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Fine Tuning of CFTR Traffic and Function by PDZ Scaffolding Proteins

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1. Introduction

Cystic fibrosis (CF) is the most common lethal genetic disorder in Caucasian population (Welsh et al., 1995). This pathology is due to mutations in the CF transmembrane conductance regulator (CFTR) encoding gene leading to alterations or loss of function of this channel (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989). CF affects several organs such as sweat glands, reproductive system and gastrointestinal tract, but the first cause of morbidity and mortality is respiratory system affections. While gene therapy for replacement of CFTR was a promising curative approach since the discovery of the CFTR gene, it turned to be more difficult than initially thought and no cure has arisen so far. Protein-protein interactions are powerful regulators of both protein trafficking and function, and can be enhanced or prevented by small molecules and short peptides (Zhang et al., 2011). Modulating these protein interactions are becoming a hopeful approach to develop new treatments for various diseases, including CF.

2. CFTR protein localization, structure and functions

CFTR protein is a chloride channel expressed at the apical membrane of polarized cells (Dalemans et al., 1992) and randomly expressed at the plasma membrane of non-polarized cells (Cheng et al., 1990). Moreover, CFTR protein has been also localized at the membrane of organelles such as endoplasmic reticulum (ER) where it is inserted upon biosynthesis (Pasyk & Foskett, 1995), Trans-Golgi network where it matures, endosomes where it interchanges with plasma membrane (Lukacs et al., 1992) and lysosomes where it is degraded (Barasch et al., 1991). CFTR is the only chloride channel of the adenosine triphosphate (ATP) Binding Cassette (ABC) transporters family. As several ABC transporters, CFTR protein is composed of two transmembrane domains (TMD) and two nucleotide binding domains (NBD). Unlike the other ABC transporters, CFTR also possesses a regulatory (R) domain. CFTR gating is controlled by two simultaneous phenomena: (i) the fixation and the hydrolysis of ATP on NBD domains (Anderson et al., 1991) and (ii) the phosphorylation of specific residues on the R domain by different kinases

such as the 3'-5' cyclic adenosine monophosphate (cAMP) dependant protein kinase (PKA) (Berger et al., 1991; Tabcharani et al., 1991). In addition to be a chloride channel, CFTR also regulates other transmembrane proteins. Thereby, in several epithelial cells, CFTR expression decreases the epithelial Na^+ channel (ENaC) (Stutts et al., 1995) and Ca^{2+}-dependent Cl^- channel (CaCC) (Kunzelmann et al., 1997; Wei et al., 1999) activities; and increases the function of the outwardly rectifying Cl^- channel (ORCC) (Egan et al., 1992; Gabriel et al., 1993), the $Cl^-/HCO3^+$ exchangers of SLC26 family (Ko et al., 2002), the Renal Outer Medullary K^+ channel (ROMK) (Loussouarn et al., 1996) and some aquaporins (AQP) (Schreiber et al., 1997; Schreiber et al., 1999). Consequently, CFTR loss of function is responsible of a general dysregulation of ion transports in cells. Moreover, the regulation of most of these ion channels by CFTR occurs through interactions with Postsynaptic density-95/ Disc large/ Zonula occludens-1 (PDZ) scaffolding proteins (Lohi et al., 2003; Mohler et al., 1999; Pietrement et al., 2008; Yoo et al., 2004).

3. CFTR interacts with PDZ proteins through its C-terminus tail

PDZ domains are highly conserved sequences of about 80-90 amino acids known to be the most abundant protein-protein interaction modules in the human genome. Their three-dimensional structure is composed of 6 β sheets and 2 α helixes forming a cavity able to receive a protein motif of 3 to 7 amino acids, generally expressed at the C-terminus cytosolic tail of the target proteins (Bezprozvanny & Maximov, 2001; Fanning & Anderson, 1999; Harris & Lim, 2001; Hung & Sheng, 2002). However, intra-protein motifs able to bind PDZ domains have also been described (Hillier et al., 1999; Paasche et al., 2005; Slattery et al., 2011). A single PDZ domain can bind several target proteins with variable affinities. Moreover, a single PDZ motif can be recognized by different PDZ domains. To date, four types of PDZ motifs have been reported (Table 1).

PDZ domain	Protein motif
Type I	S/T-x-Φ
Type II	Φ-x-Φ
Type III	Ψ-x-Φ
Type IV	D-x-V

x: any amino-acid; Φ: hydrophobic amino-acid; Ψ: hydrophilic amino-acid

Table 1. Consensus sequences linking the different PDZ domains.

A PDZ protein can have several PDZ domains and so can interact simultaneously with several partner proteins. In addition, PDZ proteins can also form homo- or hetero multimeric structure (Fouassier et al., 2000; Lalonde & Bretscher, 2009; Lau & Hall, 2001; Shenolikar et al., 2001) thus forming wide submembrane docking networks for multiple transmembrane proteins where they can interact with each other and with anchored cytosolic regulatory proteins. Therefore, PDZ proteins play an important role in protein stability at the plasma membrane (i.e. endocytosis and recycling) and function. Some PDZ proteins are ubiquitous and others have a cell type-specific expression. Considering the organ or the tissue, PDZ proteins can be implicated in cellular morphology, cellular polarity, intercellular contacts, cell migration and cell growth (Altschuler et al., 2003; Hall et al., 1998; Kocher et al., 1999; C. Li et al., 2005; 2007; Naren et al., 2003; Pietrement et al., 2008; Seidler et

al., 2009; Short et al., 1998; Singh et al., 2009; Wang et al., 1998; 2000; Yoo et al., 2004). CFTR possesses at its C-terminus a consensus motif, (D/E)-T-(R/K)-L, which belongs to the type I class of PDZ domain-binding motifs and is conserved across species (Table 2).

Species	CFTR C-terminal sequence
Human	SSKCKSKPQIAALKEETEEEVQ**DTRL**
Frog	SSKRKSRPQISALQEETEEEVQ**DTRL**
Rat	SSKQKPRTQITAVKEETEEEVQ**ETRL**
Mouse	SSKHKPRTQITALKEETEEEVQ**ETRL**
Dogfish	SSKRKTRPKISALQEEAEEDLQ**ETRL**
Sheep	SSRQRSRANIAALKEETEEEVQ**ETKL**
Bovine	SSRQRSRSNIAALKEETEEEVQ**ETKL**
Rabbit	SSKHKSRPQITALKEEAEEEVQ**GTRL**

Table 2. C-terminal sequence of CFTR protein from various species.

3.1 Role of CFTR C-terminus

Patients harbouring a C-terminal truncation of CFTR such as the deletion of the 26 last amino acids (CFTR-S1455X) have a mild CF phenotype. A first study described that a mother and her daughter, both heterozygous for CFTR-S1455X and deletion of exon 14a (del14a) mutations, exhibited no CF phenotype but only an increase in sweat Cl$^-$ concentration, while a second daughter homozygous for del14a mutant had a severe CF phenotype (Mickle et al., 1998). A second study described two sisters heterozygous for F508del, the most common CF mutation, and CFTR-S1455X with also no CF phenotype and an elevated sweat Cl$^-$ secretion (Salvatore et al., 2005). Those studies suggest that deletion of CFTR C-terminus has no major incidence on the phenotype of patients. The role of CFTR C-terminus was also studied *in vitro* using plasmid constructs where CFTR's PDZ binding motif was deleted (ΔTRL-CFTR). The resulting phenotype of ΔTRL-CFTR in polarized and non-polarized cells is controversial. In type I MDCK cells, Moyer and colleagues have demonstrated that PDZ binding motif is an apical polarization signal and its deletion decreases CFTR activity (Moyer et al., 1999; 2000). On the opposite, several teams have described that deletion of the PDZ binding motif of CFTR has no effect on its apical membrane localization nor its function in numerous cells such as BHK-21, COS-1, type II MDCK, CaCo-2, PANC-1 and primary human airway epithelial cells (Benharouga et al., 2003; Milewski et al., 2005; Ostedgaard et al., 2003). The discrepancy between these studies can be explained by additional sorting motifs (Milewski et al., 2005), but also by the use of MDCK type I cells as the unique polarized cell line by Moyer et al., whereas different non polarized cell models were used by the other groups. Indeed, MDCK type I and II cells exhibit different polarized sorting (Svennevig et al., 1995). Accordingly, CFTR polarized expression would be differentially regulated depending on the type or the origin of the cells. The role in this process of the six PDZ proteins described so far to interact with CFTR is not clearly established, but they indubitably have different functions in CFTR polarized expression.

3.2 Na$^+$/H$^+$ exchanger regulatory factor family proteins

Na$^+$/H$^+$ Exchanger Regulatory Factor (NHERF) family proteins possess multiple PDZ domains. NHERF1 (also called EBP50 for Ezrin-radixin-moesin Binding Phosphoprotein of 50 kDa), the most studied member of the NHERF family, is the first protein evoked to interact with CFTR through its PDZ domain (Hall et al., 1998; Short et al., 1998; Wang et al., 1998). The NHERF family comprises four members: NHERF1 and NHERF2 (also called E3KARP for Na$^+$/H$^+$ Exchanger 3 Kinase A Regulatory Protein) have two PDZ domains while NHERF3 (also called PDZK1, CAP70 or NaPi CAP-1) and NHERF4 (also called PDZK2, IKEPP or NaPi CAP-2) have 4 PDZ domains (Fig.1). In addition, NHERF1 and NHERF2 have in their C-terminus tail a consensus sequence for ezrin binding, allowing their anchor to the actin cytoskeleton (Reczek et al., 1997).

NHERF1 and NHERF2 have similar functions but their cell expression is generally mutually exclusive *in vivo* (Ingraffea et al., 2002). In human lungs, NHERF1 is expressed in epithelial cells while NHERF2 is expressed in alveolar cells (Ingraffea et al., 2002). However, it seems not to be the case in cell lines. The affinity of CFTR C-terminus for PDZ domains varies across NHERF family members Indeed, CFTR C-terminus tail interacts preferentially with PDZ1 domain of NHERF1 (Wang et al., 1998) but with PDZ2 domain of NHERF2 (Hall et al., 1998; Sun et al., 2000). NHERF1 C-terminus tail interacts with its own PDZ2 domain preventing NHERF1 binding to ezrin. This auto-inhibition of NHERF1 is abolished by PKC phosphorylation (J. Li et al., 2007), which promotes macromolecular complex formation. In addition, both NHERF1 and NHERF2 have been shown to form homo- and heterodimers (Lau & Hall, 2001; Shenolikar et al., 2001).

CFTR C-terminus tail can interact with three out of four PDZ domains of NHERF3 (Wang et al., 2000). To date, only one team has demonstrated an interaction between CFTR and NHERF4 in transfected sf9 insect cells (Hegedüs et al., 2003). On the opposite of NHERF1 and NHERF2, NHERF3 and NHERF4 cannot anchor CFTR to the actin network. However, as NHERF3 is able to bind NHERF1, it can indirectly link CFTR to the cytoskeleton (Lalonde & Bretscher, 2009).
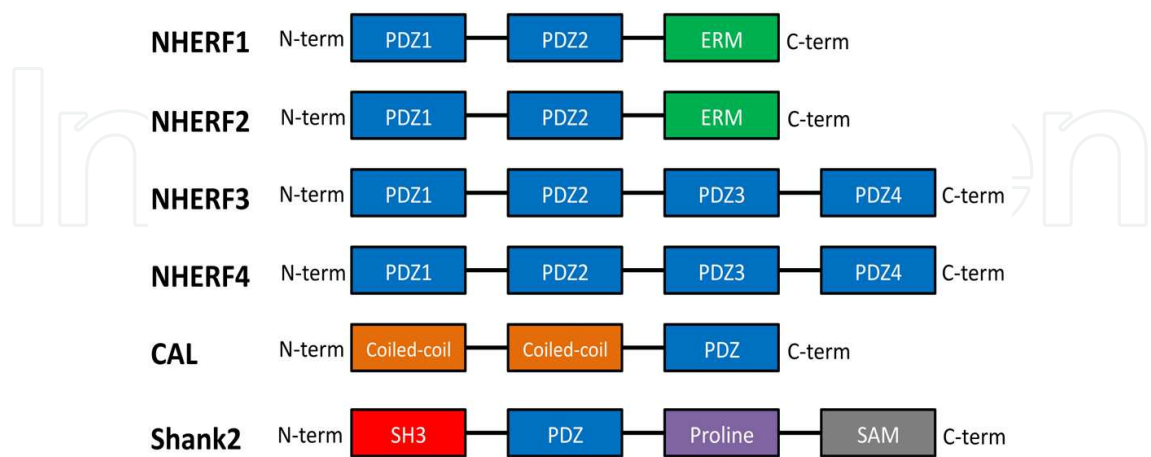
NHERF: Na$^+$/H$^+$ exchanger Regulatory Factor; CAL: CFTR Associated Ligand; ERM: Ezrin-Radixin-Moesin domain; PDZ: Postsynaptic density-95/ Disc large/ Zonula occludens-1; SAM: sterile Alpha Motif; SH3: Src Homology 3 domain; C-term: C-terminal; N-term: N-terminal.

Fig. 1. PDZ domain containing proteins interacting with CFTR C-terminus tail.

3.3 CFTR-associated ligand

CFTR-Associated Ligand (CAL, also called Golgi-associated PDZ and coiled-coil motif containing protein, GOPC, or PDZ Protein Interacting Specifically with TC10, PIST) is a protein of approximately 50 kDa containing a single PDZ domain and two coiled-coil domains (Fig.1). CAL can form homodimers independently of its single PDZ domain but through its N-terminal portion (Cheng et al., 2002; Cushing et al., 2008; Neudauer et al., 2001). CAL is ubiquitously expressed in the Golgi apparatus of human tissues. Although it has no transmembrane domain, CAL is associated with membranes by interacting with resident proteins from the Trans-Golgi network *via* its coiled-coil domain (Cheng et al., 2002). CAL can interact with CFTR C-terminus tail through its PDZ domain as determined by yeast two-hybrid assay and co-immunoprecipiation (Cheng et al., 2002).

3.4 Shank2

Shank2 (also known as Cortactin-binding protein 1, CortBP1; or Proline-rich synapse-associated protein 1, ProSAP1) contains a single PDZ domain and other sites for protein–protein interaction, including an SH3 domain, a long proline-rich region, and a sterile alpha motif (SAM) domain (Fig.1). The SAM domain is able to self-associate to form dimers (Gisler et al., 2001). Shank2 is expressed abundantly in brain as well as in kidney, liver, intestine, and pancreas. In this last tissue, it is localized to the luminal pole in pancreatic duct cells and luminal area of colonic epithelia (Du et al., 1998; Kim et al., 2004; Lee et al., 2007; Lim et al., 1999). Shank2 has been shown to be associated with CFTR through its PDZ domain in the yeast two-hybrid system and in mammalian cells (Kim et al., 2004).

4. PDZ interactions regulate CFTR trafficking

4.1 NHERF family proteins form a subapical network for CFTR plasma membrane docking

NHERF1, NHERF2 and NHERF3 can auto-assemble in a regulated fashion and form a subapical network serving as a cytoskeleton-anchored platform for the docking of multiple regulatory and transmembrane proteins. CFTR binding to this station is PDZ-dependent and results in increased stability at the plasma membrane. Indeed, a CFTR mutant truncated for its C-terminal PDZ interacting motif is highly mobile at the plasma membrane, whereas intact CFTR exhibits a greater immobile fraction or a more confined diffusion (Bates et al., 2006; Haggie et al., 2004; 2006). Moreover, C-terminal truncation of CFTR alters its endocytic/recycling dynamics. While CFTR endocytosis from the apical plasma membrane seems to be unaffected in polarized MDCK type I cells, C-terminal deletion decreases its recycling efficiency (Swiatecka-Urban et al., 2002). Despite the reported decrease in the half-life of ΔTRL-CFTR at the apical plasma membrane, the same group observed that its degradation rate was not accelerated. Albeit this should lead to an intracellular accumulation of ΔTRL-CFTR, it is not the case (Benharouga et al., 2003; Milewski et al., 2005; Ostedgaard et al., 2003), because other trafficking mechanisms may compensate for the recycling defect of ΔTRL-CFTR in order to ensure its observed apical localization. Recently, preliminary results from Bossard et al. (2010) reconcile this discrepancy by reporting a new intracellular trafficking pathway for CFTR polarized sorting. Indeed, apically endocytosed

CFTR is efficiently recycled back to the apical plasma membrane but a significant fraction is constitutively trafficked to the basolateral plasma membrane. This mistargeted pool is transiently localized at the basolateral cell surface, as it is rapidly rerouted to the apical plasma membrane. This transcytotic pathway occurs in various polarized cell lines such as canine kidney (MDCK type II), human airway (CFBE41o⁻) and pig kidney (LLC-PK1) epithelia (Bossard et al., 2010). When comparing wt- and ΔTRL-CFTR dynamics in CFBE epithelia, both proteins exhibit similar half-life and apical surface stability. However, ΔTRL-CFTR undergoes a faster endocytosis from the apical plasma membrane, a slower recycling to the apical membrane and a more intense transcytotic pathway (Fig.2). Those characteristics could compensate for ΔTRL-CFTR recycling defect and explain the comparable half-life and apical stability with its wt counterpart (Bossard et al., 2010). This new insight into CFTR intracellular trafficking uncovers a novel role of PDZ adaptors in protein sorting, and raises further questions about the cause of the mild CF phenotype observed in patients with C-terminally truncated CFTR mutants.
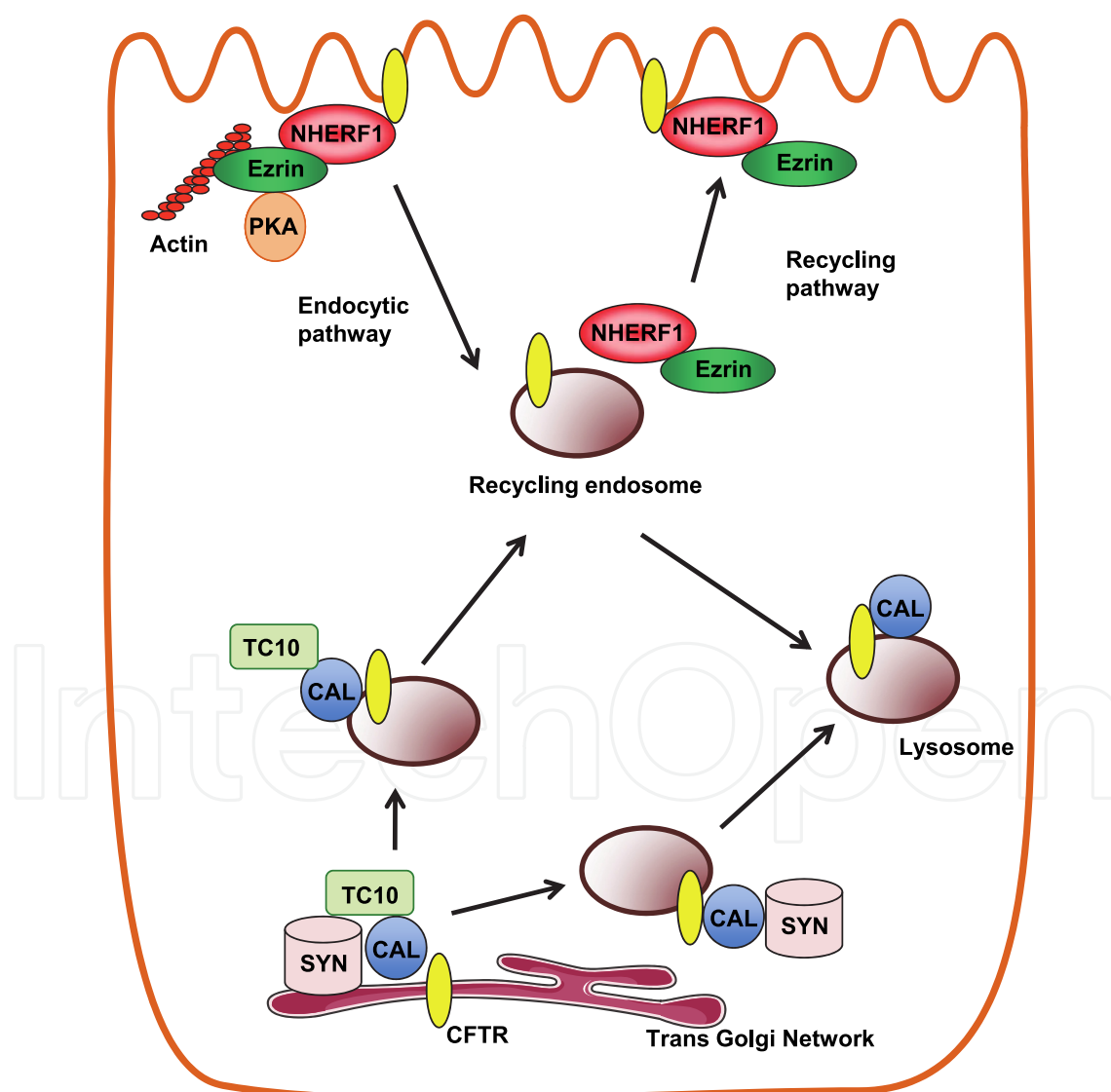

Fig. 2. Regulation of intracellular traffic of CFTR by interaction with several proteins with PDZ domains. SYN: synthaxine 6.

4.2 CAL targets CFTR to lysosomal degradation

PDZ interaction of CAL with CFTR occurs at the endosomal level and targets CFTR to lysosomal degradation (Cheng et al., 2004). This CAL-dependent sorting of CFTR is counteracted by NHERF1 binding (Cheng et al., 2002). Comparable to CFTR, both the beta1-adrenergic receptor and cadherin 23 interact with CAL, leading to their intracellular accumulation and/or degradation (Fig.2); these interactions can be competitively counteracted by binding with other PDZ domain-containing proteins (He et al., 2004; Xu et al., 2010). Likewise, CAL is an intracellular retention partner for the somatostatin receptor subtype 5 and the metabotropic glutamate receptor subtypes 1a (Wente et al., 2005; Zhang et al., 2008).

Accordingly, both inhibition of CAL protein expression and NHERF1 overexpression are efficient in promoting the cell surface expression and function of the most common disease-associated mutant, F508del-CFTR (Bossard et al., 2007; Guerra et al., 2005; Wolde et al., 2007). Interestingly, the conformational and molecular interactions between CFTR and CAL differ from those between CFTR and NHERF1, indicating that PDZ-selective inhibitors can be designed to improve CFTR mutant expression (Amacher et al., 2011; Cushing et al., 2010; Piserchio et al., 2005). CAL/CFTR interaction is modulated by the Rho family small GTPase, TC10. Its activation redistributes CAL to the plasma membrane and reverses CAL-mediated CFTR degradation (Cheng et al., 2005). Thus, CAL can play opposite roles on CFTR trafficking depending on the activation of TC10, which is a molecular switch between the degradation and exocytosis pathways. Moreover, the Q-SNARE [Q-soluble N-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor] protein syntaxin 6 interacts with both CAL and CFTR (Charest et al., 2001; Cheng et al., 2010). Syntaxin 6 binds the N-terminal half of CFTR after its interaction with CAL, and this binding mediates CFTR lysosomal degradation (Cheng et al., 2010).

4.3 Do Shank2 and NHERF4 regulate CFTR trafficking?

Only one study reports the modulation of CFTR membrane expression by Shank2. Shank2 overexpression tends to increase CFTR membrane expression and stability (Kim et al., 2004).

Since the first study showing a CFTR/NHERF4 interaction (Hegedüs et al., 2003), no more data were published. This could be explained, in part, by the fact that NHERF4 is not expressed in lungs. Its expression is restricted to the gastrointestinal tract and kidney in mouse (Gisler et al., 2001; Scott et al., 2002; Watanabe et al., 2006) without data in human. In particular, NHERF4 is localized close to or at the apical plasma membrane (Gisler et al., 2001; Scott et al., 2002; Van De Graaf et al., 2006; Watanabe et al., 2006), consistent with CFTR localization.

5. PDZ interactions regulate CFTR function

As a phosphorylation-regulated chloride channel, CFTR physical and functional proximity to kinases and phosphodiesterases has fundamental importance. NHERF1, NHERF2 and NHERF3 have all been reported to link CFTR to PKA. Indeed, NHERF1 and NHERF2 both interact with ezrin, a well known A kinase anchoring protein (AKAP) (Fig.2) (Reczek et al.,

1997; Sun et al., 2000). Likewise, NHERF3 is able to bind the dual-specific A-kinase anchoring protein 2 (D-AKAP2) with higher affinity than NHERF1 (Gisler et al., 2003). Moreover, AKAPs are also anchors for phosphodiesterases (PDE) (Dodge-Kafka et al., 2005; Willoughby et al., 2006), allowing local fine tuning of cAMP concentration for proper regulation of CFTR channel activity. The PDE4D is the most abundant PDE in airway epithelia and forms a cAMP diffusion barrier at the apical confinement where CFTR is localized (Barnes et al., 2005). As Shank2 is associated with PDE4D, thus reducing local cAMP availability, this could explain the observed inhibition of CFTR chloride activity (Lee et al., 2007).

CFTR activation by PKA is potentiated by PKC (Winpenny et al., 1995). As NHERF1 binds the Receptor for Activated C Kinase (RACK1) through PDZ1 interaction, this interaction could anchor PKC epsilon isoform in the vicinity of CFTR and facilitate its activation (Liedtke et al., 2002; 2004).

Besides anchoring cytosolic regulatory proteins, the apical docking station formed by PDZ proteins brings other transmembrane proteins closer to CFTR for reciprocal regulation. Likewise, CFTR and the ENaC display cross-functional regulation (Jiang et al., 2000). This regulation involves NHERF1 that binds the Yes-associated protein 65 (YAP65) through its PDZ2 domain and CFTR C-terminus through PDZ1 (Mohler et al., 1999). YAP65 is an anchoring protein for the cytosolic tyrosine kinase c-Yes, a member of the Src family, which has been reported to inhibit ENaC (Gilmore et al., 2001; Mohler et al., 1999). Furthermore, NHERF1 allows the adrenergic regulation of CFTR by bridging it with the beta2-adrenoceptor at the apical plasma membrane (Naren et al., 2003; Taouil et al., 2003). NHERF2 can form a molecular bond connecting CFTR to the lysophosphatidic acid type 2 receptor or the Na^+/H^+ exchanger isoform 3. The activation of those latter proteins is able to inhibit CFTR chloride current (Bagorda et al., 2002; Favia et al., 2006; C. Li et al., 2005). Moreover, NHERF3 connects CFTR to the cAMP transporter multidrug resistance protein 4 (MRP4), which enhances CFTR function (C. Li et al., 2007).

An additional mechanism for PDZ proteins to regulate CFTR activity is their ability to form CFTR homodimers as detected in the plasma membrane of mammalian cells (Ramjeesingh et al., 2003). The formation of CFTR dimers is triggered by NHERF1, NHERF2 and NHERF3 leading to an increase in CFTR channel activity (Li et al., 2004; J. Li et al., 2005; Wang et al., 2000).

The PDZ protein CAL has not been reported to have direct influence on CFTR chloride current. However, CAL-mediated lysosomal degradation of CFTR indirectly decreases CFTR channel activity by reducing its apical plasma membrane density.

Some studies have reported that annexin A5 (AnxA5) could be involved in the traffic of CFTR. Recently, in oocytes, AnxA5 inhibited CFTR-mediated whole-cell membrane conductance presumably by a mechanism independent of PDZ-binding domain at the C-terminus of CFTR but PKC-dependent and resulted from either endocytosis activation and/or exocytosis block. In contrast, in human cells, co-expression of AnxA5 augmented CFTR whole-cell currents, an effect that was independent of CFTR PDZ-binding domain. Those results suggest that AnxA5 has multiple effects on CFTR, but the effect observed is cell system-dependent (Faria et al., 2011).

6. Are PDZ proteins potential targets for drug therapy in CF?

Because PDZ proteins regulate CFTR membrane expression and/or function, several studies have investigated the potential therapeutic effectiveness of these proteins. To date, only the potential therapeutic role of NHERF1 overexpression or CAL silencing has been investigated.

6.1 NHERF1 overexpression restores F508del-CFTR plasma membrane expression

In 2005, Guerra and colleagues observed that mouse NHERF1 but not NHERF2 overexpression increased F508del-CFTR plasma membrane expression and activity in human bronchial epithelial cell line endogenously expressing F508del-CFTR (CFBE41o-) (Guerra et al., 2005) (Fig.2). Four years later, our team demonstrated in A549 and type II MDCK cells microinjected with F508del-CFTR plasmid that human NHERF1 overexpression restored F508del-CFTR apical plasma membrane expression and chloride channel activity (Bossard et al., 2007). This effect was abolished in the presence of a sense oligonucleotide complementary to NHERF1 mRNA sequence attesting that this mechanism is specific to NHERF1 overexpression (Bossard et al., 2007). Moreover, in type II MDCK cells microinjected with a CFTR double mutant: F508del and K1468X (deletion of the 12 last C-terminus amino acids, thus avoiding PDZ-based interaction), NHERF1 overexpression had no effect on F508del-K1468X-CFTR expression and activity certifying that an interaction between F508del-CFTR and NHERF1 is required (Bossard et al., 2007). Immunostaining experiments confirmed these results by demonstrating a colocalization of F508del-CFTR and NHERF1 at the apical plasma membrane (Bossard et al., 2007). Furthermore, it is important to note that NHERF1 overexpression was not a nonspecific global rescue of ER-retained proteins because it did not restore the plasma membrane expression of an unrelated trafficking defective mutant potassium channel, KCNQ1 [mutant P117L highlighted by Dahimene et al. (2006)] (Bossard et al., 2007).

Thereby, we are currently investigating the effects of NHERF1 overexpression on F508del-CFTR expression and activity *in vivo* by non viral gene transfer using block copolymers in homozygous F508del-CFTR mice (Desigaux et al., 2005).

6.2 CAL silencing restores F508del-CFTR plasma membrane expression

RNA interference targeting endogenous CAL specifically increases cell surface expression of the F508del-CFTR mutant and thus enhances transepithelial chloride currents in polarized CFBE41o- cells overexpressing F508del-CFTR (Wolde et al., 2007) (Fig.3).

Recently, it has been demonstrated that CAL interaction with F508del-CFTR can be avoided by using a blocking peptide, iCAL36 (ANSRWPTSII), which specifically targets CAL but not NHERF1, NHERF2 or NHERF3 PDZ domain (Cushing et al., 2010). The presence of iCAL36 extends F508del-CFTR half-life at the plasma membrane in the human bronchial epithelial cell line CFBE41o- (Cushing et al., 2010) (Fig.3). It is important to mention that this blocking peptide needs a delivery agent to allow its entry into the cells, thereby limiting its potential therapeutic use.

Syntaxin 6 acts at the Trans-Golgi Network where its silencing enhances the protein expression of the rescued, post-ER F508del-CFTR mutant, but not of the non-rescued, ER-trapped F508del-CFTR (Cheng et al., 2010). Thus, impairing CAL interaction with F508del-CFTR or other CFTR mutants as well as the inhibition of CAL/Syntaxin 6 interaction could represent new therapeutic tools for CF (Fig.3).
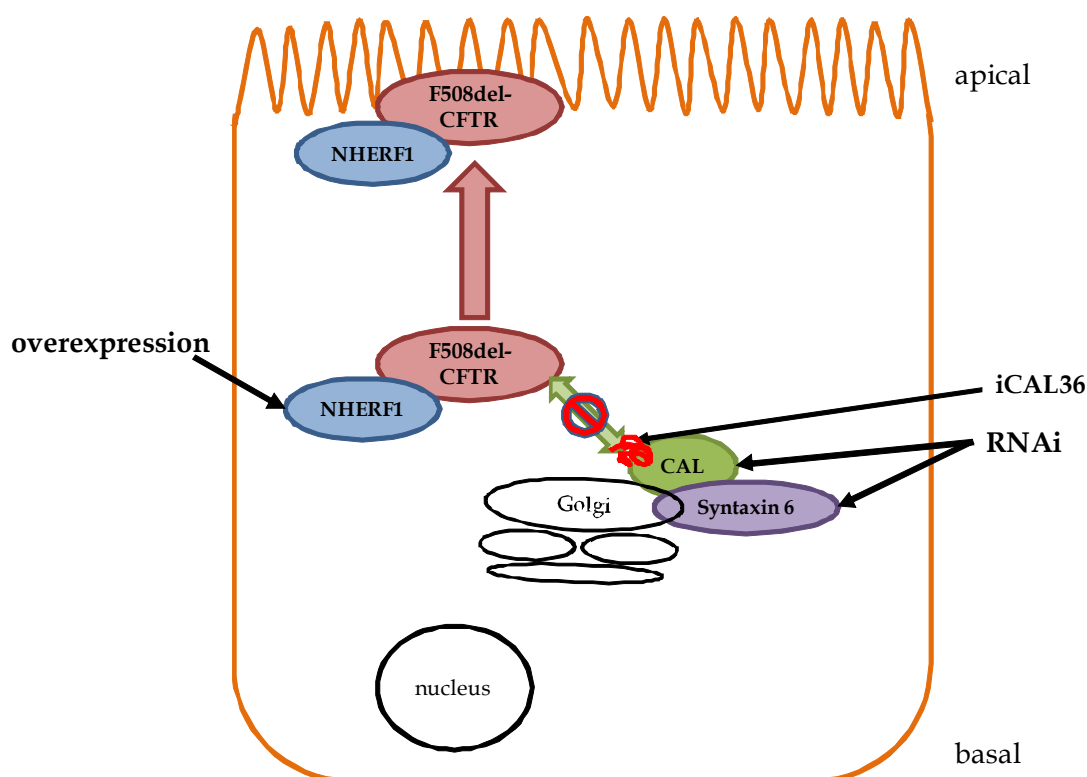
Fig. 3. Strategies for modulation of F508del-CFTR interactions with NHERF1 and CAL to enhance its apical plasma membrane expression.

6.3 Are PDZ interactions a potential drug target for CF treatment?

Since several decades, drug research has focused on finding compounds targeting receptors, ion channels, metabolic transporters and enzymes. More recently, the comprehension of the interrelated omics (genomics, proteomics, transcriptomics, metabonomics, interactomics, signalomics…) suggests that the protein-protein interactions could play a virtually universal role. Among them, PDZ-based interactions are a ubiquitous mechanism to modulate complex cellular processes. Therefore, the inhibition of a single protein-protein interaction should be highly selective in order to avoid undesirable effects.

Besides its conformational-related trafficking defect, the most frequent CFTR mutant, F508del-CFTR, also exhibits an intrinsic functional deficiency, suggesting that its potent rescue to the apical membrane might not be sufficient to restore a healthy phenotype. Consequently, PDZ protein-targeted drugs acting as a corrector treatment should be complemented with a potentiator treatment for effective therapeutic outcome. Moreover, drug or peptide candidates targeting PDZ interaction act exclusively as inhibitors, whereas PDZ interaction reinforcement (e.g. with NHERF1, NHERF2 and NHERF3) would also be suitable for CF treatment. Selective inhibition of interaction between CFTR and PDZ proteins, should likely target CAL and Shank2, which are inhibitors of CFTR expression and/or function. The physical properties of small molecule inhibitors - i.e. cell permeability, good oral bioavailability and high affinity - make them better candidates than peptidic inhibitors (including antibodies) which usually are rapidly degraded and have poor pharmacokinetics. Synthetic inhibitors can target the C-terminus (or sometimes internal)

PDZ motif or the PDZ domain of the PDZ adaptor, rendering either one unavailable for interaction. Taking CFTR as an example target, the inhibitor should avoid CFTR interaction with CAL but not with NHERF1, NHERF2 or NHERF3. This could be accomplished by competitive inhibition (i) if the small molecule inhibitor can block an interaction interface specific to CAL but not to NHERF1, NHERF2 or NHERF3, or (ii) if the affinity of CFTR C-terminus for the small molecule is similar to that for CAL, and lower than that for NHERF1, NHERF2 or NHERF3 which has already been reported (Cushing et al., 2008). It is important that the small molecules that bind CAL to inhibit CAL/CFTR interaction, should not interfere with CAL's other partners in order to prevent any possible adverse effect. In any case, the crystal structure of both interacting partners and especially the geometry of the protein-protein interaction interface can help to rationally design small molecules inhibitors. Moreover, the targeted interaction could only be material in specific organs, tissues, cell types and subcellular compartments, hence complicating the task to achieve targeted drug delivery and selective effects. Although, small molecule inhibitors of PDZ-based interactions are of great interest as research tools for understanding the involvement of these scaffolding proteins in protein trafficking and function, their usefulness as therapeutic agents is still elusive and needs further investigations.

7. Conclusion

In conclusion, PDZ domains containing proteins have a fundamental role in the regulation of CFTR trafficking and chloride channel activity. The modulation of their selective interaction with CFTR using gene therapy and/or drug treatments is an auspicious approach for the treatment of patients harboring CFTR trafficking defect mutations such as F508del, but it remains to be intensively and carefully investigated in order to assess their specificity and possible side effects.

8. Acknowledgment

9. References

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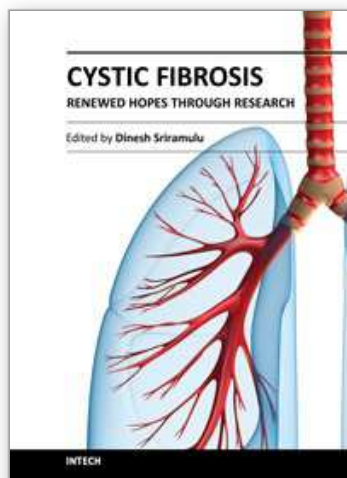
Cystic Fibrosis - Renewed Hopes Through Research

Edited by Dr. Dinesh Sriramulu

Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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