Fluorogen activating proteins report corrector-mediated restoration of mutant CFTR trafficking to the cell surface and its regulated peripheral recycling

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Numerous human diseases arise from defects in protein folding. They lead to degradation of those misfolded proteins in the endoplasmic reticulum, a process known as ER associated degradation (ERAD). Among them is Cystic Fibrosis (CF), caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), an epithelial anion channel. The most common CF mutation, F508del, disrupts CFTR folding, which blocks its trafficking to the plasma membrane. To date, most F508del CFTR corrector high throughput screening assays have relied on measurements of restored CFTR function. These methods require multiple wash steps and are susceptible to signal saturation. They rely on the recruitment of a functional CFTR to the cell surface. Immunofluorescence labeling methods to detect an epitope-tagged version of CFTR also require multiple wash and binding steps, adding variability and reducing screening throughput. Direct detection of F508del CFTR at the cell surface with a single labelling step would improve throughput and increase the dynamic range of corrector screening assays.

To address these issues, we tagged CFTR with a protein module that provides a selective fluorescence assay of CFTR's abundance at the cell surface. The protein tag is comprised of a genetically encoded fluorogen activating protein (FAP), fused to the N-terminus or inserted into the 4th extracellular loop (EL4) of CFTR. The FAP faithfully reports the trafficking of WT and F508del CFTR, and FAP WT CFTR retains functional activity. The presence of FAP CFTR at cell surface can be selectively detected by a cell impermeant fluorogen, while total cellular CFTR is detectable by a cell permeant fluorogen.

Using this approach, we determined the efficacy of corrector compounds provided from the CFFT (www.cftrfolding.org), both alone and in combination, to rescue F508del CFTR to the plasma membrane. Combinations of correctors produced additive or synergistic effects, improving the density of mutant CFTR at the cell surface up to 9-fold over single compound treatment. The results correlated closely with functional assays of stimulated anion transport performed in polarized human bronchial epithelia (HBE) that endogenously express F508del CFTR. These findings indicate that the FAP-tagged construct quantitatively reports mutant CFTR correction activity, and that this approach should be useful as a screening assay in diseases that impair protein trafficking to the cell surface.

To gain further insight into the mechanism of corrector activity, we investigated the peripheral trafficking itineraries of WT and F508del CFTR in living cells using high-speed fluorescence microscopy together with FAP-CFTR detection. Using a cell impermeant fluorogen, we directly visualized the internalization and accumulation of CFTR WT from the plasma membrane (PM) to a perinuclear compartment which co-localized with the endosomal recycling compartment (ERC) markers, Rab11 and EHD1, reaching steady-state distribution by 25 minutes. Stimulation by protein kinase A (PKA) depleted this intracellular pool and redistributed CFTR channels to the cell surface, elicited by reduced endocytosis and active translocation to the PM. Corrector or temperature rescue of F508del CFTR also resulted in targeting to the ERC and exhibited subsequent PKA stimulated trafficking to the PM. Corrector treatment (24 h) lead to persistent residence of F508del in the ERC, while thermally destabilized F508del was targeted to lysosomal compartments by 3 h. Acute addition of individual correctors acted on peripheral trafficking steps to partially block lysosomal targeting of thermally destabilized F508del CFTR. Taken together, corrector treatment redirects F508del trafficking from a degradation pathway to a regulated recycling route. Proteins which mediate this process become potential targets for improving the efficacy of current and future correctors.

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