January 2021

Current projects

⇒ Role of anoctamins in renal tubules and polycystic kidney disease

Polycystic kidney disease (PKD) causes kidney failure due to cyst enlargement. Cyst expansion is driven by the chloride channels cystic fibrosis transmembrane conductance regulator (CFTR) and by TMEM16A (anoctamin 1). Our previous work demonstrated that in fact TMEM16A has a major role in cyst growth. We described that peroxidation of plasma membrane phospholipids activates renal TMEM16A, facilitating Ca^{2+} signaling and activation of Ca^{2+} sensitive adenylate cyclase ADCY1. cAMP produced by ADCY1 further stimulates CFTR. Cyst enlargement is significantly delayed by the antioxidant idebenone and by ferrostatin-1, suggesting that during PKD activation of ferroptosis takes place, an apoptosis-independent regulated cell death pathway. Our data show that TMEM16A causes cyst expansion by increase in intracellular Ca^{2+}, which drives proliferation and fluid secretion (Fig. 1). Subsequent studies in collaboration with Dr. Björn Buchholz (Universitätsklinikum Erlangen, GER) will further analyze the precise mechanisms by which TMEM16A controls proliferation and fluid secretion, and how inhibition of TMEM16A will reduce cyst formation.

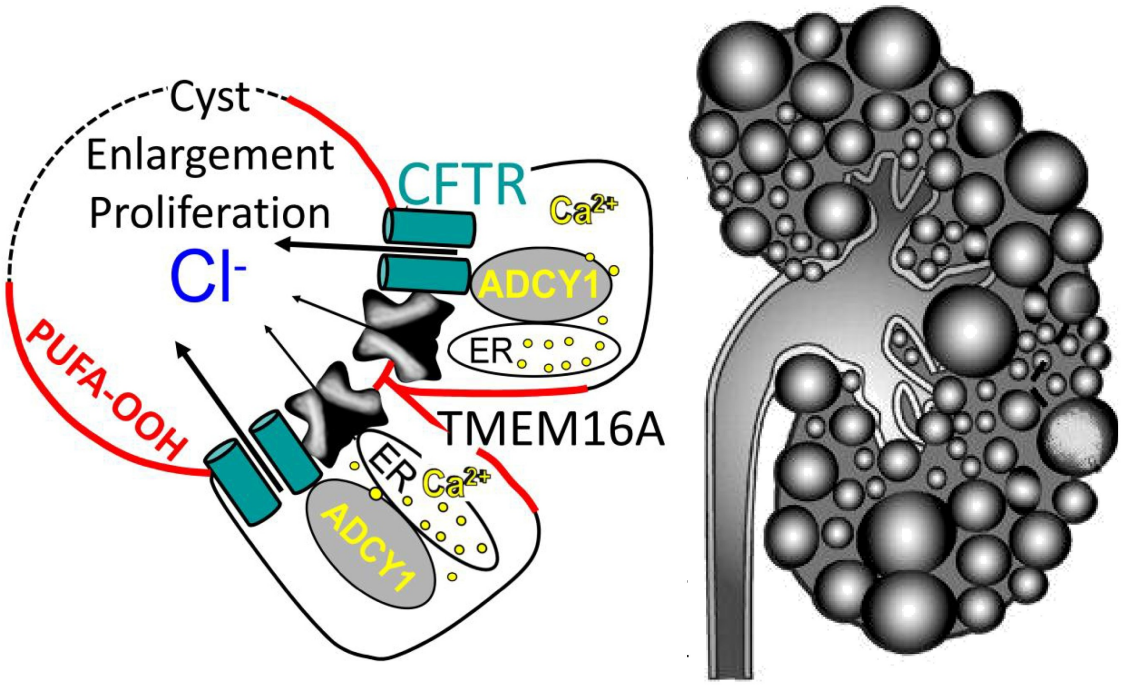


Fig. 1: TMEM16A causes an increase in intracellular Ca^{2+}, which activates CFTR via stimulation of ADCY1, thereby driving proliferation and fluid secretion.

⇒ Role of CFTR for renal HCO_3^- secretion and salt absorption

CFTR is expressed in the proximal tubular epithelial and in collecting ducts cells, particularly in intercalated type-B cells (Fig. 2). In collaboration with Prof. Dr. Jens Leipziger, University of Aarhus, Denmark, we examine whether CFTR regulates renal HCO_3^- secretion via SLC26A4 (Pendrin) in ß-intercalated cells, thereby providing a recycling pathway for Cl^- ions. CFTR may also regulate Na^+ absorption by NHE3 in proximal tubular epithelial cells (Fig. 2,3).

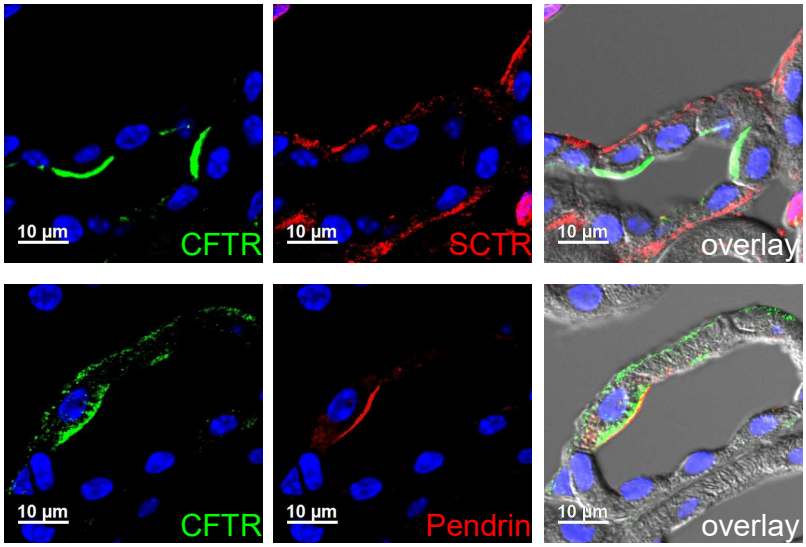


Fig. 2: Colocalization of CFTR, Pendrin (SLC26A4) and secretin receptor (SCTR) in ß-intercalated cells of mouse collecting duct.

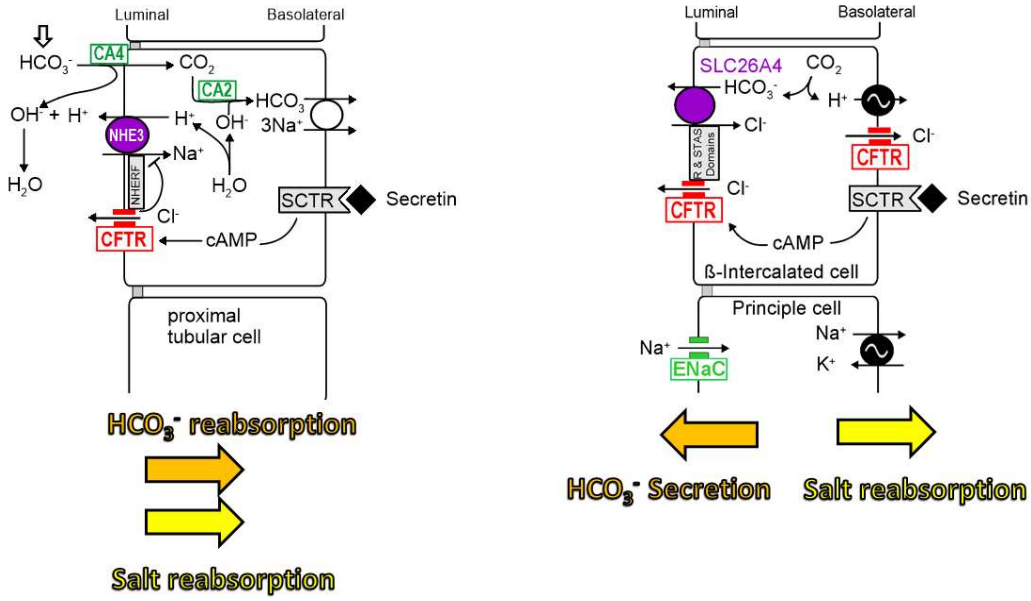


Fig. 3: Putative regulation of Na^+ absorption in proximal tubule (left) and HCO_3^- secretion in ß-intercalated cells (right) by CFTR.

⇒ Role of TMEM16A and TMEM16F in cellular exocytosis

TMEM16A is a Ca^{2+} activated Cl^- channel in intestinal and airway epithelial cells, but together with TMEM16F it also controls cellular exocytosis (Fig. 4). TMEM16A provides a mechanism

to enhance Ca^{2+} in the apical pole of mucus producing cells, which is essential for ATP-activated mucus release. TMEM16A is a regulator of exocytosis of mucus, the release of proinflammatory mediators, and facilitates membrane insertion of transmembrane proteins such as CFTR (Fig. 5). These findings may be correlated with the reported role of TMEM16A for cytoplasmic Cl^- homeostasis, control of PtdIns(4,5)P2 microdomains and membrane remodeling. Remarkably, in conditional airway and intestinal knockout mice, lymphocytes from Scott disease patients, and other overexpressing cells, CFTR is not inserted into the plasma membrane in the absence of TMEM16A and TMEM16F. We are interested in the precise mechanism how TMEM16 proteins determine intracellular Ca^{2+} signals and control the activity of CFTR (Fig. 5 upper panel), release of mucus and inflammatory mediators (Fig. 5 middle panel), and membrane insertion of CFTR (Fig. 6 lower panel).

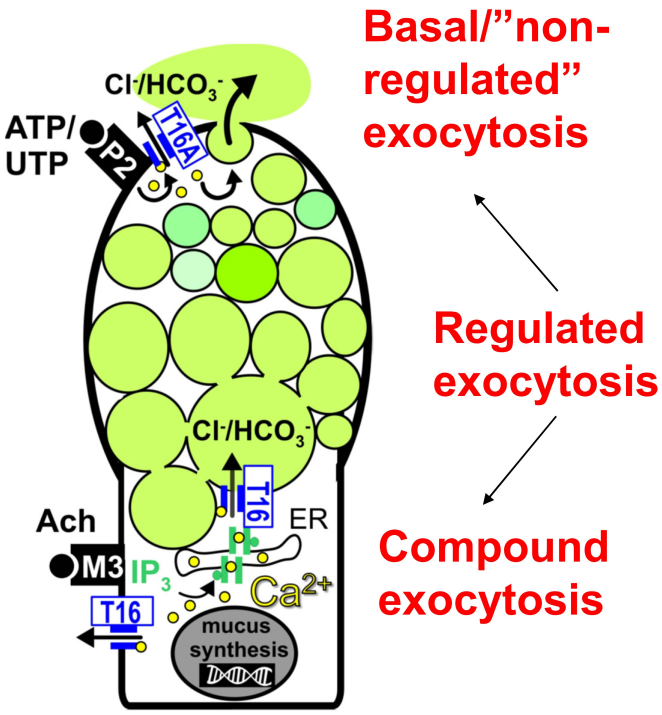
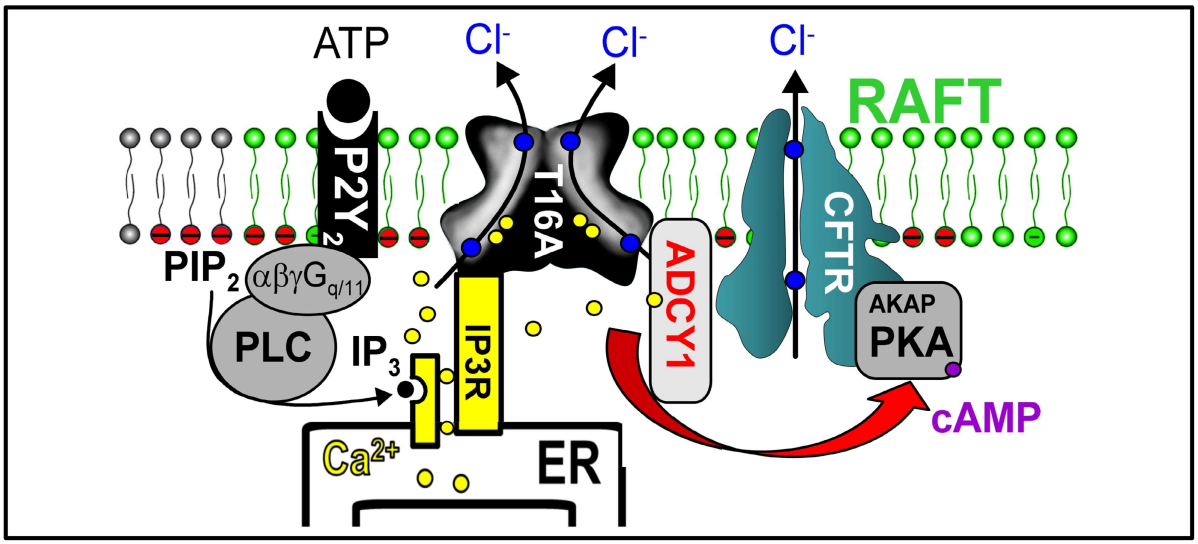


Fig. 4: Basal and compound exocytosis in mucus producing goblet cells.



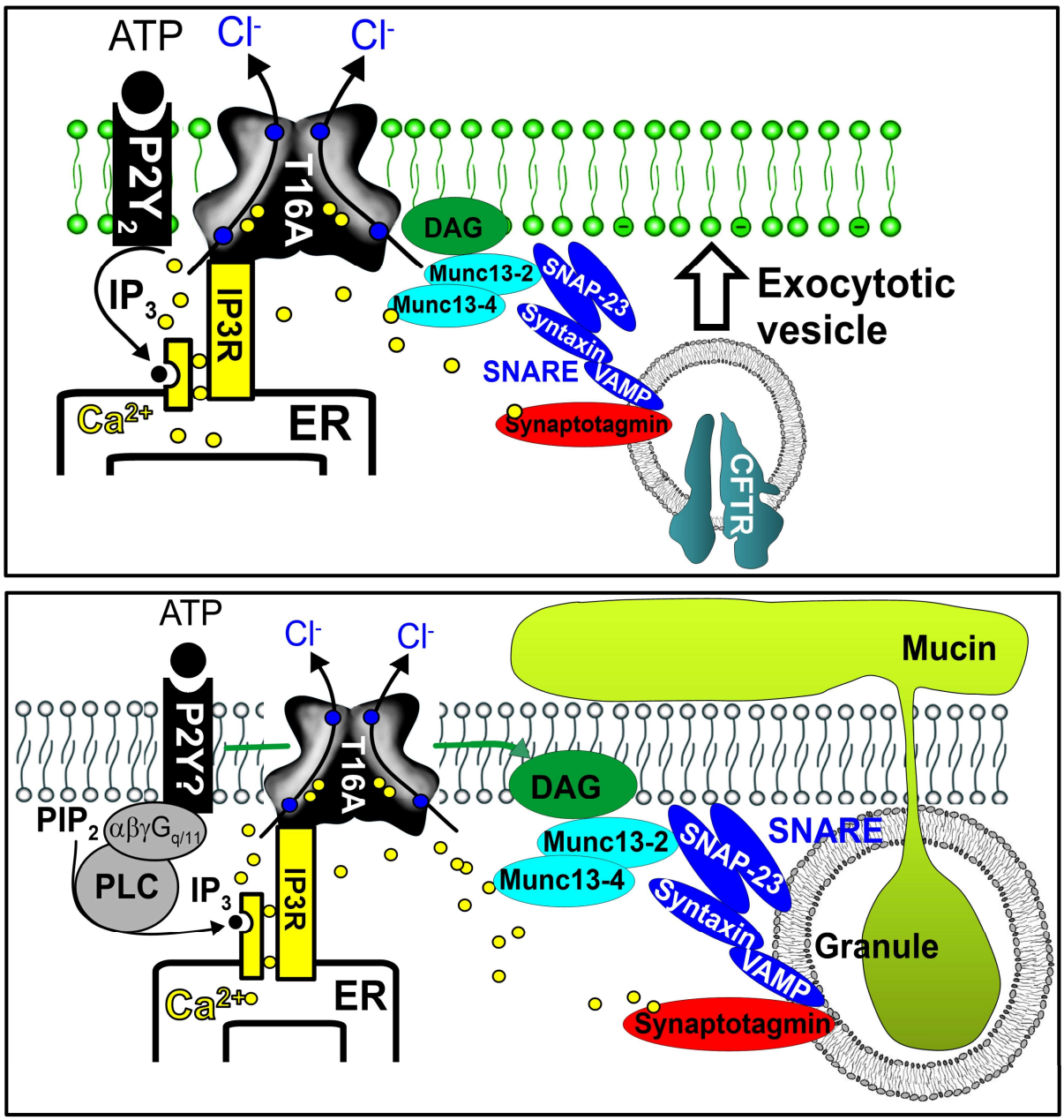


Fig. 5: Control of submembranous increase of intracellular Ca^{2+} which activates CFTR (upper panel), membrane insertion of CFTR (middle panel) and exocytic mucus release by TMEM16A (lower panel).

⇒ Treatment of Cystic Fibrosis by inhibition of TMEM16A

The inflammatory airway disease cystic fibrosis (CF) is characterized by airway obstruction due to mucus hypersecretion, airway plugging and bronchoconstriction. The cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel is dysfunctional in CF, leading to defects in epithelial transport. Although CF pathogenesis is still disputed, activation of alternative Cl^- channels is assumed to improve lung function in CF. Two suitable non-CFTR Cl^- channels are present in the airway epithelium; the Ca^{2+} activated channel TMEM16A and SLC26A9 (Fig. 6, upper panel). Activation of these channels is thought to be feasible was to improve hydration of the airway mucus and to increase mucociliary clearance. Interestingly, both channels are upregulated during inflammatory lung disease.

They are assumed to support fluid secretion, necessary to hydrate excess mucus and to maintain mucus clearance. During inflammation, however, TMEM16A is upregulated particularly in mucus producing cells, with only little expression in ciliated cells (Fig. 6 lower panel). Recently it was shown that knockout of TMEM16A in ciliated cells strongly compromises Cl^- conductance and attenuates mucus secretion, but does not lead to a CF-like lung disease and airway plugging. We examine whether inhibition of TMEM16 proteins indeed improves lung function in patients with CF or asthma.

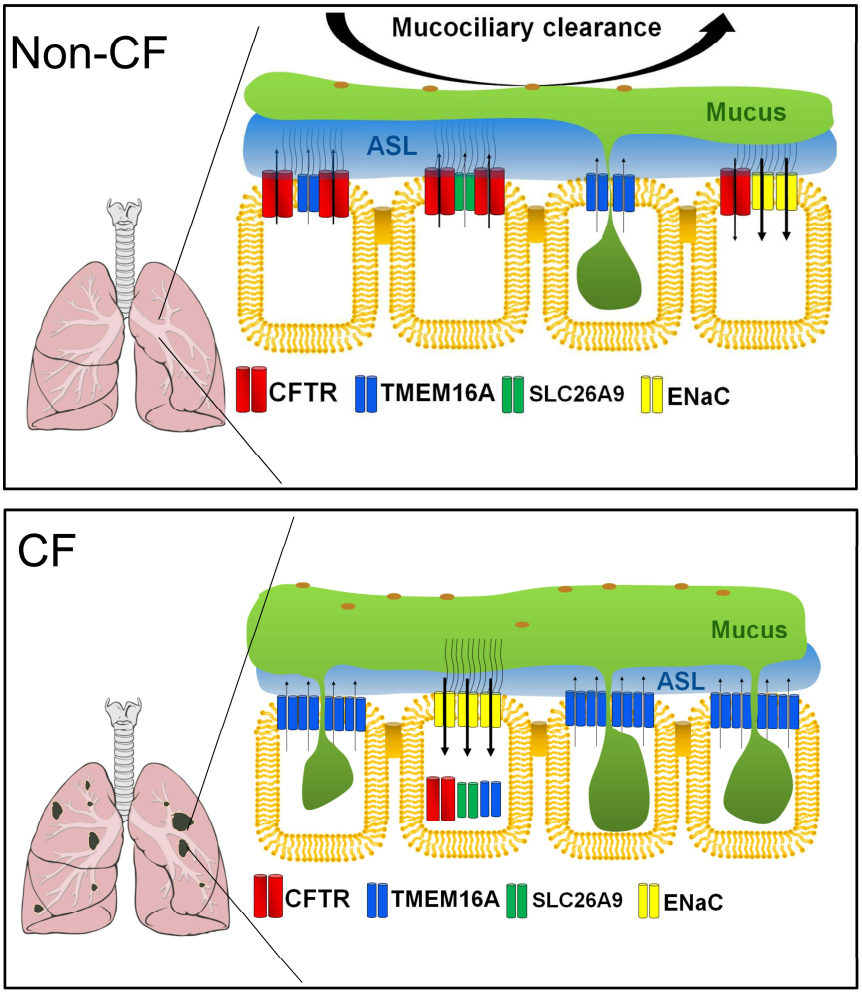


Fig. 6: Ion transport in non-CF and CF airways.

A number of studies suggest that TMEM16A is essential for mucus secretion and possibly also for mucus production. Evidence is now provided for a crucial role of TMEM16A in fusion of mucus-filled granules with the apical plasma membrane and cellular exocytosis, which is due to local Ca^{2+} signals facilitated by TMEM16A. Thus, TMEM16A supports fluid secretion by ciliated airway epithelial cells, but also causes excessive mucus secretion during inflammatory airway disease (Fig. 7 upper panels). Because TMEM16A causes mucus secretion and supports airway smooth muscle contraction, inhibition rather than activation of TMEM16A might be the appropriate treatment for CF lung disease, asthma and COPD (Fig. 7 lower panels). We examine the precise role of TMEM16 proteins in inflammatory airway disease.

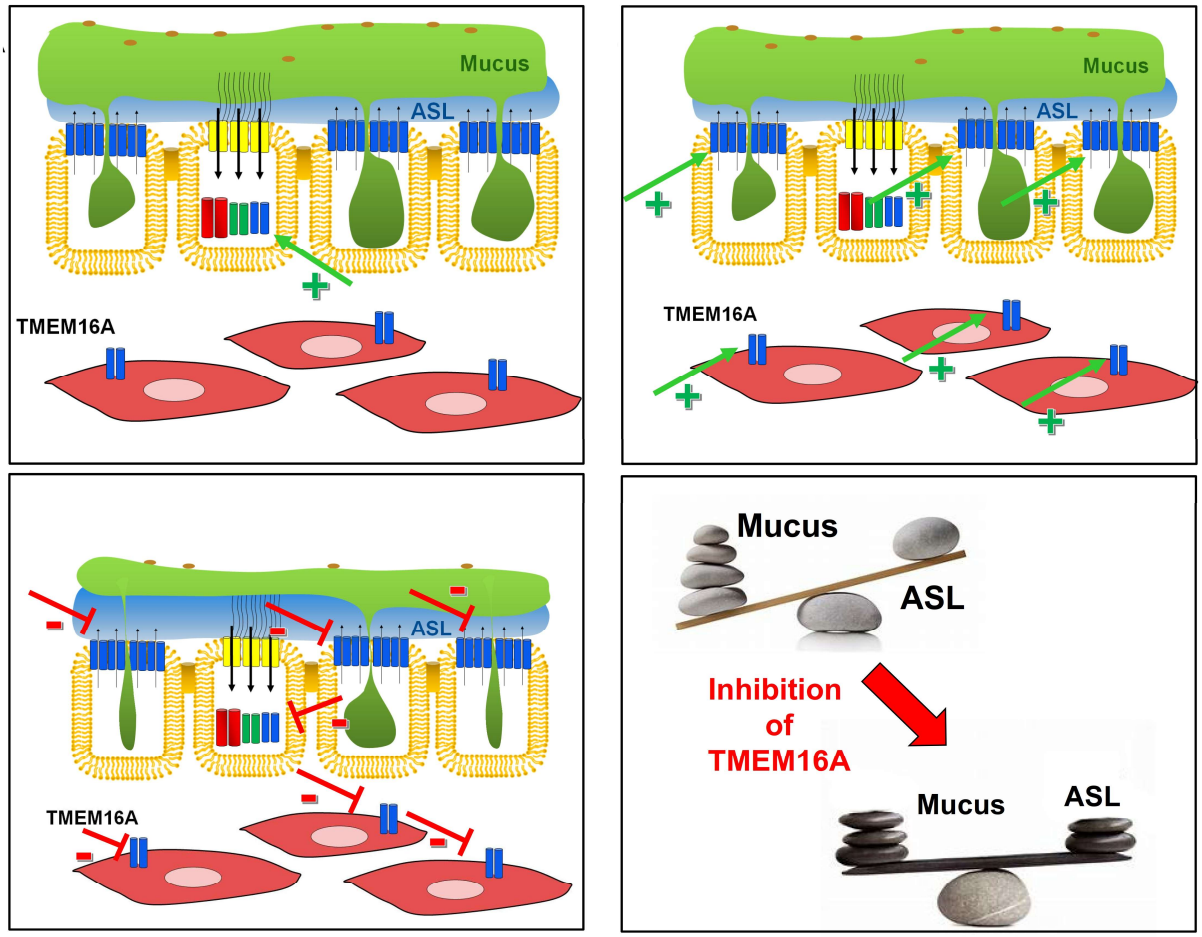


Fig. 7: Inhibition of TMEM16A as a therapy in CF.

⇒ Role of TMEM16F during inflammation and regulated cell death during ferroptosis and NETosis

When overexpressed, TMEM16A and TMEM16F produce spontaneous Cl^- currents at 37°C, even at resting intracellular Ca^{2+} levels, which can be abolished by inhibition of phospholipase A2 (PLA2). Conversely, activation of PLA2 or application of active PLA2, as well as lipid peroxidation induced by reactive oxygen species (ROS) using staurosporine or tert-butyl hydroperoxide, enhanced ion currents by TMEM16A/F and in addition activated phospholipid scrambling by TMEM16F. Thus, TMEM16 proteins are activated by an increase in intracellular Ca^{2+} or independent of intracellular Ca^{2+}, by modifications occurring in plasma and intracellular membrane phospholipids. These results may help to explain why regions distant to the TMEM16 pore and the Ca2+ binding sites control Cl^- currents and phospholipid scrambling. Regulation of TMEM16 proteins through modification of membrane phospholipids occurs during regulated cell death, such as apoptosis and ferroptosis. It contributes to inflammatory and nerve injury-induced hypersensitivity and generation of pain, and therefore provides a regulatory mechanism that is particularly relevant during disease. Leukocytes release their nuclear chromatin (left) to form an extracellular network that traps bacteria and other microorganisms (right). Anoctamins could contribute to the initial cell shrinkage required for NETosis. For this project we will use immune cells from knockout animals.

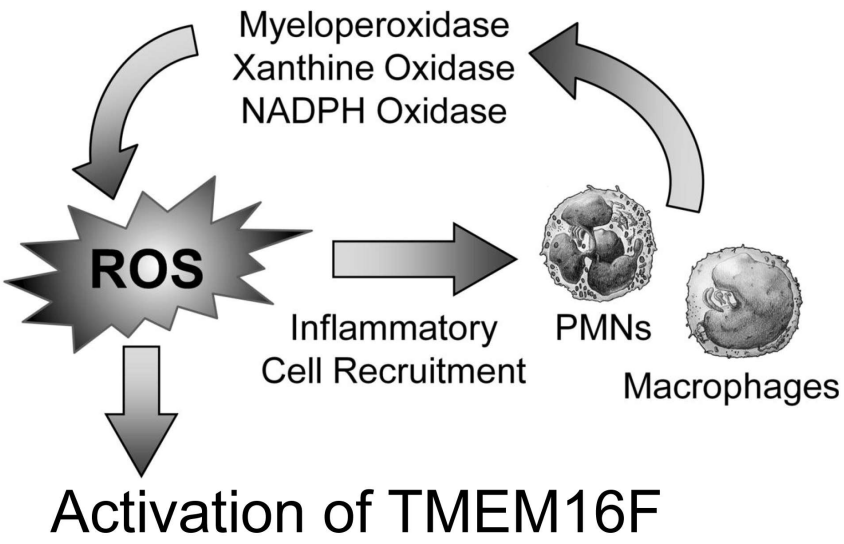
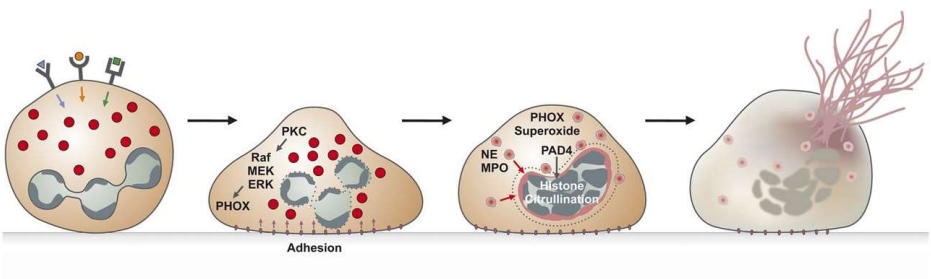


Fig. 8: Activation of TMEM16F during ROS-induced ferroptosis



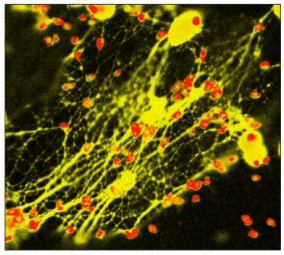


Fig. 9: Putative activation of TMEM16F during NETosis

⇒ Identification of novel proteins and small molecule compounds to regulate TMEM16A, TMEM16F, and SLC26A9

Double tagged (extracellular, intracellular) TMEM16A, TMEM16F and SLC26A9 will be stably expressed in CFBE airway epithelial cells (in collaboration with Prof. Dr. Margarida Amaral, Faculty of Sciences, BioSys - Biological Systems - Functional & Integrative Genomics, University of Lisbon, Portugal). Overexpressing CFBE cells will be used in Lisbon for automatic microscopy-based high throughput screening to identify novel target proteins controlling biosynthesis and activity of anoctamins, and to identify novel small molecules to modulate biosynthesis and activity of TMEM16A, TMEM16F, and SLC26A9. Identified target proteins and pharmacological hits will be validated (Fig. 10).

⇒ PEG formulated niclosamide for the treatment of inflammatory airway disease

Polyethylene formed microspheres and nanospheres contain niclosamide, which is known to inhibit activation of TMEM16A Cl^- channels. We examine whether these microspheres and nanospheres have an improved potency in treating inflammatory airway disease in ovalbumin-sensitized animals. Moreover, we examine the effects of incapsulated niclosamide on mucus production in cultured airway epithelial cells (Fig. 11). These studies are performed in collaboration with Prof. Dr. A. Göpferich at the department of Pharmacy of the University of Regensburg.

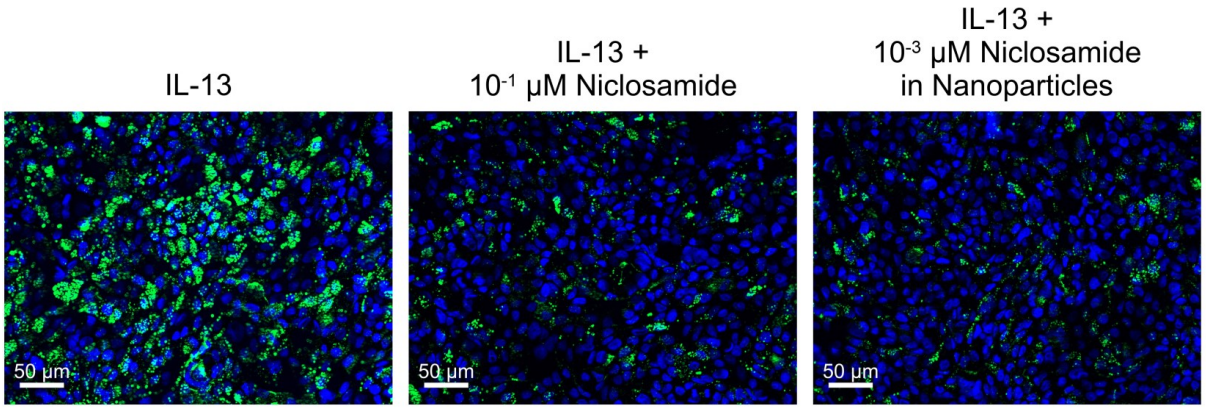


Fig. 11: Effects of incapsulated niclosamide on mucus production in cultured airway epithelial cells. MUC5AC is labelled in green.