Tissue-specific control of CFTR endocytosis by Dab2
Cargo recruitment as a therapeutic target

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Keywords: CFTR, Cystic fibrosis, ΔF508 mutation, Endocytosis, Dab2, clathrin coated vesicles, human airway epithelial cells

Clathrin-mediated endocytosis dynamically regulates cell membrane abundance of CFTR and plays an essential role in CFTR-dependent Cl− conductance in fluid-transporting epithelia. It requires two closely related, but distinct processes: assembly of the clathrin coat and recruitment of cargo proteins for endocytosis. The assembly polypeptide-2 complex (AP-2) is the prototypical endocytic adaptor responsible for optimal clathrin coat formation. Disabled-2 (Dab2) is a clathrin associated sorting protein (CLASP) that also mediates clathrin assembly and cargo selection. Both of these complexes have clearly been shown to play roles in CFTR endocytosis in cells that endogenously express the channel. However, their precise functions exhibit cell-specific differences. While Dab2 appears to play a central role in CFTR recruitment to the clathrin coat in airway epithelial cells, it does not play a direct role in CFTR endocytosis in intestinal epithelial cells. Here, we review our current understanding of the role of Dab2 in CFTR endocytosis in different tissues. Next, we present new data demonstrating the role of Dab2 in endocytosis of the most commonly mutated CFTR gene product, ΔF508-CFTR, in human airway epithelial cells. Finally we discuss the potential therapeutic implications of targeting the functional interaction between ΔF508-CFTR and Dab2.

Tissue Specificity of CFTR Trafficking

The cystic fibrosis transmembrane conductance regulator (CFTR) belongs to the family of ATP binding cassette (ABC) transporters, but forms a cAMP-activated Cl− channel that mediates transepithelial Cl− secretion in various fluid-transporting epithelia.1−3 CFTR is mutated in patients with cystic fibrosis (CF). In the airway, CFTR plays an essential role in maintaining mucociliary clearance by regulating the airway surface liquid.4,5 A central aspect of this regulation involves control of Cl− secretion across polarized epithelial cells, which is modulated at the level of both CFTR channel activity and cell membrane abundance (reviewed in6). The plasma membrane abundance of CFTR in turn depends on its biosynthetic processing and post-maturational trafficking (reviewed in6). The long plasma membrane stability of CFTR stands in contrast to its inefficient biosynthetic processing and primarily reflects efficient recycling, which is required to compensate for rapid endocytosis in clathrin-coated vesicles (CCVs).9−11

Complex protein networks control the endocytic uptake of CFTR.7 While several proteins involved in the uptake process have been identified,12 their roles are incompletely defined, and there is evidence for tissue- and cell-specific differences. Nevertheless, it is clear that clathrin-mediated endocytosis
these processes, since robust recruitment can sustain endocytosis even after a substantial fall of the CCV formation. For example, depletion of AP-2 by more than 90% results in a 10-fold reduction of CCV number. This profoundly inhibits the recruitment and endocytic uptake of cargo proteins that rely entirely on the YxxΦ motif, such as the transferrin receptor (TfR). In contrast, it has relatively modest effects on the uptake of the low-density lipoprotein receptor (LDLR), which undergoes privileged cargo recruitment independent of AP-2.

Both of Dab2 and AP-2 have clearly been shown to play roles in CFTR endocytosis in cells that endogenously express the channel. In human airway epithelial cells, the knockdown of μ2 adaptin by more than 90% resulted in only a 2-fold reduction in CFTR endocytosis, compared with the 10-fold reduction that would be expected for purely YxxΦ-mediated uptake. This suggested that CFTR experiences AP-2 independent, privileged recruitment to a diminished population of CCVs. Consistent with this interpretation, a more limited (~60%) knockdown of μ2 adaptin caused no difference in airway epithelial cells, a comparable 64% knockdown of α adaptin in HEK293 cells caused a 2-fold reduction in the endocytic uptake of CFTR. To achieve an equivalent 2-fold CFTR reduction in airway cells, a 90% knockdown of μ2 adaptin in epithelial cells was required.

Thus, in HEK293 cells, AP-2 appears to play a more critical role in CFTR endocytosis. Together, these results underscore the importance of studying protein trafficking processes in cells most representative of the appropriate physiological context.

Therapeutic Implications

Having identified the importance of Dab2-mediated recruitment in CFTR endocytosis in airway epithelial cells, we were interested in the therapeutic potential of Dab2 inhibition. Both biosynthetic processing and post-maturational trafficking are critically affected by the most common disease-associated mutation in the CFTR gene. In 70% of patient alleles, loss of Phe508 (ΔF508) leads to a temperature-sensitive processing defect in the CFTR protein (reviewed in ref. 8). Low temperature and chemical chaperones increase biosynthetic processing and rescue the cell membrane abundance of ΔF508-CFTR, and disease-modifying therapies based on correction of the biosynthetic processing of ΔF508-CFTR have been highly anticipated (23,24). Rescued ΔF508-CFTR (rΔF508-CFTR) is partially functional as a Cl- channel but is unstable in the plasma membrane due to altered endocytic trafficking. Hence, addressing the post-maturational trafficking defect of rΔF508-CFTR represents a therapeutic approach complementary to biosynthetic rescue.

Specifically, proteins involved in either endocytic uptake or lysosomal degradation should be the targets for CFTR stabilization at the apical plasma membrane. Indeed, the plasma membrane half-life, cell-membrane abundance, and net chloride-channel activity of ΔF508-CFTR can be increased by depletion or inhibition of the CFTR-associated ligand CAL, which facilitates CFTR post-endocytic degradation. Similarly, depletion of proteins involved in CFTR endocytic uptake increases its apical membrane abundance. However, these studies have focused on WT-CFTR, and our earlier work clearly showed that WT- and rΔF508-CFTR experience differential rates of endocytic uptake in airway epithelial cells. We therefore directly tested the impact of Dab2 depletion on rΔF508-CFTR. Given the sensitive dependence of these processes on cell type, we studied paired CFBE41o- cell lines stably expressing either WT- or rΔF508-CFTR.

Based on these results, targeting of Dab2 may offer an approach to investigate and control the limited plasma

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Figure 1. Schematic illustration of the Dab2 and AP-2 interactions during clathrin-mediated endocytosis. Dab2 and AP-2 mediate assembly of the clathrin coat and recruitment of transmembrane cargo proteins for incorporation into clathrin coated pits (CCPs). The Dab2 DH domain interacts with the NPF motif-containing cargo proteins and the μ2 adaptin of AP-2 interacts with the YxxΦ motif-containing cargo proteins. Our recent data demonstrate that the DH domain may recruit cargo, such as CFTR that does not have a canonical NPF motif (20). The Dab2 NPF repeats and the β2 adaptin of AP-2 bind clathrin. Moreover, Dab2 and AP-2 interact with each other by binding of the Dab2 DPF motif to the β adaptin of AP-2.

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the CFTR YDSI motif in vitro. Thus, while a ~60% knockdown of μ2 adaptin caused no change in airway epithelial cells, a comparable 64% knockdown of α adaptin in HEK293 cells caused a 2-fold reduction in the endocytic uptake of CFTR. To achieve an equivalent 2-fold CFTR reduction in airway cells, a 90% knockdown of μ2 adaptin in epithelial cells was required. Thus, in HEK293 cells, AP-2 appears to play a more critical role in CFTR endocytosis. Together, these results underscore the importance of studying protein trafficking processes in cells most representative of the appropriate physiological context.

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Based on these results, targeting of Dab2 may offer an approach to investigate and control the limited plasma
membrane stability of rΔF508-CFTR. CFTR does not contain a recognized Dab2 binding motif and the stereochemical basis of the functional interaction between Dab2 and CFTR remains unclear. Nevertheless, our data have previously shown that a peptide that binds to the Dab2 DH peptide-binding pocket can regulate CFTR abundance in the plasma membrane. Such compounds do not target the AP-2 mediated interactions and thus should not decrease the number of CCVs nor globally inhibit protein endocytosis. As a result, targeting of this interaction may provide future pharmacological approaches to correct the post-maturational trafficking defects of ΔF508-CFTR.

**Methods**

CFBE41o- cells stably expressing WT-CFTR or ΔF508-CFTR proteins were plated on collagen-coated tissue culture plates and incubated with the optimized transfection mixture containing 10 nM siRNA against human Dab2 gene (siDab2; Hs_Dab2_6 siRNA) or with a non-silencing siRNA control (siCTRL; Qiagen, Valencia, CA), using Lipofectamine 2000 (Invitrogen, Grand Island, NY), as previously described (20). The transfection medium was changed after 24 h and cells were cultured for 72 h to establish a monolayer. CFBE41o- cells expressing ΔF508-CFTR were cultured at 27°C for 36 h before experiments to increase the cell-membrane abundance of ΔF508-CFTR (11). Silencing the Dab2 gene under these conditions resulted in the corresponding protein depletion by ~70%. This level of silencing allowed sufficient clathrin coat formation to better differentiate the role of Dab2 in clathrin-coat formation vs. cargo recruitment and to prevent off-target effects that may occur with more complete gene silencing. The biochemical quantification of plasma membrane CFTR was performed by surface biotinylation, streptavidin bead capture, western blotting, and chemiluminescence, as previously described. The following mouse monoclonal antibodies were used: anti-human CFTR C-term (Millipore, Billerica, MA), anti-Dab2 p96 and anti-ezrin (BD Biosciences, Sparks, MD).

Statistical analysis of the data was performed using GraphPad Prism version 4.0 for Macintosh (GraphPad Software Inc., San Diego, CA). The means were compared by a two-tailed t-test. A p value < 0.05 was considered significant. Data are expressed as mean ± SE.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We would like to thank Karyn Hansen from the Geisel School of Medicine at Dartmouth for her technical assistance with the experiments. This study was supported by National Institutes of Health Grants R01HL090767 and R01HL090767–02S1 (to A.S.-U.) and R01-DK075309 (to D.R.M.). This work was also supported by the Pennsylvania Department of Health, Health Research Formula Fund to the Children’s Hospital of Pittsburgh of the UPMC Health System (to A.S.-U.).
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