The flipside of cystic fibrosis protein transport

Deficiency of ATP8B1 in humans causes severe progressive liver disease that is characterized by impaired bile flow. Besides liver disease, many ATP8B1 disease patients develop extrahepatic symptoms of yet unknown etiology such as pulmonary infection and defects in sweat gland function, symptoms commonly associated with cystic fibrosis (CF). CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride and bicarbonate channel that is expressed in the apical membrane of, amongst others, pulmonary and intestinal epithelial cells.

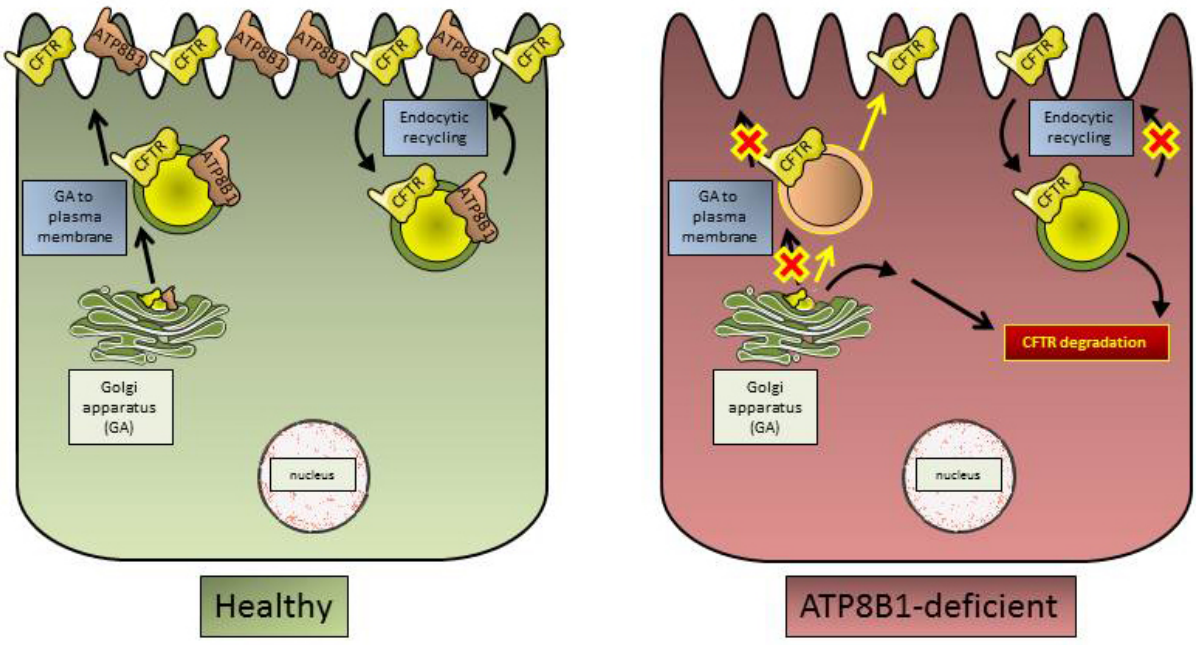

Fig. 1. In healthy epithelial cells, ATP8B1 mediates the apical targeting of CFTR either at the Golgi apparatus (GA), on route from the GA to the plasma membrane or during endocytic recycling at the apical membrane. In ATP8B1-deficient cells, apical membrane expression of CFTR is strongly reduced due to either impaired GA-to-plasma membrane transport of CFTR or to impaired endocytic recycling of CFTR (indicated by red crosses). Consequently, CFTR protein is destined for degradation. An alternative trafficking pathway (indicated by yellow arrows) may partially compensate the loss of ATP8B1 and delivers some CFTR protein to the apical membrane.

ATP8B1 localizes to the apical membrane of many epithelial cells, including hepatocytes and enterocytes. The apical membrane faces the lumen of an organ and is the membrane domain at which many excretory and absorptive processes occur. ATP8B1 is a lipid flippase, an activity that

catalyzes the transport of phospholipids from the exoplasmic to the cytosolic leaflet of biological membranes. Emerging evidence indicates an important role for lipid flippases in the biogenesis and transport of intracellular vesicles in the biosynthetic and endocytic pathways.

We assessed whether impaired CFTR function may underlie some extrahepatic phenotypes in ATP8B1 disease, and analyzed its activity in intestinal epithelial cells in which ATP8B1 protein levels were depleted by more than 70%. In these cells, CFTR activity was significantly reduced as measured by two different assays; Firstly, by measuring intracellular chloride concentrations using a genetically encoded fluorescent chloride sensor, and secondly, by determining electrical currents over the cell generated by chloride transport. Impaired CFTR activity coincided with a 70% reduction of CFTR protein levels in the apical plasma membrane, suggesting that ATP8B1 is important for correct membrane localization of CFTR. To study this hypothesis, inducible CFTR was over-expressed in control and ATP8B1-depleted intestinal epithelial cells. Chemical induction of CFTR protein levels resulted in a comparable increase in total CFTR levels in both cell lines. However, whereas plasma membrane abundance of CFTR was significantly enhanced in control cells, no increase in CFTR membrane levels was observed in ATP8B1-depleted cells. These data indicate that ATP8B1 plays an important role in the proper localization of CFTR to the plasma membrane, possibly by controlling the transport of newly-synthesized CFTR protein from the Golgi apparatus to the plasma membrane or by controlling recycling of CFTR from endosomal compartments to the plasma membrane (Figure 1). In addition, ATP8B1 protein depletion in pulmonary epithelial cells also coincided with a strong reduction of CFTR levels in the plasma membrane indicating that this flippase is an important determinant of correct CFTR localization in the lungs as well. Since the CF-like symptoms in ATP8B1 disease patients are mild, ATP8B1 most likely is not the only mediator of CFTR localization. Hence, there may be redundancy in the trafficking itineraries of CFTR that partially compensate for the loss of ATP8B1. Our study shows that the phospholipid flippase ATP8B1 is an important mediator of CFTR membrane localization in intestinal and pulmonary epithelial cells and that impaired CFTR localization may be the cause of some of the extrahepatic phenotypes observed in ATP8B1 disease.

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