experiments were performed using cells transiently transfected with G429R Rme-1, transferrin was observed in large perinuclear compartments (Fig. 3c). Such compartments colocalized with GFP-tagged G429R Rme-1 (Fig. 3, c and d), consistent with the effects of G429R Rme-1 on affecting exit but not entry into the ERC. Nontransfected cells incubated under similar conditions with DiLDL showed uptake of labeled LDL into large cytoplasmic vesicles (Fig. 3e), presumably late endosomes/lysosomes. Transient expression of G429R Rme-1 (Fig. 3, g and h) did not affect the localization of DiLDL (Fig. 3, f and h), which showed little or no colocalization with GFP-tagged G429R Rme-1 (Fig. 3h). These results are consistent with the premise that G429R Rme-1 has no effect on endocytic uptake nor on traffic to late endosomes/lysosomes but only affects exit from recycling compartments.

CFTR is targeted to Rme-1-positive compartments. Having shown that internalized CFTR recycles efficiently back to the plasma membrane, we determined whether such recycling involved trafficking of CFTR through the ERC in a similar fashion to such molecules as transferrin. In untransfected cells, CFTR was seen associated with the plasma membrane (Fig. 4a), consistent with previously published data. However, upon expression of G429R Rme-1, plasma membrane-associated staining for CFTR was markedly decreased, with a concomitant increase in intracellular CFTR localization (Fig. 4, b and d). Moreover, such intracellular CFTR was found to colocalize mostly with the recycling compartment as determined by colocalization with Rme-1 (Fig. 4, c and d). Neighboring cells that failed to take up plasmid for G429R Rme-1 retained cell surface staining for CFTR.

To confirm that CFTR was indeed trapped in the ERC in the presence of G429R Rme-1, we performed immunogold electron microscopy on cells expressing CFTR. Such studies revealed the presence of both transferrin receptor [a marker for the ERC (Fig. 5)] and CFTR within the same vesicle. CFTR also colocalized with gold-labeled anti-GFP antibodies (data not shown).

To confirm the redistribution of CFTR from a cell surface location to an intracellular compartment, cell surface biotinylation experiments were performed. The presence of band C CFTR (Fig. 6, A and B) was seen in untransfected cells, cells transfected with wild-type Rme-1, and cells transfected with G429R Rme-1, arguing against a role for Rme-1 in the biosynthetic pathway. While there was a slight reduction in the amount of cell surface CFTR in the presence of wild-type Rme-1, the expression of G429R Rme-1 caused a marked reduction in the amount of cell surface CFTR (Fig. 6, A and B), consistent with a redirection of CFTR to intracellular compartments. The reduction in cell surface CFTR in the presence of G429R Rme-1 was not.

Expression of G429R Rme-1 slows transferrin recycling. To confirm that G429R Rme-1 inhibited exit from endosomal recycling compartments in 293 cells, we transiently expressed GFP-tagged G429R Rme-1 in cells stably expressing wild-type CFTR. Endosomal recycling compartments were identified by incubating
Fig. 2. Distribution of endogenous Rme-1 in 293 cells. Cells were fixed and stained with polyclonal antibodies against Rme-1 (followed by secondary antibodies conjugated to Alexa 488) and monoclonal antibodies against endoplasmic reticulum (ER) markers (Bip) (a–c), Golgi markers (Golgin 97) (d–f), lysosomal markers (Lamp-1) (g–i), and early endosome markers (EEA1) (j–l) (followed by secondary antibodies conjugated to Cy3). Mitochondria were initially labeled in live cells using Mito-tracker (Molecular Probes), followed by fixation and staining for Rme-1 (m–o).

Fig. 3. Expression of G429R Rme-1 affects the subcellular localization of transferrin but not low-density lipoprotein (LDL). Cells stably expressing wild-type Rme-1 were incubated for 1 h in the continued presence of Cy3-labeled transferrin (a) or LDL labeled with 3,3'-di-octadecyldiacarbocyanine (DiLDL) (e). Expression of G429R Rme-1 caused a redistribution of transferrin to large cytosolic vesicles (b) that colocalized with GFP-tagged G429R Rme-1 (c). The merge of the transferrin and G429R signal shows almost complete overlap (d). Expression of G429R Rme-1 did not affect the delivery of DiLDL to late endosomes and lysosomes (f), and no colocalization between GFP-tagged Rme-1 (g) and DiLDL was seen [as shown by lack of colocalization in the merge (h)].
due to a global reduction in CFTR expression because the amount of total CFTR in cells expressing G429R Rme-1 was not significantly lower than that in untransfected cells (Fig. 6, C and D).

**Immunomagnetic isolation of CFTR containing vesicles.** Immunofluorescence microscopy revealed the presence of CFTR trapped in the ERC in the presence of G429R Rme-1. As an alternative strategy to show that G429R Rme-1 causes “trapping” of CFTR in the ERC, vesicles from CFTR/293 cells transfected with G429R Rme-1 were subject to immunomagnetic isolation by using anti-CFTR antibodies (Fig. 7). Because CFTR in ERC should be oriented such that the cytosolic domains of CFTR are facing the outside of the vesicle, we immunoprecipitated CFTR-containing vesicles from cells by using antibodies directed against the amino and carboxyl termini of CFTR. Immunoblot analysis of such isolated vesicles revealed the presence of band C CFTR. Rme-1 and transferrin receptor were also identified within CFTR-containing vesicles. In contrast, markers for early endosomes (EEA1) and lysosomes (Lamp1) showed very little signal.

**The carboxyl terminal domain of CFTR contains ERC-targeting information.** To show that CFTR enters the ERC from the clathrin-coated endocytic pathway, we monitored the traffic of internalized CFTR. Because the steady-state level of cell surface CFTR in the presence of G429R Rme-1 is extremely low and there are no high-affinity extracellular antibodies or ligands for CFTR, we decided to utilize a CFTR/transferrin receptor chimera transiently coexpressed with G429R Rme-1. TRVb1 cells lacking endogenous transferrin receptors were transiently transfected with a CFTR/transferrin receptor (TfR) chimera in which the cytoplasmic domain of the transferrin receptor was replaced with the carboxyl terminal domain of CFTR. Twenty-four hours after such transfection, some cells were also transfected with G429R Rme-1. Traffic of the CFTR/TfR chimera was monitored by using Cy3-conjugated transferrin. Cells containing CFTR/TfR (Fig. 8, a and b) or CFTR/TfR and G429R Rme-1 (Fig. 8, c–f) were incubated for 1 h in the continued presence of Cy3 transferrin (Fig. 8, a and c). Cy3 transferrin was efficiently taken up in both sets of cells. After the hour pulse with labeled transferrin, cells were subject to an hour chase in the absence of labeled transferrin and 100-fold excess of unlabeled transferrin. Cells expressing CFTR/TfR alone (Fig. 8b) showed loss of cell associated signal because the transferrin was efficiently recycled back to the cell surface and released into the medium. In contrast, cells expressing CFTR/TfR and G429R Rme-1 (Fig. 8d) failed to recycle internalized transferrin back to the cell surface. The structures within which the transferrin remained colocalized with GFP-tagged G429R Rme-1 (Fig. 8, e and f).

**DISCUSSION**

It is now clearly established that signals in the carboxyl terminal domain of CFTR direct its entrance into the clathrin-mediated endocytic pathway (4, 12,
18, 27, 35, 36). Of interest are the observations that the endocytic internalization kinetics for CFTR are rapid (12, 18, 27, 34) and that internalized CFTR appears to be fully functional (5, 17). These observations suggest the possibility that internalized CFTR may be constitutively recycled back to the cell surface. Indeed, given the rapid internalization kinetics of CFTR, biosynthetic kinetics would have to be equally rapid to maintain steady-state cell surface CFTR. However, treatment of CFTR-expressing CHO cells with either cycloheximide or brefeldin A did not cause any diminution of forskolin-stimulated CFTR function up to 24 h (16), arguing that internalized CFTR may be recycled rather than degraded. Studies by Swiatecka-Urban et al. (31) have further argued that recycling of CFTR may be important in establishing its apical polarity in epithelial cells.

Although the exit of material from the ERC and its reinsertion into the plasma membrane have been thought to be a constitutive pathway, such traffic nonetheless requires cellular machinery. The role of Rme-1 in regulating aspects of the endocytic/recycling pathway has been established by using dominant negative mutants in CHO cells and C. elegans. While Rme-1 has no effect on initial clathrin-mediated endocytic events, Rme-1 function is necessary for proteins to exit the ERC and travel back to the plasma membrane. In contrast, Rme-1 plays no role in the traffic of proteins, such as LDL, to the late endosome/lysosome pathway. Although there is only one Rme family member in C. elegans, there are four related genes in humans (8, 21, 26). As yet, the tissue distribution and expression overlap of CFTR with the different Rme-1 genes are not known.

We show that internalized CFTR enters the endocytic recycling compartment on its traffic back to the cell surface in an identical fashion to the well-described marker for endosomal recycling, the transferrin receptor. Expression of dominant negative Rme-1 has no effect on endocytosis, as shown by uptake of LDL, transferrin, and CFTR/transferrin chimeras, consistent with previously published data (8, 15). In contrast to traffic of LDL to late endosomes/lysosomes, which is unaffected by dominant negative Rme-1 and shows no colocalization with GFP-tagged G429R Rme-1, trans-

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Fig. 6. Expression of Rme-1 (G429R) but not wild-type Rme-1 causes loss of cell surface CFTR. Untransfected CFTR/293 cells, cells transfected with wild-type Rme-1, or G429R Rme-1 were subjected to cell surface biotinylation as described in MATERIALS AND METHODS. After Streptavidin precipitation, subsequent immunoblot analysis revealed the presence of band C CFTR (A) in untransfected cells and in cells transfected with wild-type Rme-1 but markedly reduced in cells transfected with G429R Rme-1. Shown in B are results of densitometric scans of data shown in A. Analysis of total cell lysate CFTR in the presence or absence of G429R Rme-1 (C and D) revealed little difference in total CFTR. Results are means ± SE of 3 separate experiments.

Fig. 7. G429R Rme-1 localizes CFTR to ERC but not early endosomes or lysosomes. CFTR-containing vesicles were subjected to immunomagnetic isolation. For subsequent immunoblot analysis, the following antibodies were employed. Rme-1 (70 kDa; a gift from M. S. Robinson), transferrin receptor (TR 85 kDa; Transduction Labs/BD Pharmingen), CFTR (180 kDa; Upstate Biotech), EEA1 (180 kDa), and Lamp-1 (Transduction Labs/BD Pharmingen).
ferrin (a marker for the recycling compartment) shows almost complete overlap with GFP-tagged G429R Rme-1. CFTR localization in dominant negative Rme-1-expressing cells shows overlap with both transferrin and GFP, arguing that internalized CFTR enters the same recycling compartment that transferrin has access to. The observation that expression of dominant negative Rme-1 caused a marked redistribution of CFTR away from the plasma membrane to being trapped in the ERC argues for a rapid endocytic rate for CFTR. Indeed, several groups have shown that CFTR is efficiently endocytosed from the cell surface (12, 18, 27, 34). Moreover, the redistribution of CFTR in the presence of G429R Rme-1 argues that maintenance of steady-state cell surface levels of CFTR is primarily due to recycling of internalized CFTR rather than insertion of newly synthesized CFTR. The lack of high-affinity antibodies to extracellular domains of CFTR, and the low level of expression of cell surface CFTR in the presence of G429R Rme-1, precluded monitoring of CFTR from the cell surface to the ERC. To confirm that entry of CFTR into the ERC was from the endocytic pathway, we monitored access of transferrin receptor/CFTR chimeras to the ERC. Transferrin receptor constructs that lack a cytoplasmic tail are internalized inefficiently (13, 27). Addition of the carboxyl tail of CFTR to tail-less transferrin receptors restores efficient internalization of the receptor and labeled transferrin reporters (27). Transfection of cells with transferrin receptor/CFTR chimeras and dominant negative Rme-1 shows that labeled transferrin is trapped within the ERC, arguing that entry into the ERC is from the endocytic rather than the biosynthetic pathway. Moreover, it argues that the carboxyl tail of CFTR does not contain any signals that direct trafficking of CFTR away from recycling pathways to lysosomal degradative compartments. While many endocytosed membrane proteins are thought to recycle to the cell.
surface via a juxtanuclear recycling compartment (23), this is not true for all proteins. For example, Kir channels, a potassium channel family involved in regulating membrane excitability (38), do not recycle to the cell surface through Rme-1-regulated compartments (19). Thus it is clear that different ion channels can traffic and recycle through diverse compartments.

Many laboratories, both commercial and academic, are currently attempting to acquire pharmacological compounds to increase the efficiency with which mutant CFTR molecules fold, exit the endoplasmic reticulum, and get inserted into the plasma membrane. The observation that even wild-type CFTR is rapidly removed from the cell surface argues for the necessity to understand recycling processes with the potential to increase the rate at which CFTR exits the recycling compartment and gets reinserted back into the plasma membrane. This is particularly true for AF508 CFTR, the most common mutation in CFTR, which even when “corrected” by growth of cells at reduced temperatures has a significantly shorter half-life in the plasma membrane than wild-type CFTR (11). Thus a clear understanding of the molecular events governing the recycling of CFTR will provide insight into ways to augment the overall residence time of pharmacologically corrected mutant CFTR molecules at the cell surface.

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DISCLOSURES

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