

# Structure and function of the cystic fibrosis transmembrane conductance regulator

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## Abstract

Cystic fibrosis (CF) is a lethal autosomal recessive genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR). Mutations in the CFTR gene may result in a defective processing of its protein and alter the function and regulation of this channel. Mutations are associated with different symptoms, including pancreatic insufficiency, bile duct obstruction, infertility in males, high sweat  $\text{Cl}^-$ , intestinal obstruction, nasal polyp formation, chronic sinusitis, mucus dehydration, and chronic *Pseudomonas aeruginosa* and *Staphylococcus aureus* lung infection, responsible for 90% of the mortality of CF patients. The gene responsible for the cellular defect in CF was cloned in 1989 and its protein product CFTR is activated by an increase of intracellular cAMP. The CFTR contains two membrane domains, each with six transmembrane domain segments, two nucleotide-binding domains (NBDs), and a cytoplasmic domain. In this review we discuss the studies that have correlated the role of each CFTR domain in the protein function as a chloride channel and as a regulator of the outwardly rectifying  $\text{Cl}^-$  channels (ORCCs).

## Key words

- CFTR
- Cystic fibrosis
- Chloride channel
- Function
- Structure
- Mutations

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## Introduction

Cystic fibrosis (CF) is a lethal autosomal recessive disorder in which abnormal regulation of epithelial  $\text{Cl}^-$  channels is associated with the pathophysiology of the disease (1,2). The hallmarks of CF include thick and dehydrated airway mucus, pancreatic insufficiency, bile duct obstruction, infertility in males, reduced fertility in females, high sweat  $\text{Cl}^-$ , intestinal obstruction, nasal polyp formation, and chronic sinusitis (3). The major causes of morbidity and mortality in CF patients are opportunistic chronic lung infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These infections

progressively destroy the lung parenchyma and lead to additional complications such as pulmonary hypertension, bronchiectasis, and right ventricular hypertrophy that ultimately lead to cardiorespiratory failure and death (4).

The cloning and subsequent studies of the CF gene have shown that its product is a low conductance, c-AMP-regulated chloride channel with properties distinct from those of other  $\text{Cl}^-$  channels expressed in epithelial cell membranes (1,5,6). The protein presents significant homology with the ATP-binding cassette family of transporters, responsible for the transit of hydrophobic drugs and small peptides, which includes the multidrug resis-

tance protein (7). Taken together, these characteristics support the hypothesis that this protein transports a substance that regulates other conductances, motivating its name, cystic fibrosis transmembrane regulator, or CFTR.

CFTR mRNA is expressed in the lung and other tissues, such as testis, duodenum, trachea and kidney (8) and was recently shown to be expressed in the human hypothalamus (9). The CFTR protein has a molecular weight of 180 kDa and is usually localized on apical cell membranes but there is evidence that it is also expressed in the basolateral membrane of sweat duct epithelia (10,11).

Morales et al. (12) have shown that the CFTR mRNA is expressed in human kidney cortex and medulla. In the rat, it is expressed along all nephron segments except glomerulus. It is hypothesized that CFTR participates in  $\text{Cl}^-$  reabsorption in these epithelia, but its role in renal function is not well understood since renal dysfunction is not observed in patients with cystic fibrosis.

Mutations in the CFTR gene reduce  $\text{Cl}^-$  permeability in several affected exocrine epithelial cells. This defect has attracted attention to the chloride transport function of CFTR and to the other  $\text{Cl}^-$  channels that may

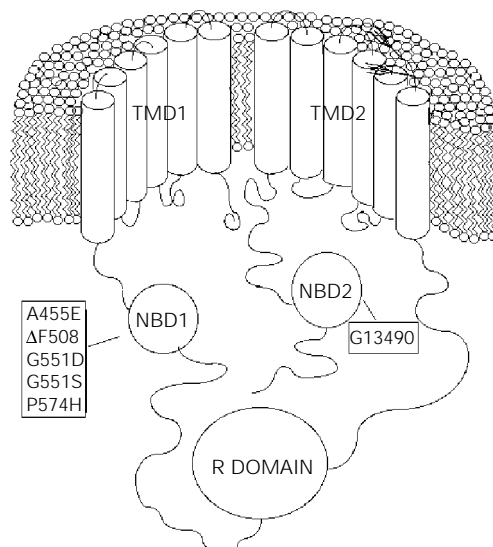
serve as redundant pathways for  $\text{Cl}^-$  secretion across the apical membrane in response to cAMP or other stimuli. The importance of each CFTR domain in the chloride channel and conductance regulator functions of the protein as well as the influence of the most common mutations in CF will be discussed in this review.

## The CFTR structure

The CF gene has been localized in human chromosome 7 and an analysis of the cDNA sequence has shown that the gene product, the CFTR protein, consists of 1,480 amino acids (13). Based on the hydropathy analysis of the amino acid sequence of CFTR, Collins et al. (14) proposed a putative model schematically illustrated in Figure 1. Beginning at the N terminus, the protein has six transmembrane-spanning domains followed by a first nucleotide-binding domain (NBD) with Walker A and B consensus sequences that bind ATP. Flanking this site, a large regulatory domain rich in cAMP-dependent kinase and protein kinase C phosphorylation sites is followed by a second set of six transmembrane-spanning domains and a second NBD that binds ATP. Morales et al. (12) described the existence of a functional alternative form of CFTR in rat trachea, lung and kidney. This alternative structure contains only the first transmembrane domain (TMD1), the first NBD and the regulatory domain (RD) and is designated TNR-CFTR. In the kidney, the TNR-CFTR is expressed in a tissue-specific manner primarily in the renal medulla and conserves the functional characteristics of the wild-type CFTR. This CFTR form is also found in human kidney medulla, but not in human kidney cortex.

The functional significance of this variant remains to be elucidated, but Morales et al. (12) raised two hypotheses: since several related members of the ATP-binding cassette family to which the CFTR belongs function in intracellular organelles as half

Figure 1 - Illustration of the predicted two-dimensional or secondary structure of the CFTR protein. The CFTR domains are shown. TMD1 and TMD2: transmembrane-spanning domains 1 and 2, respectively; R domain: regulatory domain; NBD1 and NBD2: nucleotide-binding domains 1 and 2, respectively. A summary of the selected and important mutations in NBD1 and NBD2 discussed in this review is also shown. Modified from Ref. 14.



molecules with the TMD1-NBD1 motif (15-17), there is a possibility that TNR-CFTR may have a basic function in intracellular organelles rather than in plasma membrane. Another possibility is based on the fact that this unusual form of mRNA processing occurs only in the renal medulla, a portion of the kidney with high osmolality. This tissue-specific processing of CFTR mRNA may create functional isoforms that can protect the kidney from functional defects in CF.

### Secretory chloride channel function of CFTR

Several laboratories using different techniques have definitely shown that the CFTR functions as a low conductance (7-12 pS), cAMP-regulated Cl<sup>-</sup> channel (6,18-20). This conductance is independent of the voltage and the time the voltage is applied to the membrane (18). The CFTR Cl<sup>-</sup> channel is insensitive to the Cl<sup>-</sup> channel-blocking drug, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid, but can be inhibited at least in part by other blocking agents such as diphenylamine-2-carboxylic acid, 5-nitro-2-[3-phenylpropyl-amino]-benzoic acid, and glybenclamide (18,21).

Phosphorylation by cAMP-dependent protein kinase (PKA) and ATP hydrolysis or binding are necessary for the channel to become active and remain so (19,20,22,23). Phosphorylation of the channel seems to occur only in the RD of the channel, whereas ATP binding or hydrolysis occurs in one or both of the NBD.

It is believed that the first transmembrane domain (TMD1) plays a role in the formation of the channel pore. The removal of a large portion of TMD1 including segments M1-M4 (M representing an individual membrane-spanning domain) reduces the single channel conductance of CFTR by 30%, without affecting ion selectivity, demonstrating that internal sites can function as translation initiation codons (24). These results agree

with those of Oblatt-Montal et al. (25) who showed that peptides with sequences corresponding to M1, M3, M4 and M5 do not form channels, whereas hetero-oligomers of M2 and M6 exhibit channel characteristics that emulate wild-type CFTR. McDonough et al. (26) hypothesized that both M6 and M12 interact to form the pore structure of CFTR. These studies suggest that spliced forms of CFTR could contribute significantly to overall Cl<sup>-</sup> secretion *in vivo*.

### CFTR is also a conductance regulator

The original hypothesis that CFTR would function as a conductance regulator seems to be correct, as several ion transport abnormalities found in cystic fibrosis airway epithelial cells cannot be explained by mutations in the CFTR chloride channel.

Schwiebert et al. (27) showed that CFTR regulates outwardly rectifying Cl<sup>-</sup> channels (ORCCs) by an autocrine mechanism involving ATP release and other researchers suggested that the CFTR is a dual ATP and chloride channel (28,29). However, several researchers were unable to detect ATP release by CFTR-expressing cells (30-33). These contradictory results suggest that there is some not yet understood relationship between the CFTR and ATP.

Devidas and Guggino (34) recently proposed three possible models for the release and function of ATP (Figure 2): 1) ATP, as well as Cl<sup>-</sup>, may pass through the CFTR itself; 2) ATP may pass through a channel that is different from the CFTR, but is regulated by it; 3) ATP-loaded vesicles fuse at the membrane level with the CFTR, followed by an exocytotic release of ATP via a Cl<sup>-</sup> osmotic gradient mediated through the CFTR.

Once out of the cell, ATP may interact with purinergic receptors that, once activated, may stimulate ORCCs through second messenger pathways, increasing chloride transport across the cell.

*Xenopus* oocytes lack ORCCs that interact with CFTR, which makes them an ideal model system to study CFTR functions. Recently, Schwiebert et al. (35) constructed varied mutant forms of CFTR and transfected them to *Xenopus* oocytes and in IB3-1 cells, a CF bronchial epithelial cell line extensively used to study CFTR function, in order to investigate the Cl<sup>-</sup> channel and ORCC regulatory functions of CFTR. They showed that the first TMD of CFTR is an essential part of the Cl<sup>-</sup> channel pore, necessary for proper Cl<sup>-</sup> conduction by CFTR and its selectivity. Conversely, the first NBD and the regulatory domain are essential for the ability of CFTR to regulate ORCCs. Thus, the roles of CFTR as a Cl<sup>-</sup> channel and a conductance regulator are not mutually exclusive, since one function can be eliminated

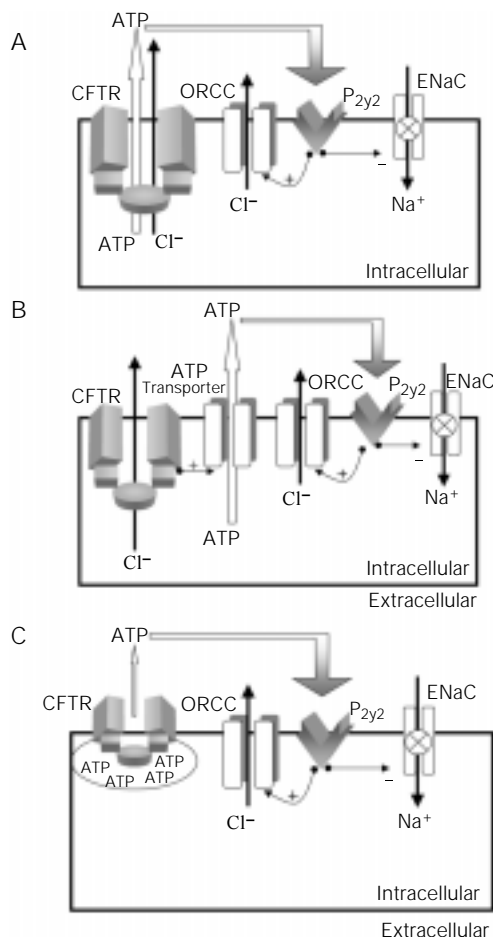
while the other is preserved. In agreement with these results, Sugita et al. (36) showed that expression of CFTR in MDCK cells results in the appearance of a novel ATP channel with different properties from CFTR. However, deletion of the CFTR regulatory domain leaves the CFTR chloride channel pore intact, while eliminating the ATP channel. They also demonstrated that phosphorylation and nucleotide-hydrolysis-dependent gating of CFTR is directly involved in gating of the associated ATP channel, suggesting that the structural changes in CFTR that control its opening and closing mechanisms have similar effects on the ATP conduction pathway.

The fact that different domains of CFTR are responsible for its Cl<sup>-</sup> channel and ORCC regulatory functions led to an improved understanding of cystic fibrosis physiopathology. The mutations expected to cause the most severe disease are those that affect the ability of CFTR to function both as a chloride channel and as a conductance regulator. Mutations that retain at least one function or only partially reduce both functions may result in less severe pulmonary disease (37). In agreement with this assumption, Fulmer et al. (38), studying two CFTR mutations that lead to different phenotypic effects in CF patients, found that a CFTR mutation that preserves the Cl<sup>-</sup> channel and ORCC regulator functions also produced less severe pulmonary disease.

### Other functions of CFTR

Epithelial Na<sup>+</sup> channels (ENaCs) are inhibited during activation of Cl<sup>-</sup> secretion in cells expressing CFTR (39,40), suggesting that when CFTR is activated by cAMP, it inhibits ENaCs. The results of three recent studies suggest that CFTR and ENaCs may interact directly by protein-protein binding (41-43). Moreover, it has been shown that Cl<sup>-</sup> transport by CFTR contributes to the inhibition of ENaCs, but the mechanism is still unclear (44).

Figure 2 - Membrane model depicting the role of ATP release in the interaction between the cystic fibrosis transmembrane conductance regulator (CFTR) and other conductances. In the polarized cells of airway epithelia the chloride exit occurs through the CFTR. A, ATP and Cl<sup>-</sup> are transported by the CFTR. ATP may interact and activate the purinergic receptor P<sub>2y2</sub> to stimulate the chloride transport via outwardly rectifying Cl<sup>-</sup> channels (ORCC) and block the reabsorption of Na<sup>+</sup> through the epithelium sodium channel (ENaC). B, ATP is released from the cell via an ATP transporter regulated by the CFTR. Once in the extracellular compartment ATP stimulates ORCC and inhibits the ENaC via purinergic receptors. C, The ATP located in intracellular vesicles fuses with the membrane containing the CFTR and the transport of ATP through CFTR occurs secondary to the osmotic chloride gradient. The ATP in the extracellular compartment stimulates the purinergic receptor to activate ORCC and inhibits the ENaC through intracellular second messengers. Modified from Ref. 34.



Besides playing a role in  $\text{Cl}^-$ , ATP and  $\text{Na}^+$  transport, CFTR also leads to a multi-drug resistance phenotype due to the extrusion of some therapeutic drugs (45,46). Moreover, it seems that CFTR and P-glycoprotein, another protein of the ATP-binding cassette family, have complementary patterns of expression (47-49). The significance of these findings is still unclear.

### **Mutations in the CFTR gene and its consequences**

In patients with CF, more than 170 different mutations have been described in the CFTR gene (3). Affected individuals have two abnormal copies of the defective gene. Subjects that have one normal and one abnormal copy of the gene are symptom-free and are not known to be at increased risk for any disease.

Although the known number of missense, nonsense and frameshift mutations within CFTR exceeds 500 (50), a 3-base pair deletion in exon 10 is responsible for approximately 70% of the mutant alleles (3). This deletion results in the loss of a single amino acid, the phenylalanine at codon 508, and is thus designated as  $\Delta\text{F508}$ . The phenotype of patients bearing the  $\Delta\text{F508}$  mutation varies considerably, but this mutation is often associated with severe disease.

The  $\Delta\text{F508}$  mutation affects the structure, function and folding of the NBD1 (51). The alteration in the structure leads to defective processing of the  $\Delta\text{F508}$ -CFTR protein through the endoplasmic reticulum, resulting in a drastically reduced level of protein to be expressed on the plasma membrane of exocrine epithelia in patients carrying the  $\Delta\text{F508}$  mutation.

Most of the non- $\Delta\text{F508}$  mutations are relatively rare, ranging from <1% to 10%, and are not symmetrically distributed (Figure 1). In the first half of the gene almost 60% of the mutations are substitutions or deletions of amino acids, while in the second

half nearly 80% are nonsense, frameshift or splice mutations. All the known CF mutations combined account for approximately 85% of all mutant chromosomes, and it is assumed that the remaining mutations may be located at the promotor or introns of the gene. In this review, we will focus on only a small number of mutations in specific functional domains of the protein that have been studied in more detail.

Besides the  $\Delta\text{F508}$ , both the G551D and G1349D missense mutations have been well studied as the cause of severe CF illness, characterized by severe respiratory disease, pancreatic insufficiency and high sweat  $\text{Cl}^-$  concentration (37). The G551D mutation occurs at the core of the Walker A binding motif for ATP in NBD1, a highly conserved residue among the ATP-binding cassette transporter family. The G1349D mutation is analogous to G551D, but is located in NBD2. Both mutations are thought to affect ATP binding in their respective NBDs.

On the other hand, the missense mutations A455E, P574H and G551S have been associated with less severe or mild pulmonary disease and/or less compromised CFTR function as a  $\text{Cl}^-$  channel (37,52,53). Smit et al. (53) explored the effect of natural and artificial mutations in the motifs of the NBDs thought to be important for ATP binding in CFTR. They showed that a severe mutation in one NBD was sufficient to critically reduce channel activity, but addition of a second severe mutation in the other NBD produced no additional defect.

Fulmer et al. (38) studied the A455E (associated with mild pulmonary disease) and G551D (associated with severe pulmonary disease) mutations concerning their effect on CFTR-ORCC regulatory interaction. They observed that the A455E-CFTR maintains about 67% of the chloride channel function while in the G551D-CFTR this function is reduced to 20%. Moreover, only the A455E mutation in CFTR retained the ORCC regulating function. These findings suggest

that the severity of pulmonary disease may be associated not only with the chloride function, but also (and perhaps mainly) with the regulatory role, rather than the channel function of CFTR.

Besides the mutations in NBDs, some mutations in TMDs have also been studied. In general, these mutations cause mild phenotypes in patients with CF (50). Several studies have examined the effects of TMD1 mutations on CFTR Cl<sup>-</sup> channel function (54,55). Their results, taken together, indicate that the  $\alpha$ -helices 5 and 6 may form the central pore of the CFTR Cl<sup>-</sup> channel. Moreover, the results obtained by Piazza-Carrol et al. (24) suggest that alternative methionines can be used as translation initiation codons to translate the CFTR-mRNA into protein.

The effects of TMD2 mutations on CFTR function have not been examined and studies performed on the regulatory domain are confusing (56-58), further studies being necessary to achieve a better understanding of

the function of the RD.

In conclusion, in addition to having a function in chloride transport, CFTR can modulate other conductances such as sodium channels (ENaCs) and chloride channels (ORCCs) probably by the mediation of ATP transport through the CFTR. The information obtained from the studies reviewed here shows the importance of the regulatory properties of CFTR at the cell plasma membrane level. The studies about the structure and function of CFTR are crucial for a better understanding of the defects involved in cystic fibrosis and for the development of alternative therapies for this complex human disease.

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