



CFTR and Ca²⁺ signaling in cystic fibrosis

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Among the diverse physiological functions exerted by calcium signaling in living cells, its role in the regulation of protein biogenesis and trafficking remains incompletely understood. In cystic fibrosis (CF) disease the most common CF transmembrane conductance regulator (CFTR) mutation, F508del-CFTR generates a misprocessed protein that is abnormally retained in the endoplasmic reticulum (ER) compartment, rapidly degraded by the ubiquitin/proteasome pathway and hence absent at the plasma membrane of CF epithelial cells. Recent studies have demonstrated that intracellular calcium signals consequent to activation of apical G-protein-coupled receptors by different agonists are increased in CF airway epithelia. Moreover, the regulation of various intracellular calcium storage compartments, such as ER is also abnormal in CF cells. Although the molecular mechanism at the origin of this increase remains puzzling in epithelial cells, the F508del-CFTR mutation is proposed to be the onset of abnormal Ca²⁺ influx linking the calcium signaling to CFTR pathobiology. This article reviews the relationships between CFTR and calcium signaling in the context of the genetic disease CF.

Keywords: cystic fibrosis, CFTR, trafficking, calcium signaling, calcium stores, pharmacology

INTRODUCTION

Cystic fibrosis (CF) is a genetic disease characterized by abnormal ion transport across the apical plasma membrane (PM) of many epithelial tissues, including the airways (Riordan, 1993). The most common CF mutation F508del-CF transmembrane conductance regulator (CFTR) is the deletion of phenylalanine at position 508 (F508del-CFTR) leading to chloride impermeability in many exocrine glands (salivary, airways, pancreatic) associated to reduced volume of the final secretory fluid (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989; Quinton, 1990). CFTR functions as a cyclic AMP-activated and ATP-gated Cl⁻ channel that is expressed at the apical membrane of a wide variety of epithelial cell types. Although the gating of the channel is affected by the F508del-CFTR deletion, the mutant protein retains some functionality as a Cl⁻ channel (Dalemans et al., 1991). Moreover, the protein has a much shorter half-life in the PM than wild-type CFTR (Heda et al., 2001). Therefore, therapeutic efforts have been aimed at overcoming the trafficking defect in order to increase the amount of functional F508del-CFTR proteins present at the cell membrane (Zeitlin, 2000; Becq, 2010). The F508del-CFTR proteins have an improper folding leading to its trapping in the endoplasmic reticulum (ER) by multiple protein chaperone interactions (Norez et al., 2006b) such as calnexin. Calnexin is a Ca²⁺-sensitive ER-resident protein that interacts with monoglucosylated, trimmed intermediates of the N-linked core glycans on newly synthesized glycoprotein (Helenius and Aebi, 2004). Although CFTR is not a Ca²⁺-dependent chloride channel, numerous studies have investigated the role of ER Ca²⁺ on CFTR trafficking and in particular in the context of the abnormal F508del-CFTR trafficking. These studies showed for example that the depletion of ER Ca²⁺ store by SERCA pump inhibitors (Thapsigargin, CPA, DBHQ. . .) leads to the partial correction of abnormal F508del-CFTR trafficking by a mechanism, at least in

part, dependent on decreasing of calnexin-F508del-CFTR interaction (Egan et al., 2002, 2004; Norez et al., 2006a). Hence, the prevention of F508del-CFTR and calnexin interactions became a valuable target to identify new pharmacological agents able to restore the abnormal F508del-CFTR trafficking. Some of them are also able to modify the Ca²⁺ homeostasis. These results demonstrated the key role of ER Ca²⁺ in protein trafficking in epithelial CF and non-CF cells.

OVERVIEW OF Ca²⁺ SIGNALING IN NON-EXCITABLE CELLS

The diverse physiological functions of Ca²⁺ are possible because of the existence of a large variety of Ca²⁺ binding proteins that respond to a rise in [Ca²⁺]_i and transmit the [Ca²⁺]_i information to specific effectors. To ensure high fidelity in reading the Ca²⁺ signal and to minimize the energy invested in the removal of Ca²⁺ from the cytoplasm at the end of the Ca²⁺ signal, cells maintain very low resting [Ca²⁺]_i, usually below 100 nM. The most elemental mechanism of increasing [Ca²⁺]_i in response to extracellular stimuli is an influx into the cell of Ca²⁺ from the extracellular space, which contains about 2 mM Ca²⁺.

The ubiquitous mechanism by which external information is translated into a [Ca²⁺]_i signal is by G-protein-coupled receptors (GPCRs). GPCRs are the largest protein family in mammals, and decode stimuli that vary from light to pheromones to peptide hormones. With such diversity, GPCRs regulate virtually any cellular function via multiple second messengers that converge on all known signaling pathways. GPCRs complexes include the receptor, a trimeric G-protein composed of G α and G $\beta\gamma$ subunits (Gilman, 1987; Freissmuth et al., 1989; Dessauer et al., 1996), an effector (PLC β in the case of Ca²⁺ signaling; Rebecchi and Pentylala, 2000), and regulators of G-protein signaling (RGS) proteins (Ishii and Kurachi, 2003; Wieland and Mittmann, 2003). In the case of Ca²⁺ signaling, the effectors are the different PLC β

isoforms, which cleave the minor lipid PIP₂, to yield two different second messengers diacylglycerol (DAG) and IP₃. IP₃ and DAG act on separate targets. DAG activates protein kinase C (PKC) and some transient receptor potential canonical (TRPC) channels, and IP₃ activates the IP₃ receptors (IP₃Rs) and the Ca²⁺ release channels in the ER (Berridge, 1993). Activation of IP₃Rs by IP₃ releases the Ca²⁺ stored in the ER to the cytoplasm. Since the ER spans the entire cell, such a system can deliver Ca²⁺ to virtually every domain and every site in the cytoplasm without compromising cellular homeostasis or creating large concentration gradients.

Replenishment of ER Ca²⁺, by SERCA pump, at the termination of the stimulation state depends on Ca²⁺ influx channels at the PM that sense the Ca²⁺ content of the ER and open in response to Ca²⁺ release from the ER to allow Ca²⁺ influx into the cytoplasm, which is used to reload the ER with Ca²⁺ (Parekh et al., 1997; Kiselyov et al., 2003; Parekh and Putney, 2005). Removal of Ca²⁺ from the cytoplasm depends on the activity of the ER and the PM Ca²⁺ pumps, SERCA, and PMCA, respectively (Shull, 2000). The Ca²⁺ signals that can last for many hours, are in the form of Ca²⁺ oscillations with particular amplitude and frequency.

AIRWAY EPITHELIA AND CALCIUM SIGNALING

Changes in cytosolic Ca²⁺ in non-excitabile cells in response to hormone, growth factor, or cytokine that activate C-type phospholipase (PLC) are biphasic. An initial transient rise is followed by less pronounced but sustained elevation (Putney, 1987; Berridge and Irvine, 1989; Berridge, 1993). The initial phase originates from the inositol trisphosphate-induced release Ca²⁺ from intracellular stores and is transient due to the activity of the membrane Ca²⁺ pumps ATPase. The sustained phase of the increase in cytosolic Ca²⁺ requires the continued activation of a Ca²⁺ influx pathway to maintain sustained response. In airway epithelial cells like numerous cell types, the intracellular Ca²⁺ increase promotes many cellular processes, as the regulation of Ca²⁺-activated Cl⁻ conductance (CaCC) in the airway epithelial (Paradiso et al., 1991; Grubb et al., 1994). Furthermore, CaCC conductance plays a role the maintenance of periciliary liquid (PCL) height in CF airway epithelia. In CF epithelia the PCL is more rapidly absorbed from airway surfaces and could not maintain a functional PCL height/volume under basal conditions. To compensate for the absence of CFTR-Cl⁻ secretion, the Ca²⁺ activated Cl⁻ conductance is thought to play an important role in the regulation of the height of CF epithelium during the motion phase (Tarran et al., 2005). These observations highlighted the role of intracellular Ca²⁺ and of CaCC conductance in CF physiopathology. Moreover, it has been well established that airway ciliary beat frequency (CBF) is strongly regulated by second messenger, such as Ca²⁺ (Lansley et al., 1992; Braiman et al., 1998; Evans and Sanderson, 1999). The CBF is a key factor for the regulation of mucociliary transport and thus for the mechanisms of defense of the respiratory tract (Satir and Sleight, 1990; Wanner et al., 1996). But still, Ribeiro et al. (2005a,b) have demonstrated that Ca²⁺ mobilization induced by G-protein-coupled specific receptors is increased in human polarized CF epithelial cell compared to control cells and that these differences appear independent of CFTR mutation, but are the consequence of cells exposition to infectious factors.

In CF epithelial cells the presence of external Ca²⁺ agonists (ATP and histamine) induces a higher Ca²⁺ mobilization compared to non-CF cells (Antigny et al., 2008a). Several components of Ca²⁺ signaling pathway are disturbed in human CF cells compared to non-CF cells, and particularly ER Ca²⁺ release and Ca²⁺ entry through the PM. At ER level, IP₃Rs Ca²⁺ release is abnormally increased in CF epithelial cells compared to non-CF cells (Antigny et al., 2009). Abnormal increase of IP₃R Ca²⁺ release in CF human epithelial cells is proposed to be the consequence of F508del-CFTR retention in ER compartment (Antigny et al., 2009). In airway epithelial cell lines, the abnormal F508del-CFTR retention provokes concentration of the ER around nucleus followed by an increase of IP₃R activity. The abnormal F508del-CFTR ER-retention induces an ER condensation (Ribeiro et al., 2005a; Antigny et al., 2009), which in turn, by causing a likely IP₃Rs clustering of the ER membrane could facilitate the formation of highly sensitive Ca²⁺ release sites.

Ca²⁺ SIGNALING IN CFTR PROTEIN CHAPERONING

The ER is a centrally located organelle, which affects most cellular functions. Its unique luminal environment consists of Ca²⁺ binding chaperones, which are involved in protein folding, post-translational modification, Ca²⁺ storage and release, and lipid synthesis and metabolism (Baumann and Walz, 2001; Berridge, 2002). The lumen of the ER contains many proteins which carry out these diverse functions (Bergeron et al., 1994; Meldolesi and Pozzan, 1998; Nicchitta, 1998; Corbett et al., 1999; Corbett and Michalak, 2000; High et al., 2000; Molinari and Helenius, 2000; Baumann and Walz, 2001; Jakob et al., 2001). Many severe diseases result from impaired function of the ER membrane and its protein folding machinery (Brooks, 1999; Jakob et al., 2001; Sherman and Goldberg, 2001). Calreticulin, a major Ca²⁺ binding chaperone in the ER, is a key component of the calreticulin/calnexin cycle which is responsible for the folding of newly synthesized proteins and glycoproteins and for the quality control pathways in the ER (Michalak et al., 2002; Gelebart et al., 2005). The function of calreticulin, calnexin, and other ER proteins is affected by continuous fluctuations in the concentration of Ca²⁺ in the ER. Thus, changes in Ca²⁺ concentration may play a signaling role in the lumen of the ER as well as in the cytosol (Michalak et al., 2002). Of note, the decreasing of calnexin-F508del-CFTR interaction by of ER Ca²⁺ depletion by SERCA pump inhibitors leads to the partial correction of abnormal F508del-CFTR trafficking (Egan et al., 2002, 2004; Norez et al., 2006a). Recently, Martino et al. (2009) demonstrated that the inflamed CF human bronchial epithelia (HBE), or normal HBE exposed to supernatant from mucopurulent material (SMM) from CF airways, exhibit ER/Ca²⁺ store expansion and amplified Ca²⁺-mediated inflammation. HBE inflammation triggers an unfolded protein response (UPR) coupled to mRNA splicing of X-box binding protein-1 (XBP-1). The link between airway epithelial inflammation, XBP-1s, and ER/Ca²⁺ store expansion was then addressed in murine airways challenged with phosphate-buffered saline or *Pseudomonas aeruginosa*. *P. aeruginosa*-challenged mice exhibited airway epithelial ER/Ca²⁺ store expansion, which correlated with airway inflammation. These findings suggest that, in

inflamed HBE, XBP-1s is responsible for the ER/Ca²⁺ store expansion that confers amplification of Ca²⁺-dependent inflammatory response.

Ca²⁺ SIGNALING AND TRPC CHANNELS IN CF CELLS

It is well established that in secretory epithelial cells, the Ca²⁺ signal is dependent on the polarity of the cells and is always initiated at the apical pole to propagate to the basal pole (Petersen and Tepikin, 2008). In non-CF cells, there is no evidence in favor of the involvement of apical CFTR activity in the regulation of Ca²⁺ homeostasis. On the contrary, in CF cells, the different Ca²⁺ responses observed (versus non-CF cells) are dependent on the presence of CFTR to the PM (Antigny et al., 2008a). How can this be explained? Among others, one possible hypothesis is that TRPC channels constitute a missing link between the abnormal Ca²⁺ levels observed in CF cells and CFTR dysfunction.

To test for this hypothesis, the involvement of TRPC channels in airway epithelial PM Ca²⁺ influx has been investigated. TRPC are known to form divalent cation selective and non-selective cation channels (Nilius and Droogmans, 2001; Clapham, 2003). In respiratory diseases, TRPC6 isoform is proposed to contribute to mucus hypersecretion (Li et al., 2003). Interestingly, it was also observed that the store operated Ca²⁺ entry (SOCE), activated by thapsigargin-ER Ca²⁺ release in CF cells is similar in non-CF cells, suggesting that STIM1 protein (Stromal interacting molecule 1; Liou et al., 2005; Roos et al., 2005; Zhang et al., 2005) and Orai1 (Feske et al., 2005; Vig et al., 2006; Yeromin et al., 2006) channels are not disrupted in F508del-CFTR expressing cells (Antigny et al., 2011). Thus, TRPC6 channel could be a major actor for supporting the abnormal Ca²⁺ entry in CF cells. Moreover, recent evidence also suggests that both wt-CFTR and F508del-CFTR PM proteins down-regulate the TRPC6-mediated Ca²⁺ influx and TRPC6 up-regulates CFTR-dependent Cl⁻ transport (Antigny et al., 2011). This reciprocal coupling has been observed not only in CF and non-CF cell lines but also in freshly isolated ciliated human epithelial cells (Antigny et al., 2011). These observations lead to the emergency of a novel model in which CFTR and TRPC6 are present within the same complex, each channel regulating the other.

However, other molecular entities could also support the abnormal Ca²⁺ entry in CF cells. For example, a more recent study suggests that F508del-CFTR cells have enhanced SOCE which would be mediated by the ER-resident Ca²⁺ sensor protein stromal interaction 1 (STIM1) and the Ca²⁺ release-activated channel Orai1 (Balghi et al., 2011). It is also proposed that the Ca²⁺ up-regulation results from the absence of CFTR at the PM but would be unrelated to the stimulation of TRPC6 channels because TRPC6 has not been detected in the cell line CF (Balghi et al., 2011) whereas it is present in freshly isolated ciliated human epithelial cells (Antigny et al., 2011).

Thus, further experiments will be needed to clearly understand the molecular mechanisms leading to PM Ca²⁺ influx in epithelial CF and non-CF cells and the role of TRPCs. Several TRPCs candidates are now knocking at the door such as TRPC6 and TRPC1 as recently shown in isolated pancreatic acini, a tissue in which STIM1 regulates TRPC1 (Hong et al., 2010).

Ca²⁺ SIGNALING IN CF INFLAMMATION

Cystic fibrosis airways are characterized by persistent infections and an excessive inflammatory response (Muhlebach et al., 1999; Chmiel et al., 2002; Muhlebach and Noah, 2002; Boucher, 2004). In patients with CF, lack of CFTR-Cl⁻ channel function leads to progressive pulmonary damage frequently associated with a severe and persistent neutrophil-dominated endo-bronchial inflammation and bacterial infection (Tirouvanziam, 2006). The molecular mechanisms connecting abnormal CFTR function in airway epithelial cells to excessive lung neutrophilic inflammation have not been fully elucidated.

Abnormal CFTR function activity results in airway surface dehydration and a modification of the properties of mucus clearance that participate to the susceptibility of chronic infection with pathogens such as *P. aeruginosa*, *staphylococcus aureus*, and *Haemophilus influenza* (Pier et al., 1996, 1997; Chmiel and Davis, 2003; Donaldson et al., 2006; Boucher, 2007). In response to bacterial infection, CF airways epithelial cells secrete many inflammatory mediators into the airway lumen (Levine, 1995; Polito and Proud, 1998; Diamond et al., 2000). An exaggerated inflammatory response has been demonstrated *in vivo* and in *in vitro* studies. Interleukin-8 (IL-8) levels were increased in bronchial submucosal glands from patients homozygous for F508del-CFTR mutation compared to control subjects, and IL-8 release is 13-fold higher in cultured CF than in normal human bronchial glands (Tabary et al., 2000). Tabary et al. (2000) have also demonstrated that IL-8 secretion is higher in primary human bronchial gland cells derived from F508del-CFTR patients compared to non-CF bronchial gland cells. Then, it has been shown that the intracellular Ca²⁺ increase is an intermediary step in the signal transduction events linking cell stimulation by inflammatory factors to NF-κB (Nuclear Factor Kappa B) activation (Ribeiro et al., 2005a; Tabary et al., 2006). Moreover, the correction of F508del-CFTR abnormal localization by an incubation of CF cells at 26°C promoted a decrease of NF-κB activity via decrease of Ca²⁺ release (Tabary et al., 2001).

Ca²⁺ AND INFECTION IN CF

Two major explanations of the mechanisms of dysregulation of intracellular Ca²⁺ homeostasis in CF are the “infection-driven” and the “mutated CFTR-dependent” mechanistic hypothesis.

Lung mucus production is an adaptive mechanism whose function is to provide a barrier between lung cells and noxious stimuli in the inspired air. These include viruses, bacteria, dust particles, and air pollution. Relevant stimuli for mucus production in CF include bacterial pathogens and inflammation (Oliver et al., 2000). Persistent mucus hypersecretion is often associated with CF. One of the stimulatory mechanisms triggered by bacterial pathogens that contribute to mucin overproduction in CF involves Ca²⁺ signaling (McNamara and Basbaum, 2001). Indeed, the protein flagellin is a major structural component of bacterial flagella in both Gram positive and Gram negative bacteria and it has been shown that *P. aeruginosa* flagellin can elicit host cell responses through binding to a glycolipid receptor, asialoGM1 (ASGM1; Feldman et al., 1998). McNamara et al. demonstrated that ASGM1 ligation stimulates transcription of the mucin MUC 2 and this process involves the release of ATP extracellular followed by activation of cell surface ATP receptors and then Ca²⁺ mobilization

(McNamara and Basbaum, 2001). Flagellin increases the association between flagellin receptors ASGM1 and Toll-like receptor 2 as well as 5 (TLR2 and TLR5) to stimulate the release of ATP. ATP binds and activates a G-protein-coupled nucleotide receptor on the cell surface, leading to Ca²⁺ mobilization (Adamo et al., 2004; McNamara et al., 2006). Finally, it is predicted that the synergistic effects of ATP and other [Ca²⁺]_i-raising agonists to augment activation by flagellin will be larger in CF cells than in non-CF cells, potentially contributing to hyperinflammation in CF airways (Fu et al., 2007). More recently, functional studies performed in HBE cells exposed to *P. aeruginosa* demonstrate that phospholipase C-β3 (PLCβ3), by regulating intracellular calcium transients, play a relevant role in amplifying the expression and release of IL-8, the major chemokine recruiting neutrophils in CF airway lungs (Bezzetti et al., 2011). Balghi et al. (2011) have demonstrated that elevated Ca²⁺ signaling in CF cells, is caused by an increase in the exocytotic insertion of Orai1 into the PM and the formation of more STIM1/Orai1 complexes during store depletion. This phenomenon induces the increased SOCE in CF cells and enhances IL-8 secretion; therefore it may contribute to the hyperinflammatory state that characterizes CF (Balghi et al., 2011).

Pseudomonas aeruginosa relies on quorum sensing molecules such as the autoinducer *N*-3-oxododecanoyl homoserine lactone (3O-C12) to drive the expression of numerous genes related to virulence (Erickson et al., 2002), biofilm formation (Singh et al., 2000), and antibiotic resistance (Möker et al., 2010) when colonizing the CF lung. The lactone 3O-C12 has been shown to trigger Ca²⁺ release from the ER in airway epithelial cells (Schwarzer et al., 2001). The autoinducer 3O-C12 has been demonstrated to induce proinflammatory cytokine production in airway epithelial cells in a calcium-dependent manner, and that dysregulated calcium storage or signaling in CF cells results in an increased production of proinflammatory cytokines (Mayer et al., 2011).

In addition, the expansion of ER observed in CF cells (Ribeiro et al., 2005b; Antigny et al., 2008b) is reversible, in that the removal of the SMM from infected/inflamed bronchi of CF patients normalized the size of the intracellular stores of CF cells and, consistently, non-CF cells show progressive expansion of intracellular calcium stores after long term incubation *in vitro* with SMM (Ribeiro et al., 2005a). Regarding host defense mechanisms that clear airway surfaces, the raised intracellular Ca²⁺ release due to ER expansion may provide an adaptive response for both the normal and CF airways. A higher Ca²⁺ mobilization may be particularly useful to CF patients, who depend solely on Ca²⁺-dependent Cl⁻ channel to compensate for the absent cAMP-mediated Cl⁻ secretion in CF. However, these data suggested that the increased ER size and Ca²⁺ storage, following chronic exposure to bacterial factors, is independent of the intrinsic F508del-CFTR defect (Ribeiro et al., 2005a). On contrary, the effect of *Cftr* genotype on the apoptotic response of airway epithelial cells to *P. aeruginosa*, indicated that HBE cells expressing F508del-CFTR underwent significantly delayed apoptosis compared to cells expressing wt-CFTR (Cannon et al., 2003). Moreover, mice with wild-type *Cftr* alleles had apoptotic cell in their lungs after *P. aeruginosa* infections, whereas mice homozygous for the F508del- or G551D-*Cftr* alleles showed little apoptosis in response to acute infection

(Cannon et al., 2003). Then, CFTR-associated defects in apoptosis may contribute to the pathogenesis of the lung disease in CF.

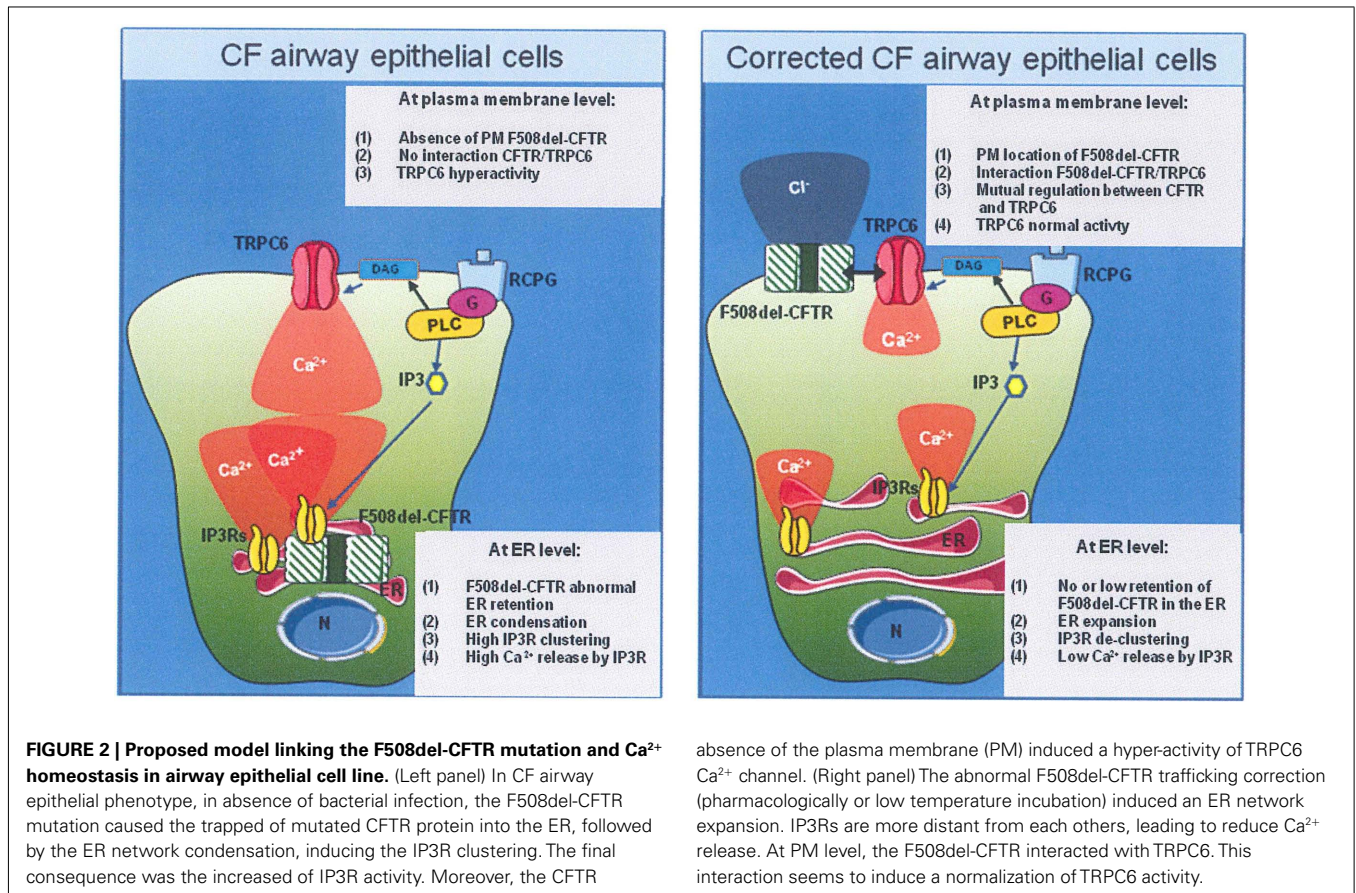
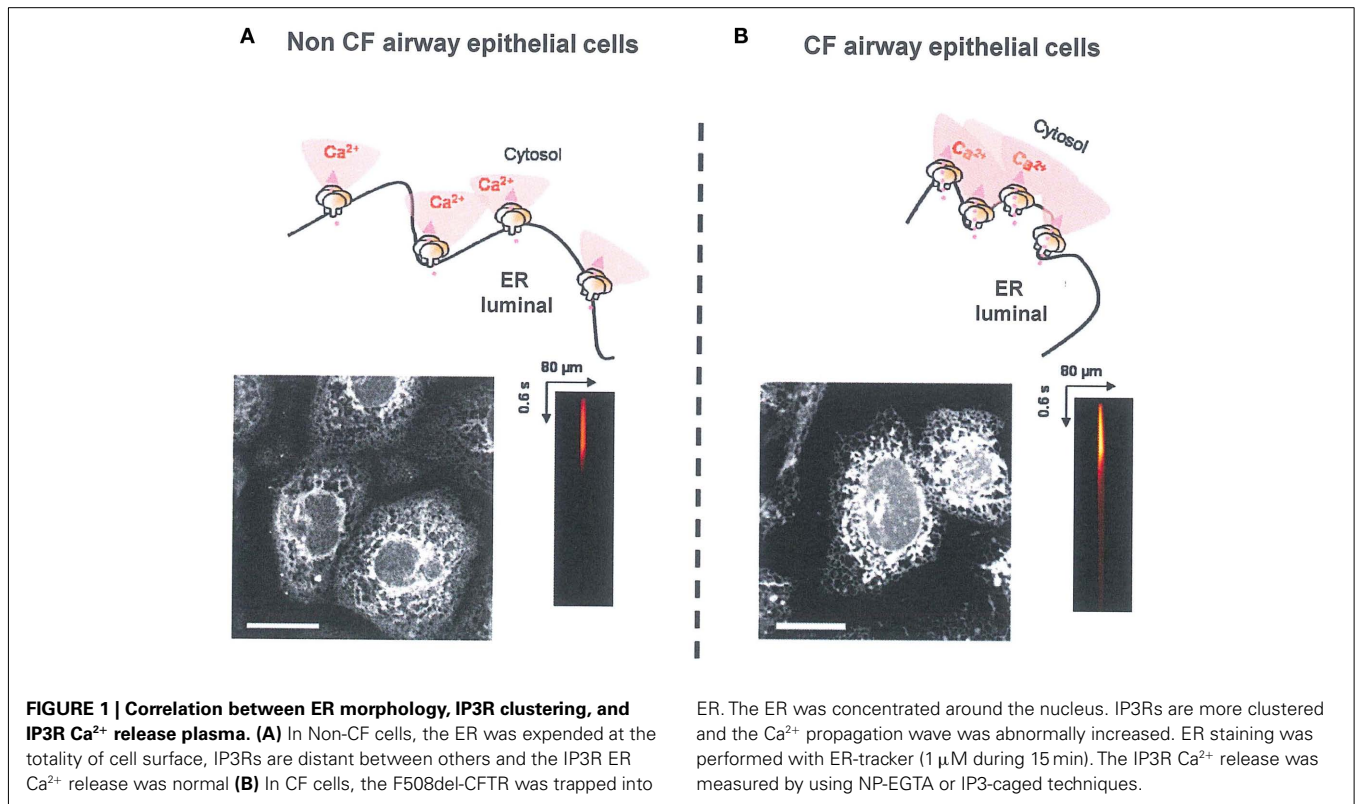
In conclusion, both major hypothesis are not mutually exclusive, at least since the “mutated CFTR-dependent” mechanism could be worsened in the progression of the disease by the “infection-driven” expansion of the intracellular calcium stores.

CALCIUM HOMEOSTASIS AND F508del-CFTR RESCUE

At all hot spots of Ca²⁺ signaling such as global Ca²⁺ response, IP3R-dependent Ca²⁺ release, and TRPC6-dependent-Ca²⁺ influx, a normalization of these Ca²⁺ responses was observed by the rescue of F508del-CFTR, after pharmacological treatment or after 24 h at low temperature (27°C; Antigny et al., 2008a, 2009, 2011). For example, the daily treatment of CF cells for 2 months with low concentration of the corrector miglustat (*N*-butyldeoxynojirimycin), an inhibitor of the α-1,2 glucosidase, results in progressive, stable reversible, and sustained correction of F508del-CFTR trafficking (Norez et al., 2009). This progressive correction is also accompanied by a down regulation of Ca²⁺ homeostasis (Norez et al., 2009). These works demonstrated a good correlation between the presence of F508del-CFTR at the PM and the normalization of global Ca²⁺ mobilization induced by histamine stimulation.

At the ER level, the IP3R hyper-activity and the ER condensation observed in CF airway were also normalized by the correction of abnormal F508del-CFTR trafficking. Moreover, using CF cells which co-expressed endogenous F508del-CFTR (trapped in the ER) and exogenous wt-CFTR (localized to the PM), the abnormal IP3R activity observed in CF human epithelial cells appears to be the consequence of the retention of endogenous F508del-CFTR in ER compartment (Antigny et al., 2009). In addition to requiring IP3, IP3R are regulated in a biphasic manner by direct interaction with Ca²⁺, i.e., activation at low concentrations (up to 0.3 μM) and inhibition at higher concentrations (0.5–1 μM; Bezprozvanny et al., 1991). These different regulations of IP3Rs by local Ca²⁺ concentrations are involved in the complex feedback regulation of the Ca²⁺ release (De Smedt et al., 1997). According to the results of Ribeiro et al. (2005a,b), even in absence of bacterial infection, the ER network is concentrated around the nucleus and expanded throughout the nucleus in corrected and non-CF epithelial cells. This expansion or condensation of ER network is responsible for the variation in local IP3Rs Ca²⁺ dependent activity (Antigny et al., 2009). In corrected CF cells, IP3Rs are more distant from each other, leading to reduce propagation of the Ca²⁺ wave (Figure 1).

At the PM level, TRPC6 exacerbated activity in CF epithelial cells could be the result of PM CFTR absence caused by CFTR mutations and not dependent of CFTR activity (Antigny et al., 2011). The down regulation of TRPC6 activity by PM CFTR is reminiscent of the interaction observed for the epithelial Na⁺ channel (ENaC) and CFTR in airway epithelia (Guggino and Stanton, 2006). Indeed, although still controversial (Itani et al., 2011) CF disease is also characterized by an Na⁺ hyperabsorption via the apical activity of ENaC channels (Donaldson and Boucher, 2007). CFTR plays a critical role (not fully understood) in the regulation of ENaC activity via intermolecular interaction (Stutts et al., 1995; Donaldson and Boucher, 2007). Moreover, as for Ca²⁺ signaling



(TRPC6 activity for example) the ENaC activity is also normalized by the correction of abnormal trafficking of F508del-CFTR (Noel et al., 2008). Therefore, CFTR seems to control the activity of both ENaC and TRPC6 channels.

The Ca²⁺ permeable TRPC cation channels are of particular interest in the pathophysiology of CF for several reasons. First, TRPC6 may contribute to mucus hypersecretion, a feature found in many respiratory diseases, including CF (Li et al., 2003). Second, the misregulation of TRPC6 channels may be relevant to CF disease because it might contribute to the increased Na⁺ absorption described in CF airway epithelial, as described with TRPV4 channel (Arniges et al., 2004).

CONCLUSION

The relationship between CFTR and Ca²⁺ signaling in human epithelial cells is far from being fully understood. Some studies showed that the Ca²⁺ signaling exacerbation in human primary epithelial cells is independent of CFTR and would be the consequence of the presence of infectious factors. But other studies proposed that perturbation of Ca²⁺ signaling in CF airway epithelial cells is a direct consequence of CFTR mutation and misprocessing. Also, more recent data now suggested a good correlation between

CFTR-related defects and abnormal Ca²⁺ signaling in CF airway epithelial cell.

A novel working hypothesis is now emerging, in which CFTR and TRPC6 channels are both present within a multiprotein membranous complex, each channel down regulating the other; i.e., CFTR down-regulates TRPC6-dependent-Ca²⁺ influx and TRPC6 up-regulates CFTR-dependent Cl⁻ transport. In addition STIM1, Orai1, and other TRPCs could also contribute to this signaling pathway. In addition STIM1, Orai1, and other TRPCs could also contribute to this signaling pathway. A scheme linking the putative sequence of Ca²⁺ signal perturbations to F508del-CFTR mutation is proposed in **Figure 2**.

Despite the progresses made in recent years to understand the role of Ca²⁺ signaling in CF, further experiments will be needed to dissect and understand the complexity of the regulation of CFTR protein activity and biogenesis by Ca²⁺ and to precise the molecular interactions between CFTR and TRPC proteins as well as their consequences for the physiopathology of CF.

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