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## Molecular studies of CFTR interacting proteins

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**Abstract** Transport via the cystic fibrosis transmembrane conductance regulator (CFTR) is activated by its interactions with cytoplasmic cofactors, such as cAMP-activated protein kinases. CFTR activity is also known to couple to other ion channels and transporters. Although the genetic cause of human cystic fibrosis by CFTR mutations has been well established, little is known about the protein machinery that plays a role in linking the CFTR to other regulatory or ion-conducting proteins. Several regions of CFTR proteins are highly conserved among different species. The conserved motifs are thought to determine various aspects of channel and mediate interactions with other regulatory proteins. The C-termini, which are not required for functional expression of the CFTR chloride conductance, are also highly conserved. Several proteins that interact with the conserved C-terminus have now been identified. They contain several distinct protein interaction domains, which may be involved in the assembly of macromolecular CFTR channel complexes *in vivo*. Molecular understanding of these proteins may provide important insights into CFTR function in cystic fibrosis.

**Keywords** CFTR · Protein interactions · PDZ · Regulation

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [5, 11, 12, 18, 20, 22]. In Caucasian populations, mutated CFTR genes

have been found in one in every 25 people. The disease is characterized by a wide variety of clinical symptoms including chronic pulmonary obstruction, bacterial colonization in the airway, pancreatic enzyme insufficiency, elevated sweat electrolytes, and reduced fertility in males (see a recent review by Zielenski and Tsui [38]). The cloning of the CFTR gene and the identification of its mutations have promoted extensive investigation in order to determine the physiological and genetic link of CF phenotypes to CFTR genotypes. Detailed structural and functional studies of the CFTR protein in heterologous expression systems have suggested that different mutations cause a variety of defects ranging from protein folding to the function of CFTR.

CFTR is thought to form a chloride channel activated by cAMP through phosphorylation of activated kinases. The CFTR activity is also known to couple to other ion channels and transporters [1, 37]. The genetic cause of human CF by CFTR mutations has been well established [18, 23, 38]. Critical questions concerning CFTR and its roles in CF are concentrated on the regulation and subcellular localization of the CFTR channel. There are several lines of evidence supporting the theory that CFTR-interacting proteins play critical roles in determining the proper expression and function of CFTR. For example, it is clear that phosphorylation by cAMP-activated kinases is essential for activation of the CFTR channel [7, 13, 19, 36]. However, it remains unclear whether the kinase(s) directly interacts with the CFTR as part of the channel complex or through some currently unknown anchor or scaffold proteins. In addition, more than 500 mutant alleles have been reported and some have been biochemically characterized, including several mutations that result in truncation of the CFTR protein (see a recent review by Zielenski and Tsui [38]). Interestingly, many C-terminal truncations, including those generated artificially by site-directed mutagenesis [15] and those from CF-like patients [14], can still produce a functional CFTR channel in heterologous systems, such as *Xenopus* oocytes. These data suggest that the C-terminal domain of CFTR is not essential for channel formation. This

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raises the possibility that the function of these mutant proteins is compromised *in vivo*, possibly because of a lack of binding sites at the C-terminus of CFTR for the regulatory proteins.

Proteins interacting with the C-terminal regions have been characterized in several other systems. Recent studies have shown that proteins containing PDZ domains play a critical role in determining the localization of their interacting target proteins (see reviews by Kennedy [10] and by Sheng [25]). PDZ domains are protein interaction modules that generally bind to the C-termini of target proteins. Because different PDZ domains often possess distinct peptide binding specificity [27, 29], a tandem arrangement of several PDZ domains in one protein allows for multivalent interactions to organize a macromolecular complex. Such domain organization is exemplified by a scaffold protein known as INAD, which is composed of five PDZ domains. Mutations of INAD resulted in the mislocalization of its binding proteins including NORPA (a phospholipase C), TRP (a putative store-operated calcium channel) and INAC (a *Drosophila* eye-specific protein kinase C) [3, 32]. Conversely, mutation at the binding sites of INAD, such as the C-terminus of NORPA, also lead to the loss of signal coupling without perturbation of phospholipase C (PLC) enzymatic activity [33].

In secretory epithelial cells, the CFTR protein is specifically localized in the apical membrane, where the CFTR activities are regulated by cytoplasmic proteins such as cAMP-activated protein kinases [13], actin [6, 9] and syntaxin [17]. In addition, increasing evidence suggests that other ion channels are functionally coupled with CFTR activity [24, 30, 31]. With the exception of the reported direct binding of syntaxin to the N-terminal domain of CFTR [17], the biochemical link between the CFTR and other functionally associated proteins is unknown. Recent studies have shown that deletion of the three C-terminal residues of CFTR abolishes its restricted apical localization [16].

We have identified a protein interacting with the CFTR C-terminus. This CFTR associated protein (CAP) has molecular weight of 70 kDa. CAP70 contains four distinct PDZ domains. Because three PDZ domains recognize the C-terminal residues of CFTR, CAP70 is well suited to serve as a scaffold that functions by linking CFTR to other proteins including regulatory factors and/or the coupled ion channels. The tandem organization of four PDZ domains in CAP70 provides considerable diversity for potential interactions with other proteins. Identification of the CAP70 binding partners and testing of their functional roles will facilitate both a basic understanding of CFTR function and clinical investigation of the physiological cause of CF.

Na<sup>+</sup>-H<sup>+</sup> exchanger regulatory factor (NHE-RF), also known as EBP50 (ezrin binding protein of 50 kDa [21]), is the first protein to be identified to bind CFTR. This protein contains two PDZ domains (PDZ1 and PDZ2) of distinct peptide binding specificity, where the binding consensus of PDZ1 matches the C-terminus of CFTR [34]. More detailed analyses have shown that the PDZ1

domain mediates specific and high-affinity binding to the CFTR C-terminus [8, 26, 34]. In addition to CAP70 and EBP50, CAL (for CFTR-associated ligand) was recently reported to interact with CFTR [2]. This protein contains two coil-coil domains and one PDZ domain, which presumably mediates the binding of CAL to CFTR. What would be the functional distinction among these proteins? In the kidney, CAP70 is found exclusively in the cortex, whereas EBP50 is found in both medulla and cortex. Considering that the presence of CFTR was reported in both medulla and cortex [28], the differential distribution of CAP70 and EBP50 offers a means to allow for the molecular heterogeneity of tissue-specific CFTR regulation. Indeed, except for containing PDZ domains, CAP70, EBP50, and CAL share no similarity. CAP70 possesses four PDZ domains with considerable binding diversity: EBP50 is thought to couple with protein kinase A modulation via its interaction with ezrin [4, 35], whereas CAL is thought to oligomerize via its coil-coil domain. It is known that the CFTR protein is expressed in a number of tissues where its function is coupled with different ion-transport processes. The heterogeneity in tissue distribution and domain organization of the CFTR-binding proteins allows them to act differentially in different cells or combinatorially within the same cell. This would provide an advantageous mechanism to accommodate the diverse regulation of the CFTR protein in terms of its targeting and macromolecular organization.

The polarized ion transport in epithelial cells is mediated by a large group of membrane ion channels, transporters, and exchangers. The restricted apical or basal lateral localization of these proteins is essential for establishing the polarity. Interestingly, the CAP70 protein is considerably more abundant than CFTR in both kidney and liver. This result raises the possibility that CAP70 has additional functions in epithelial cells by binding to a variety of integral membrane proteins. Because both CAP70 and EBP50 are localized on the apical surface of epithelial cells, an attractive model would be that these and other proteins are organized underneath the apical membrane. Such a macromolecular structure, perhaps similar to post-synaptic density (PSD) in neurons, provides a high density of protein-binding sites to the C-termini of apical membrane proteins, thereby contributing to the establishment and maintenance of the cellular polarity.

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