

Cystic Fibrosis Transmembrane Conductance Regulator Trafficking Is Mediated by the COPI Coat in Epithelial Cells*

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Cystic fibrosis (CF) is caused by defects in the CF transmembrane conductance regulator (CFTR) that functions as a chloride channel in epithelial cells. The most common cause of CF is the abnormal trafficking of CFTR mutants. Therefore, understanding the cellular machineries that transit CFTR from the endoplasmic reticulum to the plasma membrane (PM) is important. The coat protein complex I (COPI) has been implicated in the anterograde and retrograde transport of proteins and lipids between the endoplasmic reticulum and the Golgi. Here, we investigated the role of COPI in CFTR trafficking. Blocking COPI recruitment to membranes by expressing an inactive form of the GBF1 guanine nucleotide exchange factor for ADP-ribosylation factor inhibits CFTR trafficking to the PM. Similarly, inhibiting COPI dissociation from membranes by expressing a constitutively active ADP-ribosylation factor 1 mutant arrests CFTR within disrupted Golgi elements. To definitively explore the relationship between COPI and CFTR in epithelial cells, we depleted β -COP from the human colonic epithelial cell HT-29Cl.19A using small interfering RNA. β -COP depletion did not affect CFTR synthesis but impaired its trafficking to the PM. The arrest occurred pre-Golgi as shown by reduced level of glycosylation. Importantly, decreased trafficking of CFTR had a functional consequence as cells depleted of β -COP showed decreased cAMP-activated chloride currents. To explore the mechanism of COPI action in CFTR traffic we tested whether CFTR was COPI cargo. We discovered that the α -, β -, and γ -subunits of COPI co-immunoprecipitated with CFTR. Our results indicate that the COPI complex plays a critical role in CFTR trafficking to the PM.

Cystic fibrosis (CF)² is caused by inherited mutations in the gene encoding the cystic fibrosis transmembrane conductance

regulator (CFTR), a cAMP-regulated chloride channel expressed in epithelial cells (1). Ten million Americans carry a defective *cftr* gene. The most prevalent mutation causing cystic fibrosis, $\Delta F508$, is due to misfolding of the CFTR protein. Misfolded CFTR displays an abnormal trafficking, and consequently the CFTR protein is retained in the endoplasmic reticulum (ER) and is absent from the plasma membrane (PM) of epithelial cells. Therefore, understanding the trafficking pathway of CFTR from the ER to the PM is important. Recent studies suggested that CFTR processing is independent of components involved in conventional trafficking (2). It was reported that in some cells like baby hamster kidney cells, CFTR follows a non-conventional trafficking pathway where CFTR is transported from the ER to late Golgi/endosomal compartments before recycling back to Golgi compartments for oligosaccharide processing (3). Earlier studies show that the COPII machinery is required for CFTR to be exported out of the ER (4, 5). A di-acidic exit code is present in the CFTR molecule that interacts with the Sec23-Sec24 cargo selection complex present in COPII vesicles (5). However, the trafficking pathway of CFTR beyond the ER exit sites remains poorly explored.

In addition to COPII, the coat protein complex I (COPI) is implicated in secretory traffic. COPI mediates retrograde transport that selectively recycles membrane and soluble proteins as well as lipids from the *cis*-Golgi complex to the ER (6). Additionally, COPI coat proteins have multiple functions in intra-Golgi trafficking and in maintaining the normal structure of the mammalian interphase Golgi complex (7). COPI vesicles are also involved in the anterograde transport of proteins (8). The observation that COPI vesicles participate in several distinct transport directions has long been difficult to rationalize. The recent discovery of different populations of COPI-coated vesicles strongly suggests that COPI vesicles have different functions (9–11). COPI coats are comprised of heptameric coatomers, each containing seven subunits (α -, (140 kDa), β -, (110 kDa), β' -, (102 kDa), γ -, (98 kDa), δ -, (61 kDa), ϵ -, (36 kDa), and ξ -COP (20 kDa)) that bind reversibly to membranes (12). Each heptamer contains two distinct subcomplexes, the F-COPI subcomplex (β , γ , δ , ξ) and the B-COPI subcomplex (α , β' , ϵ) (13, 14). COPI is recruited to membrane by a small GTPase of the Ras superfamily, the ADP-ribosylation factor (ARF1). ARF1 cycles between an inactive GDP-bound state and an active GTP-bound state (15). Two families of proteins regulate the activation status of ARFs. ARFs are activated by a GDP/GTP exchange catalyzed by guanine nucleotide exchange fac-

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² The abbreviations used are: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; ARF1, ADP-ribosylation factor 1; COPI, coat protein complex I; ER, endoplasmic reticulum; GBF1, Golgi brefeldin A-resistant factor; PM, plasma membrane; siRNA, small interfering RNA; WT, wild-type; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary.

tors. ARFs are deactivated by GTP hydrolysis facilitated by GTPase-activating proteins. Vesicle formation requires ARF in the active GTP-bound form and is therefore dependent on the activity of guanine nucleotide exchange factors. Vesicle disassembly is dependent on the hydrolysis of GTP on ARF and requires active GTPase-activating proteins.

Yoo *et al.* (3) found that in non-epithelial cells, CFTR trafficking could be COPI-dependent (*i.e.* HeLa and human embryonic kidney 293 cells) or COPI-independent (*i.e.* baby hamster kidney and Chinese hamster ovary (CHO) cells). In animals, CFTR is mostly expressed in epithelial cells, and the intracellular trafficking of CFTR might differ between epithelial and non-epithelial cells. In this regard, endogenous CFTR is processed very efficiently in epithelial cells, whereas its processing is inefficient in transfected cells (16).

This study addresses the role of COPI complex in the trafficking of CFTR. We mostly focus on the human colonic epithelial cells, HT-29Cl.19A, that endogenously express CFTR. We now report that transfection of dominant negative guanine nucleotide exchange factor and ARF mutants that interfere with the conventional trafficking of proteins between the ER and the Golgi prevented CFTR from reaching the PM. In addition, down-regulation of COPI subunit β -COP expression in HT29Cl.19A cells by siRNA (1) impaired CFTR trafficking to the PM and (2) reduced cAMP-activated chloride currents. Finally, we show that COPI subunits co-immunoprecipitate with CFTR, suggesting that these molecules are in a complex. Taken together, these observations implicate COPI vesicles in CFTR trafficking in epithelial cells.

EXPERIMENTAL PROCEDURES

Tissue Culture and Transient Transfection—The human adenocarcinoma cell line HT-29Cl.19A, HeLa, and the Cos-7 cells were grown at 37 °C under a 5% CO₂ atmosphere in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) bovine growth serum and containing penicillin/streptomycin. The colonic epithelial cells were transfected with 10 nM siRNA to β -COP (Ambion) or control siRNA that have limited sequence similarity to known genes (Ambion) with Lipofectamine 2000 (Invitrogen) following the manufacturer's instruction. Cos-7 cells were co-transfected with WT-CFTR cDNA (8 μ g/10-cm dish) and siRNA to β -COP (Ambion) or control siRNA (Ambion) using Lipofectamine 2000 (Invitrogen) following the manufacturer's instruction.

Immunoprecipitation and Immunoblotting—The cells were lysed in phosphate-buffered saline (PBS)-1% Triton X-100 containing a mixture of protease inhibitors (Roche Applied Science). The lysates were subjected to immunoprecipitation using C-CFTR monoclonal antibody (mAb 24-1; R&D Systems) or β -COP monoclonal antibody (clone maD; Sigma) cross-linked on A/G-agarose beads in the presence of 1% bovine serum albumin. After extensive washings, the bound proteins were analyzed by Western blot after transfer to polyvinylidene difluoride membranes (Bio-Rad). After blocking with 5% dry milk, the primary antibody was added at the required dilution (1/2,000 for C-CFTR, R&D Systems; 1/2,000 for β -COP, Sigma; 1/2,000 for α -COP, Affinity BioReagents; 1/500 for γ -COP, Santa Cruz Biotechnology; 1/2,000 for SNAP-23 produced for

us by Genemed Synthesis; and 1/2,000 for Na⁺/K⁺-ATPase, Santa Cruz Biotechnologies). The secondary antibodies horseradish peroxidase-conjugated (Pierce) were used at 1/10,000. The signal was detected using West Pico (Pierce).

Immunofluorescence—HeLa cells were co-transfected with untagged wild-type CFTR and either Myc-tagged inactive GBF1/E794K mutant, hemagglutinin-tagged wild-type ARF1, or hemagglutinin-tagged constitutively active Arf1/Q71L mutant. After 24 h, cells were fixed with 3% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 in PBS for 10 min, washed with PBS three times for 2 min, and blocked in 0.4% fish skin gelatin for 5 min. Cells were then incubated with anti-NBD1 polyclonal antibodies (to detect CFTR, provided by Dr. Bebek, University of Alabama at Birmingham) and either monoclonal anti-hemagglutinin antibodies (to detect WT-ARF1 and ARF1/Q71L) purchased from Santa Cruz Biotechnology or monoclonal anti-Myc antibodies (to detect WT-GBF and GBF1/E794K) purchased from Invitrogen or monoclonal anti-GM130 purchased from BD Transduction Laboratories.

Transepithelial Short Circuit Currents—HT-29Cl.19A cells were cultured on permeable membranes (Transwell; Costar). Three days after transfection the filters were mounted in Ussing chambers. Short circuit current (*I*_{sc}) measurements were done as previously described (37). Briefly, Ringer's buffer (115 mM NaCl, 25 mM NaHCO₃, 2.4 KH₂PO₄, 1.24 K₂HPO₄, 1.2 CaCl₂, 1.2 mM MgCl₂, and 10 mM D-glucose, pH 7.4) was added in the basal compartment of the Ussing chamber, and low chloride (1.2 mM NaCl and 115 mM sodium gluconate replacing 115 mM NaCl) was added to the apical chamber. Bath solutions were vigorously stirred and gassed with 5% CO₂. Solutions and chambers were maintained at 37 °C. Forskolin (20 μ M) was added to both sides of the monolayer to evaluate cAMP-stimulated currents. Glibenclamide (200 μ M) was added to the apical side at the end of the experiment to block CFTR channels.

Cell Surface Biotinylation—The Cos-7 cells were rinsed with PBS to eliminate the proteins present in the medium. Because the biotinylation reagent cross-links primarily through lysine residues, residual serum will inhibit cell surface biotinylation. Cell surface proteins were labeled with EZ-Link NHS-SS Biotin (Pierce) for 30 min at 4 °C. Following biotinylation, cells were rinsed with PBS-bovine serum albumin for 10 min at 4 °C to quench any residual NHS-SS biotin. At the end of the experiments, the cells were lysed with PBS-1% Triton X-100 and protease inhibitors (Roche Applied Science). Biotinylated proteins were incubated with streptavidin beads and subjected to Western blot. Biotinylated CFTR was detected using a CFTR monoclonal antibody (mAb 24-1; R&D Systems).

Metabolic Pulse-Chase Labeling of CFTR—After washing in PBS, HT-29Cl.19A cells expressing CFTR were incubated in Dulbecco's modified Eagle's medium without methionine and cysteine for 60 min at 37 °C. Subsequently, the cells were metabolically labeled in the same medium with 0.2 mCi/ml [³⁵S]cysteine and [³⁵S]methionine for 60 min at 37 °C. Then the cells were incubated in Dulbecco's modified Eagle's medium containing 10% bovine growth serum and 10 mM methionine and cysteine. Labeled proteins were chased for the indicated

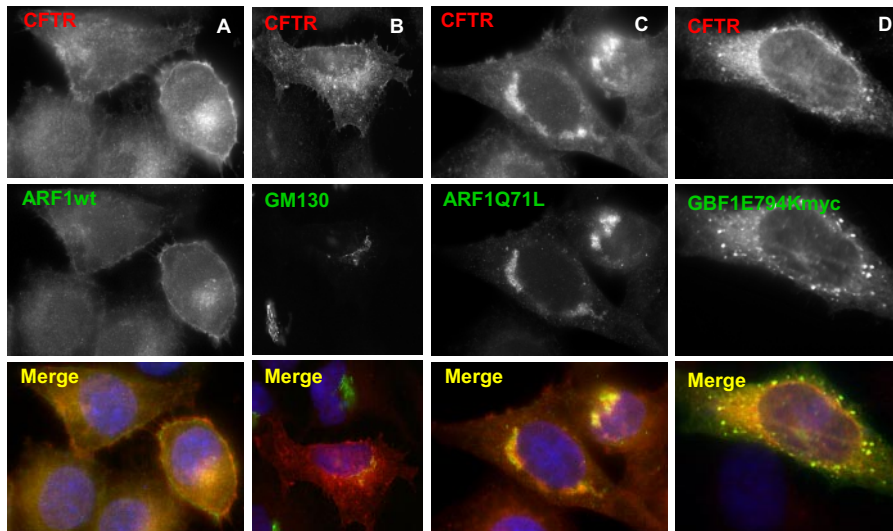


FIGURE 1. Inhibiting COPI function blocks CFTR traffic. HeLa cells were co-transfected with green fluorescent protein-tagged wild-type CFTR and either Myc-tagged GBF1/E794K inactive mutant that causes COPI dissociation from membranes, wild-type ARF1, or the Arf1/Q71L mutant that stabilizes COPI on membranes. After 24 h, cells were processed for immunofluorescence with anti-Myc antibodies or GM130 antibody, a Golgi marker. CFTR was visualized by green fluorescence. Expression of wild-type ARF1 has no effect on trafficking of CFTR to the PM. Expression of mutant GBF1 or ARF1 arrests CFTR traffic in pre-Golgi and Golgi compartments, respectively.

times (0, 30 min, 1 and 2 h). At the end of the experiment, the cells were lysed and subjected to immunoprecipitation as described under “Immunoprecipitation.” The radioactivity associated with CFTR was quantified using phosphorimaging (Bio-Rad).

RESULTS

Expression of Dominant Negative Mutant Proteins That Alter COPI Dynamics Inhibits CFTR Traffic—We and others showed that the COPII machinery is required for CFTR trafficking (4, 5). The COPII machinery is closely linked to COPI machinery, raising the possibility that COPI also participates in CFTR trafficking. COPI function is experimentally modulated in intact cells by regulating the levels of active ARF. Low levels of active ARF dissociate COPI from membranes. Low levels of active ARF can be achieved through expressing an inactive mutant of the ARF guanine nucleotide exchange factor GBF1 (GBF1/E794K) (17). In such cells, COPI dissociates from the membranes. High levels of active ARF stabilize COPI on membranes. Abnormally high levels of activated ARF are established by expressing the GTP-restricted mutant of ARF (ARF1/Q71L). In such cells, COPI is stabilized on the membrane.

We examined the trafficking of CFTR in HeLa cells by co-transfecting wild-type CFTR and either GBF1/E794K or ARF1/Q71L. We showed that expression of GBF1/E794K dissociates COPI from membranes and collapses the Golgi and relocates Golgi proteins to the ER or to punctate peripheral structures that contain GBF1/E794K. As shown in Fig. 1D, GBF1/E794K localizes to punctate elements dispersed throughout the cell. Importantly, CFTR co-localizes with GBF1/E794K in these puncta and is not detected on the PM. This effect is specific since expression of wild-type GBF1 does not affect CFTR trafficking (data not shown). In the absence of GBF1 mutant, CFTR is detected at the PM with some CFTR co-localizing with the

Golgi marker GM130 (Fig. 1, A and B). This suggests that CFTR trafficking requires COPI association with membranes.

Expression of ARF1/Q71L stabilizes COPI coat on membranes and exaggerates Golgi structure and the accumulation of PM-destined proteins within the enlarged Golgi. ARF1/Q71L is also stabilized on the enlarged Golgi. As shown in Fig. 1C, expression of ARF1/Q71L causes the accumulation of CFTR in globular structures containing ARF1/Q71L. This effect is specific since expression of wild-type ARF has no effect on CFTR detected on the PM (Fig. 1A). Together, our data suggest that COPI function is essential for CFTR trafficking at pre-Golgi and Golgi stages of the secretory pathway.

Down-regulation of β -COP Protein Expression in HT-29Cl.19A

Cells—To establish the role of COPI complex on CFTR biogenesis in physiologically relevant epithelial cells, the expression of β -COP protein was down-regulated using silencing RNA (siRNA) in the colonic epithelial cells HT-29Cl.19A. The human colonic epithelial cells HT-29Cl.19A, which express endogenous wild-type CFTR, represent a good model to study transport in epithelial cells (18). Three siRNAs (10 nM) targeting different exons of β -COP mRNA and control siRNA that has limited sequence similarity to known genes were transfected into HT-29Cl.19A cells. Seventy-two hours later, the expression of the β -COP protein was decreased by 70–90% compared with control siRNA (Fig. 2A). The silencing is specific because no decrease in the expression of the membrane-associated protein SNAP-23 was observed after introduction of the anti- β -COP siRNA. A time course of β -COP silencing revealed that the strongest inhibition in β -COP protein synthesis occurred between 48 and 72 h (Fig. 2B). The expression of β -COP protein increased after 96 h, demonstrating the transient effect of the siRNAs. The subsequent experiments were performed 72 h after transfection using siRNA targeting exons 3 and 4 of β -COP.

Down-regulation of β -COP Protein Expression Alters CFTR Trafficking—Our studies in HeLa cells expressing GBF1 and ARF1 mutants suggested that CFTR trafficking required COPI function. However, other studies suggest that CFTR follows a non-conventional pathway, possibly bypassing COPI-mediated traffic (3). It was therefore important to carefully address the question of whether or not COPI was involved in CFTR trafficking in epithelial cells. For this purpose, we investigated the effect of the down-regulation of β -COP expression on CFTR trafficking in HT-29Cl.19A cells. As shown in Fig. 3A, depleting β -COP for 72 h resulted in a $51.8 \pm 13.6\%$ decrease in mature (Band C) CFTR and a 3-fold increase in immature (Band B) CFTR. The fact that $\sim 50\%$ of mature CFTR could still be

CFTR Trafficking by COPI Coat

detected is probably due to the long half-life (~48 h) of CFTR at the plasma membrane (19, 20). Importantly, under the same conditions of β -COP depletion, the steady-state expression of the PM protein Na^+/K^+ -ATPase was not affected (Fig. 3C). Cargo-specific functions for the COPI coat have been described

previously in yeast (21–23). Importantly, it shows that β -COP depletion does not produce general cell death.

To explore the relationship between COPI and CFTR traffic, we performed metabolic pulse-chase experiments in control and β -COP-depleted cells. In cells transfected with control siRNA, band B CFTR (immature CFTR) decreased over time and correlated with the appearance of band C representing terminal glycosylation of CFTR (mature CFTR) (Fig. 3D and data not shown). In contrast, in β -COP-depleted cells, the immature B band persisted without the mature C band. No degradation of CFTR was observed as immature (band B) CFTR was stable over a 2-h period (Fig. 3D). These results suggest that CFTR traffics to the Golgi to produce mature C band is COPI-dependent.

Down-regulation of β -COP Protein Expression Inhibits CFTR Currents in HT-29Cl.19A Cells—The functional site for CFTR is the PM. So, to monitor the physiologically relevant delivery of CFTR to the PM, we measured the cAMP-activated short circuit currents using Ussing chambers. In these experiments, HT-29Cl.19A cells were grown on filters to form a monolayer. CFTR chloride currents were activated by addition of forskolin. As observed in Fig. 4A, an increase in short circuit currents in response to forskolin was observed in control cells. At the end of the experiment, CFTR short circuit currents were inhibited by addition of the CFTR inhibitor glibenclamide. In contrast, depletion of β -COP from HT-29Cl.19A cells resulted in 60% decrease of short circuit currents (Fig. 4B). The down-regulation of β -COP protein expression did not affect the resistance, suggesting that the integrity of the monolayer was not altered (data not shown).

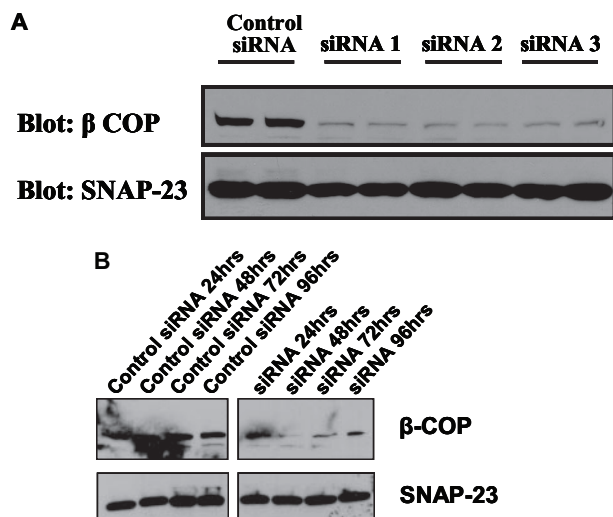


FIGURE 2. Depletion of β -COP in HT-29Cl.19A epithelial cells. A, colonic epithelial cells HT-29Cl.19A were transfected with three different anti- β -COP siRNAs. Each condition was done in duplicate. After 3 days, the cells were lysed and proteins were analyzed by immunoblotting. All three siRNAs reduced β -COP levels by 70–90%. The protein levels of SNAP-23 were unaffected. B, time course of β -COP depletion. HT-29Cl.19A cells were transfected with control or anti- β -COP siRNAs. After 24, 48, 72, and 96 h, the cells were lysed and 40 μ g of proteins were analyzed by immunoblotting. The protein levels of SNAP-23 were unaffected by siRNA to β -COP, whereas levels of β -COP decreased.

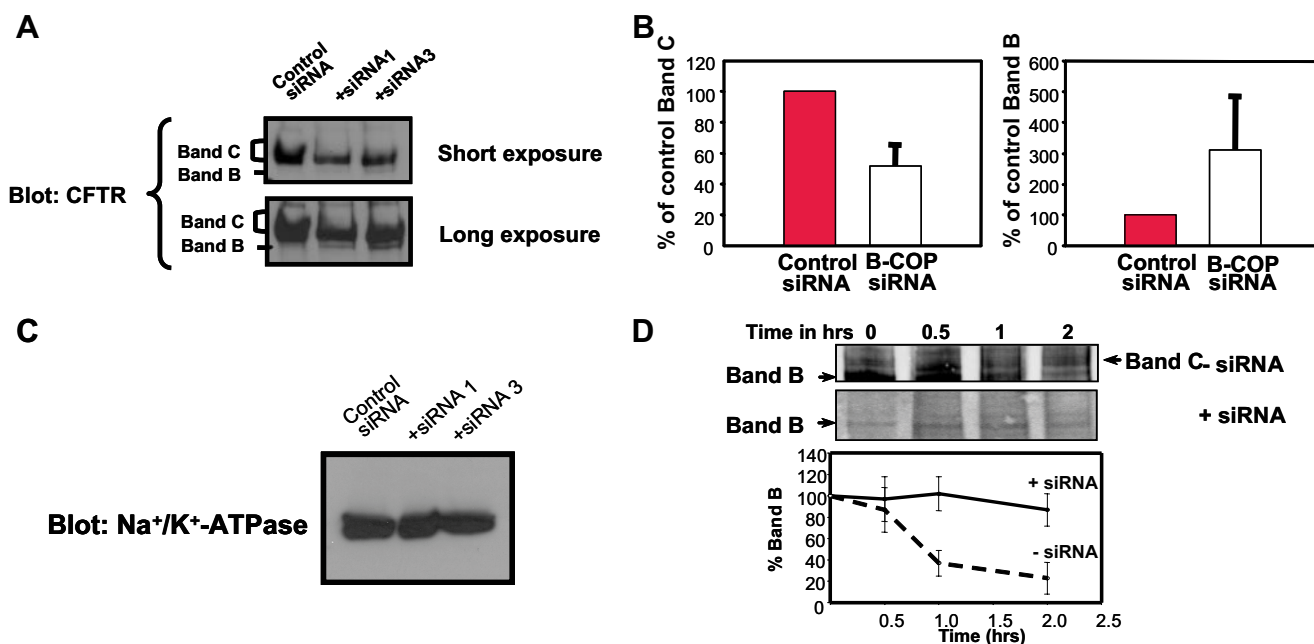


FIGURE 3. Depletion of β -COP inhibits CFTR traffic in HT-29Cl.19A cells. A, HT-29Cl.19A cells were transfected with control or anti- β -COP siRNA. After 72 h, cells were lysed and the lysates were probed for CFTR by Western blotting. Depletion of β -COP decreased the level of mature (Band C) CFTR and increased levels of immature (Band B) CFTR. B, bar graph representation of mature (Band C) and immature (Band B) CFTR after analysis of experiments analogous to those shown in panel A with siRNA 3 (average of six experiments). Values show average and S.E. C, cell lysates from panel A were Western-blotted with antibodies against the α -subunit of Na^+/K^+ -ATPase. Depletion of β -COP does not affect the Na^+/K^+ -ATPase. D, HT-29Cl.19A cells were transfected with control or anti- β -COP siRNA. After 72 h, cells were pulse-labeled with [³⁵S]Met/Cys and then chased for the indicated times. At each time, cells were lysed and CFTR was immunoprecipitated and subjected to fluorography. The levels of band B and band C were evaluated by densitometry. Depletion of β -COP decreased the processing of immature CFTR to the mature CFTR, reflecting a block in CFTR traffic at the ER-Golgi interface (average of three experiments). Values show average and S.E.

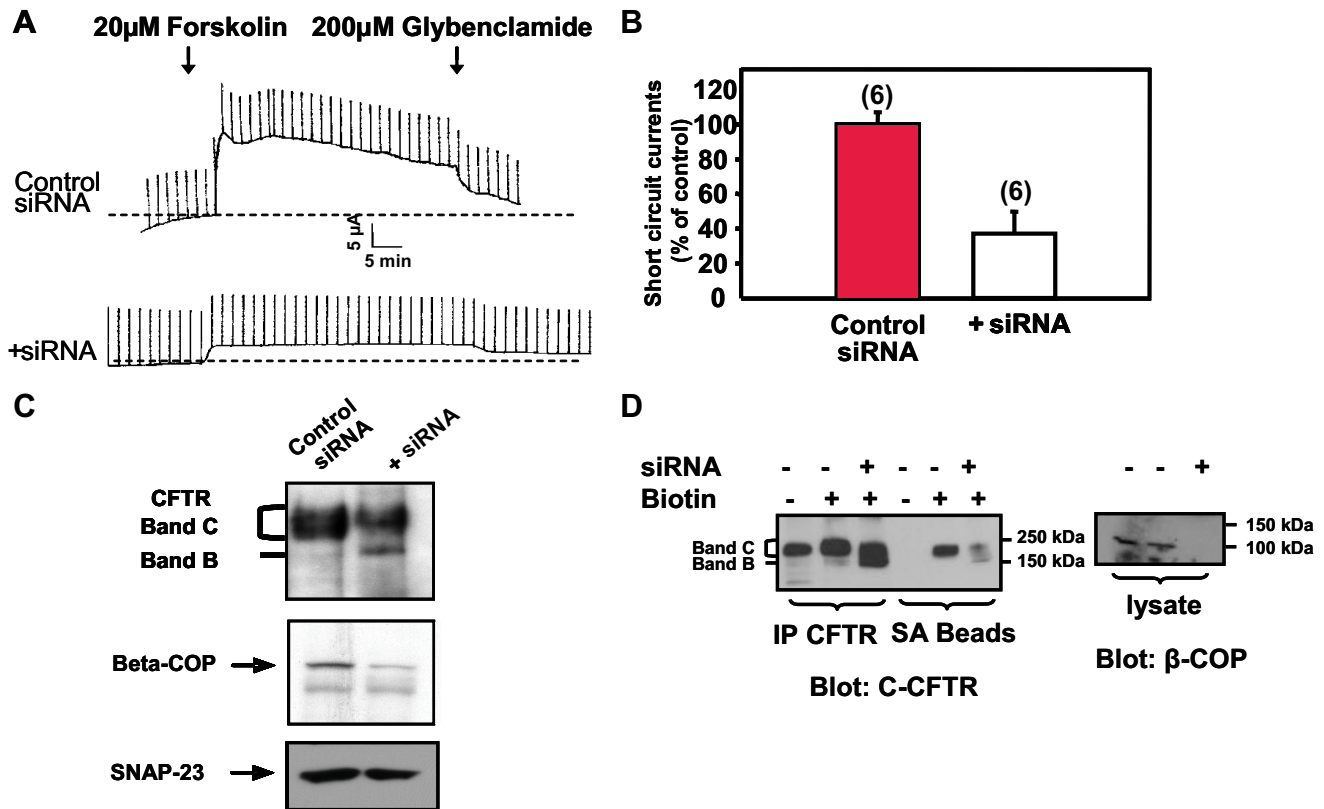


FIGURE 4. Decrease of surface delivery of CFTR in β -COP-depleted cells. *A*, HT-29Cl.19A cells were grown on filters and transfected with control or anti- β -COP siRNA. After 72 h, cAMP-stimulated short circuit currents mediated through CFTR were measured using Ussing chambers. β -COP depletion prevents CFTR delivery to the cell surface. *B*, bar graph representation of the average short circuit currents of six experiments. Values show average and S.E. *C*, Western blot analysis of CFTR, β -COP, and SNAP-23 from the cells subjected to Ussing chambers in *panel A*. *D*, depletion of β -COP expression inhibited CFTR maturation in Cos-7 cells. Cos-7 cells were co-transfected with control siRNA or siRNA to β -COP and WT-CFTR. After 3 days, cell surface proteins were biotinylated and pulled down with streptavidin beads. Biotinylated CFTR was detected by Western blot using a monoclonal anti-CFTR antibody. Expression of β -COP was also evaluated and was reduced after transfection of siRNA to β -COP. Depletion of β -COP decreased mature (*Band C*) CFTR and increased immature (*Band B*) CFTR.

A decrease in the resistance would have suggested that the monolayer was leaky and would have resulted in an increase in short circuit currents since we applied a chloride gradient. At the end of the experiments the cells were lysed and the levels of CFTR, β -COP, and SNAP-23 were assessed by Western blot. Fig. 4C shows that transfection of anti- β -COP siRNA decreased protein expression of β -COP in differentiated HT-29Cl.19A cells grown on filters by $\sim 80\%$ and was comparable with the one observed when the cells were grown on plastic. In those conditions, we observed a decrease in mature CFTR (*Band C*) and an increase in immature (*Band B*) CFTR, as previously observed when the cells were grown on plates (see Fig. 3D). The amount of SNAP-23 was unchanged in β -COP-depleted cells.

To confirm that β -COP depletion prevented delivery of CFTR to the PM, trafficking of CFTR to the PM was examined by biotinylation of cell surface proteins. It was difficult to detect CFTR present at the cell surface of HT-29Cl.19A cells due to the low expression of CFTR in those cells. Therefore, the experiments were done in Cos-7 cells that are easily transfected, allowing high expression of CFTR. In this experiment, cell surface proteins were labeled using biotin, and biotinylated CFTR was revealed by Western blot after pulling down biotinylated proteins by streptavidin beads. In control cells, ample CFTR was recovered on the streptavidin

beads (Fig. 4D). In contrast, CFTR trafficking to the cell surface was inhibited in β -COP-depleted cells (Fig. 4D). No CFTR was detected by Western blot in the absence of biotin, showing that non-biotinylated CFTR did not bind the streptavidin beads. These results suggested that COPI is essential for CFTR trafficking to the cell surface.

COPI Subunits Co-immunoprecipitate with CFTR—To investigate the mechanistic parameters of COPI function in CFTR trafficking, we tested the possibility that CFTR is COPI cargo. We therefore examined whether CFTR and COPI were recovered in a complex. Using a CFTR antibody that recognized the C-terminal tail of CFTR to immunoprecipitate lysates of HT-29Cl.19A cells, we recovered the α -, β -, and γ -COP subunits of the COPI coat (Fig. 5A). None of the COPI subunits was detected when immunoprecipitated with nonspecific IgG. Pretreatment of the HT-29Cl.19A cells with brefeldin A, which prevents COPI assembly by interfering with the activation of ARF1, diminished the interaction between CFTR and β -COP (Fig. 5B). Reciprocal immunoprecipitation with an antibody to β -COP subunit was performed but no CFTR signal could be detected, probably due to the low levels of immature CFTR present in the cells. Taken together, these results strongly suggest that COPI is involved in CFTR traffic at the ER-Golgi interface.

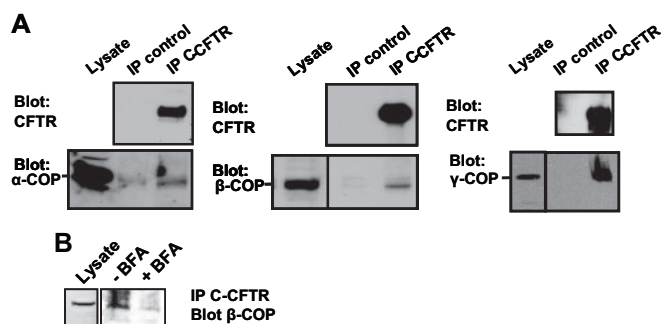


FIGURE 5. COPI subunits co-immunoprecipitate with CFTR. A, HT-29Cl.19A lysates were immunoprecipitated with control or an antibody against the C terminus of CFTR antibody cross-linked to protein A/G-agarose beads. The precipitated proteins were examined by Western blotting with the indicated antibodies. α -, β -, and γ -COPI subunits co-precipitate with CFTR. These experiments were performed at least three times, and similar results were obtained. B, treatment of HT-29Cl.19A cells with brefeldin A decreased the interaction of β -COP with CFTR. Cells were treated with 10 μ g/ml brefeldin A for 3 h. The proteins co-immunoprecipitated using a C-CFTR antibody were examined by Western blotting with anti- β -COP antibody.

DISCUSSION

Here we demonstrate for the first time that CFTR trafficking requires the COPI coat. We took advantage of the finding that COPI coat is recruited to membranes by the small GTPase ARF1 that cycles between an inactive GDP-bound and an active GTP-bound state (24). The guanine nucleotide exchange factor GBF1 activates ARF1 by catalyzing the exchange of GDP to GTP (25, 26). We used dominant negative mutants GBF1/E794K and ARF1/Q71L that impair COPI vesicle function (24, 27). The mutant GBF1/E794K can bind ARF-GDP but does not catalyze GDP displacement and thus does not promote the recruitment of the coatomer, whereas ARF1/Q71L is a GTP-restricted mutant that leads to the rapid accumulation of COPI-coated vesicles (28). We found that expression of the dominant negative GBF1/E794K mutant that causes the dissociation of COPI from membrane prevented CFTR trafficking to the PM. Similarly, expression of the constitutively activated ARF1/Q71L mutant that stabilizes COPI on membranes also prevented CFTR from reaching the PM. Expression of these mutants prevented CFTR from reaching the PM of HeLa cells.

COPI vesicles are involved in the anterograde transport as well as the retrograde transport of proteins and could play a role in sorting proteins. Proteins can move from the ER-Golgi intermediate compartment to the Golgi complex either in COPI-coated vesicles or through direct tubular connections. Protein cargo of COPI-coated vesicles includes (i) luminal ER-resident proteins that have at their C terminus the motif KDEL (single letter amino acid code, HDEL in yeast) that specifies interaction with the KDEL receptor (29), and (ii) proteins that have a K(X)KXX motif (where X represents any amino acid) at their C terminus exposed to the cytosol (22). Upon binding, these proteins are returned to the ER. In addition to roles in anterograde and retrograde traffic, COPI vesicles sequester misassembled proteins to prevent their delivery to the plasma membrane. In this regard, COPI vesicles are involved in regulating the trafficking of misfolded presenilin-1 and major histocompatibility complex class 1 (30, 31). The potassium channel KCNK3 harbors a di-basic motif that is recognized by the COPI subunit β -COP (32). Similarly, upon misfolding of the G protein-cou-

pled V2 vasopressin receptor, the β -COP subunit of COPI interacts with the RXR motif present in the cytosolic loop 3 of the receptor, resulting in the retention of the protein in the ER (33). Thus, the association of these proteins with COPI vesicles prevents the delivery of misfolded proteins to the PM.

The most common mutation causing CF, Δ F508-CFTR, encodes a protein that does not traffic to the PM. However, Δ F508-CFTR has been reported to be present in the ER-Golgi intermediate compartment in the human pancreatic adenocarcinoma cells, CFPAC, suggesting that the mutant protein exits the ER and traffics to the ER-Golgi intermediate compartment but does not move beyond that compartment (34). We found that β -COP co-immunoprecipitate with both WT- and Δ F508-CFTR in transfected human embryonic kidney 293T cells.³ However, the transient or stable nature of their association remains to be determined. Interestingly, CFTR harbors four RXR motifs and deletion of these motifs allows the rescue of the processing of the Δ F508-CFTR mutant (35). We can therefore speculate that Δ F508-CFTR is a COPI cargo for retrograde transport to the ER whereas WT-CFTR is a cargo for anterograde transport toward the PM. The recent findings that two types of COPI vesicles are present in cells provides a rationale for their participation in bidirectional transport (10, 11).

The involvement of COPI in CFTR traffic was supported by our findings that CFTR was not processed in cells depleted of β -COP. Under those conditions, we observed that CFTR trafficking was altered. Steady-state amount of CFTR exhibited a decrease in band C, representing mature CFTR that trafficked to the Golgi to acquire complex glycosylation, and an increase in band B, representing immature CFTR that is arrested in traffic before the Golgi. These results were confirmed using metabolic pulse-chase experiments and revealed that immature CFTR was not converted to mature CFTR after a 2-h period in HT-29Cl.19A cells depleted of β -COP. The effect seems to be specific to CFTR since down-regulation of β -COP had no effect on the trafficking of the membrane protein Na^+/K^+ -ATPase. However, we cannot eliminate the possibility that the trafficking of other membrane proteins is also affected under similar conditions. These results suggest that CFTR trafficking to the Golgi is dependent on COPI vesicle function.

Equally important, down-regulation of β -COP expression decreased cAMP-activated chloride currents. Our results agree with a recent study finding that CFTR channel function was abrogated in non-polarized CHO cells when COPI vesicles were inactivated (36). The authors also suggested that CHO cells incubated at a temperature that abrogated COPI function did not affect CFTR present at the cell surface. Yoo *et al.* (3) found that CFTR trafficking could be COPI-dependent in HeLa and human embryonic kidney 293 cells or COPI-independent in baby hamster kidney and CHO cells, suggesting that the trafficking of CFTR can vary depending on the cell line. CFTR is a chloride channel present at the apical membrane of epithelial cells, and absence of CFTR from the PM causes CF. Therefore, understanding the intracellular trafficking of CFTR in epithelial cells is essential, and in this study we focused on human epithelial

³ Rennolds, J. and E. Cormet-Boyaka, unpublished data.

lial cell line HT-29Cl.19A. Here we show that CFTR maturation was abrogated in epithelial cells that were depleted in β -COP.

A possible relationship between CFTR and COPI-mediated traffic was suggested by the Balch laboratory (2) documenting that 25% of total CFTR in CHO cells is associated with COPI structures. Here we show for the first time that α -, β -, and γ -COP subunits were co-immunoprecipitated with CFTR, using a monoclonal antibody that recognizes the C-terminal tail of CFTR. Reciprocally, CFTR was recovered with β -COP in HT-29Cl.19A. These results suggest that CFTR interacted with COPI coatomer as a cargo for sorting into COPI vesicles.

In summary, our data show that the COPI complex plays a critical role in the trafficking of CFTR. Further studies are required to identify the protein interacting with CFTR that binds to COPI complex or to determine whether CFTR interacts directly with COPI complex.

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**Cystic Fibrosis Transmembrane Conductance Regulator Trafficking Is Mediated
by the COPI Coat in Epithelial Cells**

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