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Macromolecular complexes of cystic fibrosis transmembrane conductance regulator and its interacting partners

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) is the product of the gene mutated in patients with cystic fibrosis (CF). CFTR is a cAMP-regulated chloride channel localized primarily at the apical or luminal surfaces of epithelial cells lining the airway, gut, exocrine glands, etc., where it is responsible for transepithelial salt and water transport. CFTR chloride channel belongs to the superfamily of the ATP-binding cassette (ABC) transporters, which bind ATP and use the energy to drive the transport of a wide variety of substrates across extra- and intracellular membranes. A growing number of proteins have been reported to interact directly or indirectly with CFTR chloride channel, suggesting that CFTR might regulate the activities of other ion channels, receptors, or transporters, in addition to its role as a chloride conductor. The molecular assembly of CFTR with these interacting proteins is of great interest and importance because several human diseases are attributed to altered regulation of CFTR, among which cystic fibrosis is the most serious one. Most interactions primarily occur between the opposing terminal tails (N- or C-) of CFTR and its binding partners, either directly or mediated through various PDZ domain-containing proteins. These dynamic interactions impact the channel function as well as the localization and processing of CFTR protein within cells. This review focuses on the recent developments in defining the assembly of CFTR-containing complexes in the plasma membrane and its interacting proteins.

Keywords: Cystic fibrosis transmembrane conductance regulator; Macromolecular complex; PDZ protein; Interacting partner

Abbreviations: ABC, ATP-binding cassette; AFM, atomic force microscopy; AKAP, A-kinase anchoring protein; AMPK, AMP-activated kinase; AP-2, adaptor protein complexes 2; β_2AR, β 2 adrenergic receptor; BRET, bioluminescence resonance energy transfer; C, carboxyl; CAL, CFTR-associated ligand; CAP70, CFTR-associated protein 70; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; cGKII, cGMP-dependent protein kinase II; E3KARP, NHE3 kinase A regulatory protein; EBP50, ezrin-radixin-moesin binding phosphoprotein-50; ENaC, epithelial Na^+ channel; ER, endoplasmic reticulum; ERM, ezrin-radixin-moesin; FRET, fluorescence resonance energy transfer; IKEPP, intestinal and kidney-enriched PDZ protein; MERM, merlin-ezrin-radixin-moesin; mNBD1, mouse NBD1; MSD, membrane-spanning domain; N, amino; NBD or NBF, nucleotide binding domain/fold; NHERF, Na/H exchanger regulatory factor; PDZ, postsynaptic density-95, discs large, zona occludens-1; PDZK1, PDZ domain containing 1; R, regulatory; ROMK, renal outer medullary potassium channel; SNAP-23 or 25, soluble NSF attachment protein of 23 or 25 kDa; SNARE, soluble NSF attachment protein receptor; TM, transmembrane.

Contents

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1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is the product of the gene mutated in patients with cystic fibrosis (CF), and this lethal genetic disease affects 1 in every 2500 Caucasians in the United States. CFTR is an integral membrane glycoprotein composed of 1480 amino acids (Welsh et al., 1995).

1.1. Cystic fibrosis transmembrane conductance regulator localization and function

CFTR is primarily localized to the lumen-facing, or apical, membranes of epithelial cells in the airway, intestine, reproductive tissues, and exocrine glands (such as sweat glands, exocrine pancreas, and salivary glands). It functions as a cAMP-regulated Cl^- channel that is responsible for transepithelial salt and water transport (Quinton, 1983; Anderson et al., 1991; Bear et al., 1992). As its name implies, in addition to functioning as a conductor of Cl^-, CFTR also acts as a conductance regulator, exerting modulatory influences over a plethora of other ion channels, transport proteins, and processes, such as the epithelial Na^+ channel (ENaC; Knowles et al., 1983; Boucher et al., 1986; Stutts et al., 1995), the outwardly rectifying chloride channel (Gabriel et al., 1993; Jovov et al., 1995; Schwiebert et al., 1995), apical K^+ channels from renal epithelial cells renal outer medullary potassium channel (ROMK) 1 and ROMK2 (McNicholas et al., 1996, 1997; Cahill et al., 2000), aquaporin water channels (Schreiber et al., 1999), Cl^-/HCO_3^- exchangers (Lee et al., 1999), and ATP release mechanisms (Reisin et al., 1994; Sugita et al., 1998; for review, see Schwiebert et al., 1999).

1.2. Cystic fibrosis transmembrane conductance regulator topology and crystal structure

1.2.1. Cystic fibrosis transmembrane conductance regulator topology

CFTR is a symmetrical, polytopic protein that belongs to the superfamily of the ATP-binding cassette (ABC) transporters, which bind ATP and use the energy to drive the transport of a wide variety of substrates across extra- and intracellular membranes (Dean et al., 2001). CFTR consists of 2 repeated motifs, each composed of a hydrophobic membrane-spanning domain (MSD) containing 6 helices (transmembranes [TM]) and a cytosolic hydrophilic region for binding with ATP, that is, nucleotide binding domain (NBD; also called nucleotide binding fold [NBF]; Fig. 1; Riordan et al., 1989). These 2 motifs are linked by a cytoplasmic regulatory (R) domain, which contains a number of charged residues and multiple consensus phosphorylation sites (substrates for various protein kinases, such as PKA, PKC, cGMP-dependent protein kinase II [cGKII], etc.). Both the amino (N) and carboxyl (C) terminal tails of this membrane protein are cytoplasmically

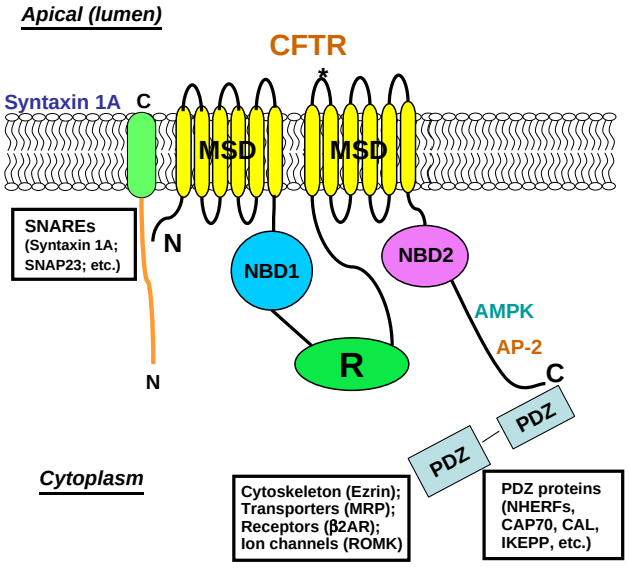

Fig. 1. Schematic illustration of putative CFTR topology and its interactions with various binding proteins. CFTR, a member of the ABC transporter superfamily, is a symmetrical, polytopic membrane glycoprotein. CFTR consists of 2 repeated motifs, each composed of a membrane-spanning domain (MSD) containing 6 helices and a cytosolic nucleotide binding domain (NBD), which can bind and hydrolyze ATP. These 2 identical motifs are linked by a cytoplasmic regulatory (R) domain, which contains a number of charged residues and multiple consensus phosphorylation sites (substrates for PKA, PKC, cGMP-dependent protein kinase II, etc.). The CFTR chloride channel can be activated through the phosphorylation of the R-domain by various protein kinases and ATP binding to and hydrolysis by the NBD domain. Both the amino (N) and carboxyl (C) terminal tails of this membrane protein are cytoplasmically oriented and mediate the interaction between CFTR and a wide variety of binding proteins, as discussed in this review. Asterisk denotes glycosylation sites (adapted from Naren & Kirk, 2000).

oriented and mediate the interaction between CFTR and a variety of binding proteins, as will be discussed in Section 3.

1.2.2. Cystic fibrosis transmembrane conductance regulator crystal structure

Attempts have been made to solve the 3-dimensional structure of CFTR. Recently, the high-resolution crystal structures for mouse NBD1 (mNBD1) have been determined (Lewis et al., 2004). The crystal structures of mNBD1 have features that distinguish them from other ABC proteins in that they have added regulatory segments, a foreshortened subdomain interconnection, as well as an unusual nucleotide conformation (Lewis et al., 2004). Human NBD1 is expected to be very similar in structure to mNBD1, as these molecules share 78% sequence identity. More recently, a low-resolution (~2 nm) structure of recombinant human full-length CFTR has been reported using conventional purification techniques and the formation of 2-dimensional crystalline arrays (Rosenberg et al., 2004). The crystallized CFTR shows an overall architecture for 2 different conformational states and demonstrates a strong structural homology to another eukaryotic ABC transporter (P-glycoprotein). The 2 conformational states of the human CFTR can be observed in the presence of nucleotide, suggesting the open and closed states of the chloride channel (Rosenberg et al., 2004). However, the high-resolution structure of human full-length CFTR by X-ray diffraction is yet to be determined before further insight can be gained into the mechanism of CFTR action.

1.3. Cystic fibrosis transmembrane conductance regulator pathophysiology

The biological significance of the CFTR chloride channel is demonstrated by the fact that several human diseases are attributed to altered function of CFTR, among which cystic fibrosis and secretory diarrhea are the 2 major disorders.

1.3.1. Cystic fibrosis

Cystic fibrosis (CF) is a lethal autosomal recessive human genetic disease and is most common among the Caucasians. CF is caused by the loss or dysfunction of the CFTR Cl^- channel activity resulting from the mutations (sequence alterations) that decrease either the biosynthesis or the ion channel function of the protein (Cheng et al., 1990; Welsh & Smith, 1993). The absence or dysfunction of CFTR chloride channel leads to aberrant ion and fluid homeostasis at epithelial surfaces in a variety of tissues or organs, including the lung, pancreas, gastrointestinal tract, liver, sweat glands, and male reproductive ducts (Zielenski & Tsui, 1995). In the lung, the defect in chloride transport is coupled with hyperabsorption of sodium, as well as the generation of thick and dehydrated mucus and subsequent chronic bacterial infections (such as *Pseudomonas aeruginosa*). This leads to bronchiectasis and progressive airway destruction. Other symptoms include, but are not limited to, pancreatic insufficiency, meconium ileus, and infertility (Welsh et al., 1995). The pulmonary manifestations of CF are responsible for substantial morbidity, and more than 90% of CF-related mortality. Currently, lung transplantation is the only effective therapy for these patients.

The most common CF mutation is the deletion of 3 nucleotides, resulting in the deletion of a single phenylalanine (F) residue at position 508 (ΔF508) on the protein molecule (Kerem et al., 1989; Davis et al., 1996), and is responsible for ~70% of CF alleles (Tsui, 1995). The ΔF508 CFTR mutant is associated with a severe form of the disease, with more than 90% of CF patients having at least 1 ΔF508 allele. It is estimated that approximately half of the CF patients are homozygous for the mutation ΔF508. This allele encodes an unstable and inefficiently folded CFTR protein, the major consequence being the failure of the mutant protein to be correctly processed and delivered to its proper cellular location in the plasma membrane (Cheng et al., 1990; Thomas et al., 1992; Welsh & Smith, 1993). As a result, the mutant protein is retained in the endoplasmic reticulum (ER) and rapidly targeted for degradation (Cheng et al., 1990; Ward et al., 1995). The investigations into the mechanisms underlying the biosynthesis, trafficking, and degradation of ΔF508 CFTR have already provided a

unique opportunity to understand the pathogenesis of this inherited disorder at the molecular and cellular levels (for review, see Kopito, 1999).

1.3.2. Secretory diarrhea

Another major disorder involving CFTR is secretory diarrhea, which is caused by excessive activation of this chloride channel in the gut (Field et al., 1972; Gabriel et al., 1994). The importance for the role of CFTR in secretory diarrhea is demonstrated by the fact that bacterial toxins fail to induce secretory diarrhea in CF mice (Gabriel et al., 1994; Cuthbert et al., 1995). Intestinal colonization by pathogenic microorganisms is a major cause for acquired secretory and inflammatory diarrhea (Hyams, 2000; Paton et al., 2000). Several species of bacteria induce secretory and inflammatory diarrhea, including *E. coli*, *Shigella flexneri*, *Salmonella typhimurium*, and *Vibrio cholerae*. CFTR resides at the apical membranes of secretory epithelial cells lining the lumen of the gut, where it is normally inactive (Frizzell & Halm, 1990). Due to the exposure to enterotoxins, intracellular second messengers (cAMP and/or cGMP) are generated, that lead to overstimulation of the secretory pathway by activating luminal CFTR (for reviews, see Sears & Kaper, 1996; Barrett & Keely, 2000). The activation of the CFTR channel by excessive phosphorylation (due to excessive cAMP and/or cGMP) leads to Cl^- secretion across the epithelium, which, in turn, increases the electrical and osmotic driving forces for the parallel flows of Na^+ and water, respectively. Therefore, the net result is the robust secretion of fluid and electrolytes across the epithelium into the gut lumen, namely, secretory diarrhea and the resultant dehydration, which can be fatal if untreated (Fig. 2; Field, 1971; Clarke et al., 1992). In parallel, electroneutral absorption by Na^+/H^+ exchanger and electrogenic absorption by ENaC are inhibited (Yun et al., 1997; Kunzelmann & Schreiber, 1999). Cholera toxin and heat-labile *E. coli* toxin induce intestinal fluid secretion by excessive increase in intracellular cAMP, due to irreversible activation of the adenylate cyclase resulting from the ADP ribosylation of the α-subunit of a stimulatory G protein, Gsα, by the toxins (Kimberg et al., 1971; Kantor, 1975). Moreover, cholera toxin increases intracellular cAMP in both crypts and villus epithelial cells, and thus, both compartments are likely to contribute to the generation of secretory diarrhea (De Jonge, 1975). Other toxins, such as heat-stable *E. coli* toxin or *Y. enterocolitica* toxin, enhance intracellular cGMP, leading to the stimulation of cGMP-dependent protein kinase II (cGKII), an apical membrane-targeted kinase that efficiently phosphorylates CFTR, resulting in the activation of Cl^- secretion in crypts and apical membranes of the intestine (Fig. 2; French et al., 1995; Swenson et al., 1996; Lohmann et al., 1997; Vaandrager et al., 1998, 2000).

Because CFTR plays the central role in certain forms of secretory diarrhea as described above, it has been argued that patients heterozygous for the CF defect have a genetic

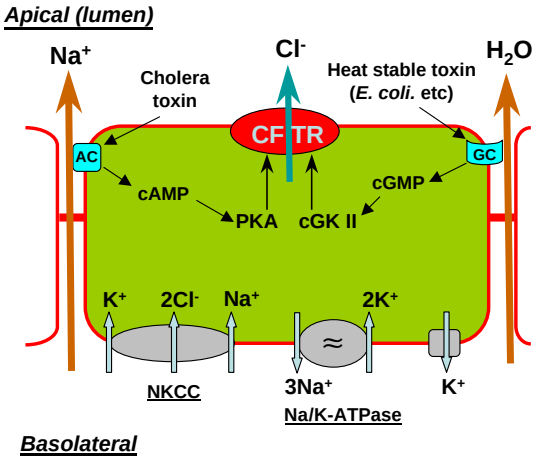

Fig. 2. A model of secretory epithelial cell and secretory diarrhea. Cholera toxin or heat-stable enterotoxin can increase the intracellular cAMP or cGMP levels by activating the membrane-localized adenylate cyclase (AC) or guanylate cyclase (GC). An increase in the intracellular cAMP or cGMP leads to the phosphorylation of the R-domain of CFTR by PKA or cGMP-dependent protein kinase II (cGKII), which, in turn, activates the CFTR chloride channel, resulting in Cl^- secretion into the lumen. As a consequence, Na^+ and water are effuxed into the lumen through the paracellular transport mechanism. Therefore, the net result is the secretion of fluid and electrolytes across the apical surface into the gut lumen. Cl^- is taken up from the basolateral (blood) side by the $Na^+-K^+-2Cl^-$ cotransporter (NKCC). K^+ recycles through basolateral K^+ channels, and Na^+ is pumped out of the cell by the Na^+-K^+-ATPase.

advantage because of their limited secretory responses to infections by some bacteria or viruses (Baxter et al., 1988; Taylor et al., 1988; Cuthbert et al., 1994, 1995; Gabriel et al., 1994; Quinton, 1994; Grubb & Boucher, 1999). On the other hand, it seems reasonable to propose that blocking luminal CFTR Cl^- channels would be the appropriate treatment for these forms of secretory diarrhea. Several studies reported new approaches to identify promising specific blockers of CFTR by high throughput screening (Schultz et al., 1999; Galietta et al., 2001). A chromanole compound 293 B was reported to block basolateral cAMP-dependent KvLQT1 K^+ channels, which play essential roles in maintaining the electrical driving force for luminal Cl^- secretion (Lohrmann et al., 1995). These blockers could also be useful for the treatment of secretory diarrhea, because they inhibited the equivalent short-circuit current induced by prostaglandin E2, vasoactive intestinal polypeptide, adenosine, cholera toxin, and cAMP in the distal rabbit colon from both the mucosal and the serosal sides of the epithelium and show fairly low IC_{50} values (Lohrmann et al., 1995). Gabriel et al. (1999) identified a novel inhibitor of cAMP-mediated fluid and chloride secretion, SP-303, which is derived from the latex of the plant *Croton lechleri*. This naturally occurring latex has been used by the indigenous people of South America to treat various kinds of watery diarrheas, including diarrhea caused by cholera. Gabriel et al. (1999) demonstrated that SP-303 is effective against in vivo cholera toxin-induced fluid secretion and in vitro cAMP-mediated Cl^- secretion. A potent and selective

small-molecule CFTR inhibitor ($CFTR_{inh}$-172) was identified recently by Verkman's group (Ma et al., 2002; Thiagarajah et al., 2004) by high-throughput screening. $CFTR_{inh}$-172 inhibited CFTR-mediated chloride transport, as well as intestinal fluid secretion induced by cholera toxin and STa *E. coli* toxin in animal models. Most recently, we found that lysophosphatidic acid, a naturally occurring phospholipid in blood and food, efficiently inhibited cholera toxin-induced CFTR-dependent secretory diarrhea in mice (Li et al., submitted for publication).

1.4. Scope of the review

The goal of this article is to review new findings and developments in our understanding of the cellular and molecular aspects of protein–protein interactions involving CFTR chloride channel. It is beyond the scope of this review to discuss all the interactions and their regulation involving CFTR. Therefore, our focus is on the recent progress made in the area of CFTR-containing complexes in the plasma membrane and a few of its interacting proteins.

2. Molecular assembly of cystic fibrosis transmembrane conductance regulator in plasma membrane

2.1. Cystic fibrosis transmembrane conductance regulator quaternary structure

Controversy remains regarding the functional form of CFTR in the plasma membrane. Investigations applying biochemical techniques (co-immunoprecipitation of different CFTR mutants or CFTR with different tags), electrophysiological techniques (patch-clamp studies), as well as electrophoretic studies (using nondenaturing gels), argued that the functional form of CFTR is a monomer. Marshall et al. (1994) concluded that CFTR existed primarily as a monomer in the membrane because coexpressed full-length and C-terminal truncated forms of CFTR could not be co-immunoprecipitated with an antibody recognizing an epitope at the C terminus in detergent-solubilized extract (Nonidet P-40, Triton X-100, digitonin, or CHAPSO). Ramjeesingh et al. (2001) found that CFTR overexpressed in insect Sf9 cells migrated on nondissociative size-resolving electrophoretic and chromatographic gels with mobilities corresponding to both monomers and multimers (primarily dimers). They did not observe any significant difference as to the channel function and ATPase activity between the monomeric and dimeric forms of CFTR, suggesting that CFTR monomers are fully active. Riordan's group (Chen et al., 2002) concluded that CFTR behaved as a monomer based on their biochemical and functional studies. They observed that CFTR migrated as a monomer through a sucrose velocity gradient after being solubilized by a variety of different detergents (Nonidet P-40, Triton X-100, or SDS) from microsomal membranes of cells expressing heterologous or endogenous CFTR. CFTR solubilized from membranes of cells in which 2 differentially epitope-tagged forms of CFTR were coexpressed, failed to co-immunoprecipitate. Coexpression of CFTR variants with very different unitary conductances (TM6 mutants) failed to yield intermediate conducting channels, implying that the monomeric CFTR polypeptides might assemble to form a single pore (Chen et al., 2002). This group recently demonstrated that the low-resolution (~2 nm), 3-dimensional electron microscopical structure of CFTR was monomeric (Rosenberg et al., 2004). It is possible that the purification of detergent-solubilized protein and crystallization might affect CFTR oligomeric behavior. In addition, the CFTR oligomer formation in vivo may be mediated by intercalating proteins that may not copurify under detergent-solubilized conditions.

An increasing body of evidence argues for the existence of a dimeric CFTR in the plasma membrane. The cross-sectional area of the freeze-fracture particles corresponding to the area of the transmembrane domains of the recombinant CFTR in *Xenopus* oocytes showed that the CFTR intramembraneous particle area corresponded more closely to 24 than to 12 packed helices. This corresponded to approximately twice as many TMs as present in a CFTR monomer, suggesting that the CFTR channel may be dimeric in the membrane (Eskandari et al., 1998). A heterodimer consisting of wild-type and mutant (R-domain partially deleted) CFTR sequences linked in tandem was proposed because it showed a channel with intermediate mixed gating properties (Zerhusen et al., 1999). Subsequently, bivalent but not monovalent PDZ domain-containing proteins (e.g., CAP70/PDZ domain containing 1 [PDZK1] or Na/H exchanger regulatory factor 1 [NHERF1]/ezrin-radixin-moesin binding phosphoprotein-50 [EBP50], etc.; discussed under Section 3.2) were shown to enhance CFTR channel gating with a stoichiometry suggesting that the promotion of dimer formation increased channel activity resulting from the cross-linking of the C-terminal tails of the dimeric CFTR (Wang et al., 2000; Raghuram et al., 2001). Both studies suggest that not only can CFTR exist as a dimer, stabilized by the multiple PDZ-binding domains within the PDZ proteins (of NHERF1 or CAP70), but also that CFTR functions more efficiently in this dimeric state. By applying atomic force microscopy (AFM) to inside–out oriented membrane patches of CFTR-expressing *Xenopus* oocytes after cAMP stimulation, Schillers et al. (2004) detected ring-like structures with bipartite symmetry, which is consistent with the model of a CFTR dimer. From the AFM molecular mass analysis of the intramolecular domains, they concluded that 2 CFTR molecules lined up in parallel, tail-by-tail, forming a pore in its center.

By employing chemical cross-linking with bifunctional amino reactive reagents, we recently found that the dominant CFTR species observed in the plasma membrane

employing SDS-PAGE and velocity gradient centrifugation had the approximate size of 300–330 kDa (Li et al., 2004). Our results suggest that the CFTR probably exists as a dimer in the plasma membrane. Interestingly, in the absence of the cross-linking regent, the protein (CFTR) extracted in detergents (e.g., Triton-X-100, Nonidet P-40, SDS, etc.) migrated as a ~150-kDa protein (Chen et al., 2002; Li et al., 2004), suggesting that the detergents probably destabilize the higher order CFTR-containing complex. The protein (CFTR) could be cross-linked into a higher order complex in the absence of the PDZ motif (i.e., using cells expressing $CFTR_{his10}$ or mutant CFTR lacking last 3 amino acids, CFTR-ΔTRL; Li et al., 2004), suggesting that the PDZ motif probably does not contribute to the higher order complex. As an extension of the previous work suggesting that CFTR interacts with PDZ domain-containing proteins (NHERF1 and NHERF2, see Section 3.2 for details), we quantitated the percentage of CFTR engaged in these interactions and found that a surprisingly low percentage participated in PDZ-mediated interactions (<2%). We also demonstrated that the dimerization of CFTR was regulated by PKA-dependent phosphorylation and did not depend on the active form of the channel (inhibitors of the CFTR channel, such as DPC and glybenclamide, did not have an effect on the dimerization). In addition, the analysis of this detergent solubilized, cross-linked complex following separation by 2D electrophoresis revealed that G_{β} and syntaxin 1A also exist in this complex (Li et al., 2004).

However, additional information is needed to support the hypothesis that the most active form of CFTR exists as a dimer and that both monomers contribute to the function of the channel. Especially needed are high-resolution structural data defining how CFTR is assembled into a structural and functional unit in the plasma membrane.

2.2. Cystic fibrosis transmembrane conductance regulator assembly in plasma membrane

Based on our studies (Li et al., 2004) and other reports, we hypothesize a model describing the molecular assembly of CFTR in the plasma membrane (Fig. 3). (i) CFTR in epithelial cells can be clustered to distinct microdomains (in apical membrane) by PDZ-based interactions, as reported for other channels (Short et al., 1998). (ii) CFTR

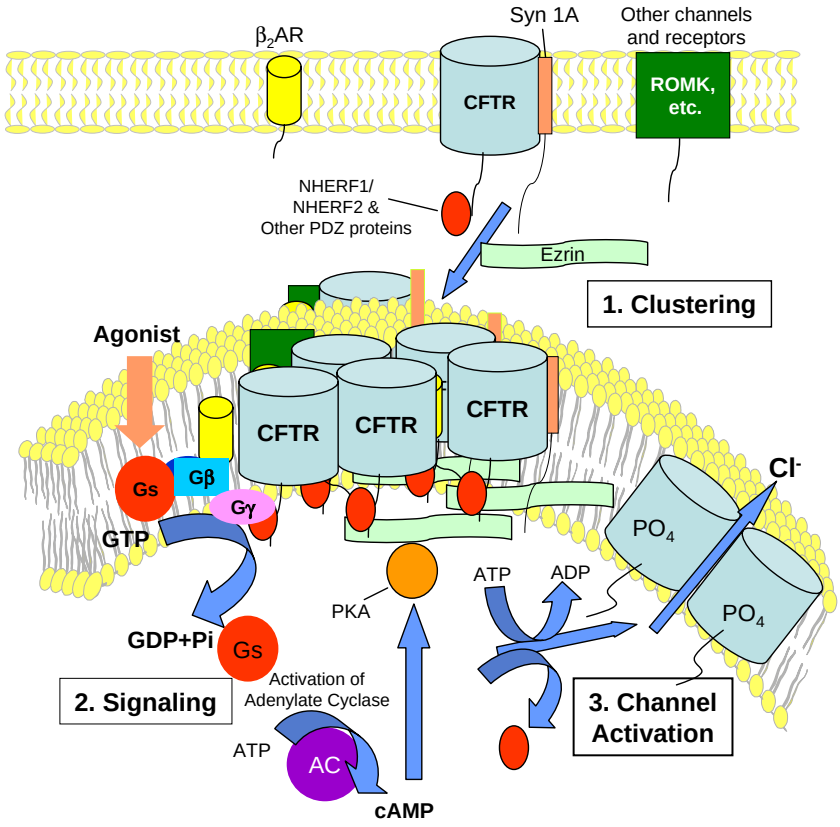

Fig. 3. Proposed mechanism underlying the molecular assembly of CFTR in the plasma membrane. CFTR in the epithelial cells can be clustered to distinct microdomains (in apical membrane) by PDZ-mediated interactions. CFTR polypeptides may form multimeric complexes in such microdomains, and a large portion of CFTR is likely to exist as PDZ protein-independent dimers. Various PDZ domain-interacting partners, such as other channels (e.g., ROMK), receptors (e.g., β_2AR), and cytoskeleton proteins (e.g., ezrin), can be in the complex as well. Other related proteins that do not interact directly with the PDZ motif, rather through other intermediary proteins (e.g., Gβ), can be in the complex as well and are likely to be involved in signaling. Upon signaling, a local increase in cAMP level leads to the phosphorylation of the CFTR Cl^- channel, resulting in rapid dimerization of the channel, which probably translocates the dimers away from the complex leading to the Cl^- efflux from the channel.

polypeptides may form multimeric complexes in such microdomains, and a large portion of CFTR is likely to exist as PDZ protein-independent dimers. (iii) Various PDZ domain-interacting partners, such as other channels (ROMK, discussed in Section 4.2), receptors (e.g., β_2AR; Naren et al., 2003), and cytoskeleton proteins (e.g., ezrin; Sun et al., 2000a), can be in the complex as well. (iv) Other related proteins that do not interact directly with the PDZ motif, rather through other intermediary proteins (e.g., G_β; Naren et al., 2003), can be in the complex as well and are likely to be involved in signaling. (v) Upon signaling, a local increase in cAMP leads to the phosphorylation of the CFTR Cl^- channel, resulting in rapid dimerization of the channel, leading to the Cl^- efflux from the channel (Naren et al., 2003).

Knowledge about the quaternary structure of a protein is essential to the understanding of its mechanism of action. Recently, there has been considerable interest in determining both the oligomeric structure of CFTR at the cell surface and its mechanism, as this knowledge is expected to be relevant for a better understanding of pathogenesis in CF patients (with disease-causing mutations in the CFTR gene). There is reason to believe that the dimerization (or oligomerization) is a more general mechanism which may be of interest, not just to researchers in the CF field, but also to those studying membrane proteins, such as ion channels and other members of the ABC superfamily of transporters (Aguilar-Bryan et al., 1998), many of which are relevant to human disease. It has been reported that P-glycoprotein (an ABC transporter) oligomerizes at the ER (Poruchynsky & Ling, 1994). We speculate that one of the critical reasons for the failure of ΔF508 mutant CFTR to exit from the ER into cell surface membrane may be that ΔF508 cannot form a functional dimer during its biogenesis but aggregates and is therefore trapped in the ER. Results from our studies may help understand how to rescue mutant CFTR by targeting its oligometric structure (especially the dimeric forms). It is plausible to use wild-type CFTR to rescue the ΔF508 protein to the cell surface through dimerization between wild-type CFTR and mutant CFTR or combined with the use of ER Ca^{2+} pump inhibitors (such as thapsigargin), which deplete ER Ca^{2+} stores and interfere with activity of some chaperone proteins, thus forcing ΔF508 mutant CFTR to exit to plasma membrane (Egan et al., 2002). By using a transcomplementation strategy, Cormet-Boyaka et al. (2004) recently reported that ΔF508 CFTR mutant can form mature, functional chloride channels in cell surfaces when coexpressed with several other CFTR mutants or with specific polypeptide fragments of the wild-type CFTR. It is possible that this transcomplementation can force the formation of functional dimer by various CFTR constructs (including ΔF508 CFTR) before they exit from ER to the cell surface.

Future works that would enhance our knowledge about CFTR oligomerization extended from the present studies include, but are not limited to, the (1) determination of the molecular and structural basis for CFTR dimerization, (2) assessment of the existence of dimers at the surface of living cells using techniques such as FRET (fluorescence resonance energy transfer; Selvin, 2000) or bioluminescence resonance energy transfer (BRET; Xu et al., 2003); (3) assessment of the role of dimerization on biosynthesis or cell surface stability of CFTR. Other functionally relevant proteins of the complex associated with the multimer should be identified through mass spectrometry among all proteins analyzed in the 2D-gels following co-immunoprecipitation; (4) some key experiments to support the proposed model for the PKA phosphorylation-induced dimerization. CFTR lacking the R-domain (ΔR-CFTR) could be tested. An alternative method is to test the effect of a previously described peptide inhibitor of PKA (PKI), which prevents phosphorylation of the R-domain, on CFTR dimerization (Ma et al., 1996).

3. Interactions between cystic fibrosis transmembrane conductance regulator and its binding partners

Accumulating evidence has been documented to demonstrate the existence of direct or indirect interactions between CFTR and a wide variety of proteins, including transporters, ion channels, kinases, phosphatases, and cytoskeletal elements. Among these reported interactions, many are mediated through a physical interaction between these binding proteins with the opposing tails of the CFTR chloride channel, as will be discussed briefly in Sections 3.1 to 3.2.

3.1. Cystic fibrosis transmembrane conductance regulator interactions at its amino (N) terminal tail

It has been demonstrated that syntaxin 1A and soluble NSF attachment protein of 23 kDa (SNAP-23) can bind to CFTR and down-regulate its function (Fig. 1; Naren et al., 1997, 1998; Peters et al., 1999; Cormet-Boyaka et al., 2002). Both syntaxin 1A and SNAP-23 (also referred to as target soluble NSF attachment protein receptors [t-SNAREs]) are expressed in part at the apical surface of epithelial cells where CFTR is known to reside (Naren et al., 2000; Cormet-Boyaka et al., 2002). The binding of syntaxin 1A to CFTR is direct and occurs with a stoichiometry of 1:1. The interacting regions have been mapped to the third helical domain of syntaxin 1A (a.a. 194–266) and the N-terminal tail of CFTR (a.a. 1–79; Naren et al., 1998). The membrane anchoring of syntaxin 1A is essential for the functional down-regulation observed on the channel (Naren et al., 1997). Disrupting the protein–protein interaction (or the membrane anchor of syntaxin 1A) can augment CFTR activity in airway epithelial cells by at least 2- to 4-fold (Naren et al., 1997). SNAP-23 can also bind to the N-terminal tail of CFTR and down-regulate its function. Interestingly, the physical and functional interactions between CFTR and SNAP-23 are synergistically affected

by syntaxin 1A, which results in a much greater inhibition of the channel (compared with syntaxin 1A inhibition or SNAP-23 by itself; Cormet-Boyaka et al., 2002).

The debate goes on as to whether the inhibitory effects of these t-SNAREs on CFTR is due to the altered or reduced number of channels at the surface (trafficking) or is due to a direct effect of the complex on the ability of the channel to open (gating). Peters et al. (1999) have reported that the coexpression of CFTR and syntaxin 1A in *Xenopus* oocytes leads to a decreased number of channels at the apical surface. They showed a decrease in the cAMP-dependent membrane capacitance measurement (which is an indirect measure of exocytosis or outbound traffic, and a direct measure of cell surface area) and a decrease in the immunofluorescent detection of the channel at the surface (an index of total surface pool of CFTR). It is possible that the SNARE interaction in the N-terminal tail of CFTR could contribute to the number of channels at the cell surface by having an influence on the trafficking (Peters et al., 1999). On the other hand, a direct channel regulation at the level of gating using epithelial cell lines (HT29-CL19A [colonic] and 16HBE14o-[bronchial]) was suggested by Chang et al. (2002) in excised inside–out patches. These cell lines endogenously express both syntaxin 1A and CFTR. It is possible that the physiological relevance of such direct inhibition of the channel by syntaxin 1A may be important in inhibiting the channel especially in compartments, where its function may not be required (e.g., endosomes). At this stage, it definitely would be useful to have information as to if and how syntaxin 1A regulates CFTR function in vivo.

From the pathophysiology point of view, that is, individuals suffering from CF, an important question would be "do these t-SNARE interactions with CFTR have any relevance to the disease". It has been demonstrated that ΔF508 CFTR (~70% of CF patients suffer from this form of mutation; Welsh et al., 1995), once pushed out of the ER (by overexpression and reducing temperature to 30 °C), behaves like wild-type CFTR in its ability to bind syntaxin 1A (Naren et al., 1998). It has also been shown that in oocytes syntaxin 1A inhibits ΔF508 CFTR Cl^- current activity. Reagents that disrupt the syntaxin 1A–CFTR interaction (i.e., BONT/C1 and fusion proteins) can potentiate ΔF508 CFTR activity in coexpressing *Xenopus* oocytes as well as in an epithelial cell line (LLC-PK1) that expresses recombinant ΔF508 CFTR (Naren et al., 1998). Of course, ΔF508 CFTR is a processing mutant for which the rate-limiting step in functional activity is release from the ER. It is reported that small amounts of ΔF508 CFTR can reach the surfaces of epithelial cells (Kalin et al., 1999). The channel, once at the surface, will interact with syntaxin 1A (syntaxin 1A is at least 10-fold in excess over CFTR in mouse tracheal and intestinal cells). Any reagent that can disrupt protein–protein interaction between syntaxin 1A and CFTR will be beneficial to this class of CF patients as well. Such syntaxin 1A-disrupting reagents will be beneficial to ~5% of CF patients suffering from the partial loss of function CFTR mutants (e.g., G551D, R117H, etc.; Welsh et al., 1995). This mutant form of CFTR traffics normally to the cell surface and syntaxin 1A down-regulates its function further. Therefore, it is likely that partial loss of function CFTR mutants that traffic normally may have a much-reduced activity. A therapeutic approach would be to see if these interactions can be disrupted specifically without altering other SNARE interactions. This may also be beneficial in patients harboring ΔF508 mutant forms of CFTR when used in combination with drugs that will push the mutant protein out of the ER.

3.2. Cystic fibrosis transmembrane conductance regulator interactions at its carboxyl (C) terminal tail

Whereas the amino terminal tail couples CFTR to the membrane traffic machinery, the opposing extreme carboxyl terminal tail binds to proteins that possess binding modules referred to as PDZ (for postsynaptic density-95, discs large, zona occludens-1) domains.

3.2.1. PDZ domains and PDZ proteins

PDZ domains are conserved protein-interaction modules of ~80–90 amino acids in length that fold to form peptide-binding clefts and typically mediate interactions with the carboxyl termini of target proteins that terminate in consensus PDZ-binding sequences (also referred to as PDZ-motif; Fig. 4; Fanning & Anderson, 1999; Bezprozvanny & Maximov, 2001; Hung & Sheng, 2002). Proteins that possess PDZ domains (PDZ domain-containing proteins, or PDZ proteins, for short) are often multivalent (i.e., they contain multiple PDZ domains) and thus can promote homotypic and heterotypic protein–protein interactions in a variety of tissues, as will be discussed in Section 3.2.2.

PDZ proteins that are primarily localized to the apical surfaces of epithelial cells include NHERF1 (Na/H exchanger regulatory factor 1; also called ezrin-radixin-moesin binding phosphoprotein-50 [EBP50]), NHERF2 (also called NHE3 kinase A regulatory protein [E3KARP]), CFTR-associated protein 70 (CAP70), also called PDZ domain containing 1 (PDZK1) and IKEPP (intestinal and kidney-enriched PDZ protein, also referred to as PDZK2), etc. NHERF1 and NHERF2 are closely related (~50% amino acid identity; Weinman et al., 1995; Reczek & Bretscher, 1998). Both NHERF1 and NHERF2 contain 2 PDZ domains and a C-terminal domain (ezrin-radixin-moesin [ERM] domain) that mediates association with MERM proteins (merlin-ezrin-radixin-moesin), while both CAP70 and IKEPP contain 4 tandem PDZ domains (Fig. 4; Fanning & Anderson, 1999; Bezprozvanny & Maximov, 2001; Scott et al., 2002). Many studies have reported the association of these PDZ proteins with a wide variety of ion channels, receptors, transporters, and signaling proteins in the apical surfaces of cells, suggesting that apical membrane PDZ proteins could facilitate the formation of

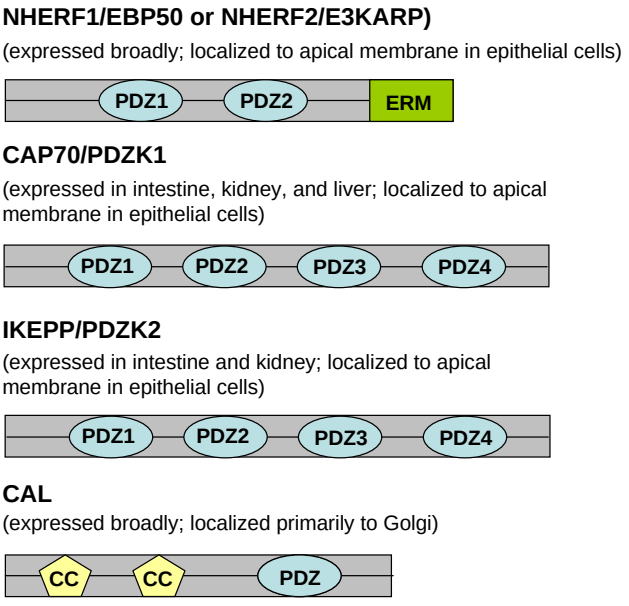

Fig. 4. Schematic representation of PDZ domain-containing proteins that can bind CFTR protein. Five different PDZ domain-containing proteins (PDZ proteins, for short) have been reported to bind to the C-terminal tail of the CFTR protein mediated through their PDZ domains with various affinities: NHERF1/EBP50, NHERF2/E3KARP, CAP70/PDZK1, CAL, and IKEPP/PDZK2. CAL is primarily localized to the Golgi apparatus, whereas the rest are localized to the apical membranes of epithelial cells. NHERF1 and NHERF2 are closely related and share ~50% sequence identity. NHERF1 and NHERF2 contain 2 PDZ domains, both of which can bind CFTR, as well as a C-terminal domain (ERM domain) that mediates association with MERM proteins to link CFTR to the actin cytoskeleton. Both CAP70 and IKEPP contain 4 tandem PDZ domains, and PDZ3 and PDZ4 of CAP70 are reported to bind 2 CFTR molecules simultaneously. CAL possesses only 1 PDZ domain and 2 coiled-coil (CC) domains that associate CAL to the membrane. The tissue distributions of these CFTR interacting proteins are not identical, suggesting that CFTR might interact with different PDZ proteins in different tissues (adapted from Cormet-Boyaka et al., 2003).

multiprotein complexes clustered within microdomains that modulate trafficking, transport, and signaling in polarized epithelial cells (Hall et al., 1998; Short et al., 1998; Kocher et al., 1999; Wang et al., 2000; Altschuler et al., 2003).

3.2.2. Interactions between cystic fibrosis transmembrane conductance regulator and the PDZ proteins

Five different PDZ proteins have been reported to bind to the C-terminal tail of the CFTR polypeptide with various affinities: NHERF1, NHERF2, CAP70, CFTR-associated ligand (CAL), and IKEPP (Fig. 4; Hall et al., 1998; Short et al., 1998; Sun et al., 2000b; Wang et al., 2000; Cheng et al., 2002; Scott et al., 2002; Hegedus et al., 2003). Four of these proteins (NHERF1, NHERF2, CAP70, and IKEPP) possess multiple PDZ domains, whereas CAL has only 1 PDZ domain. In addition, NHERF1 has been reported to self-associate into dimers (Shenolikar et al., 2001). As stated above, NHERF1, NHERF2, CAP70, and IKEPP also have been reported by many groups to be localized to the apical membranes of epithelial cells where CFTR localizes, while CAL is primarily localized to the Golgi. The tissue distributions of these CFTR interacting proteins are not identical, suggesting that CFTR might interact with different PDZ proteins in different tissues.

The PDZ-motif within CFTR that is recognized by PDZ domains in the above-described PDZ proteins is the last 3 amino acids of the CFTR protein (i.e., 1478-TRL-1480 in human CFTR; Hall et al., 1998; Short et al., 1998; Sun et al., 2000b; Wang et al., 2000; Cheng et al., 2002; Scott et al., 2002; Hegedus et al., 2003). PDZ domains have been categorized into 3 classes based on target sequence specificity, and these CFTR-interacting PDZ domains belong to class I with consensus target sequence of $-(S/T)-X-\Phi$ (referred to as PDZ-motif, where X = any amino acid, and Φ = hydrophobic amino acid; Harris & Lim, 2001; Hung & Sheng, 2002). Interestingly, CFTR can bind to more than 1 PDZ domain of both NHERFs and CAP70, albeit with varying affinities (Wang et al., 2000; Raghuram et al., 2001). This multivalency with respect to CFTR binding appears to be functionally significant, raising the possibility of PDZ proteins to facilitate the formation of macromolecular complex, as will be discussed in the following sections.

The ERM domain within the C-terminal tails of NHERF1 and NHERF2 tethers these proteins to the cortical cytoskeletonal elements via binding to ezrin in a phosphorylation-dependent manner (Reczek & Bretscher, 1998). On the basis of these observations, it has been proposed that interactions between CFTR and NHERF1 could anchor CFTR chloride channel to the apical membrane cytoskeleton (Short et al., 1998). In addition, Moyer et al. (1999) have reported that the deletion of the C-terminal TRL sequence or replacing the terminal leucine (L) with alanine (A) of CFTR protein results in the mislocalization of CFTR in epithelial cells, suggesting that an intact PDZ recognition sequence (PDZ motif) is important for the proper and efficient localization of CFTR to the apical surfaces of epithelial cells. However, this PDZ motif is also argued not to be sufficient. Other C-terminal sequences, in addition to a PDZ-binding motif, are also reported to be required for localizing CFTR to the apical plasma membrane (Milewski et al., 2001, 2005). Interestingly, the clinical phenotype of individuals harboring the CFTR deletion mutant lacking the last 26 amino acid residues exhibit moderately elevated sweat chloride concentration without obvious pancreatic and pulmonary phenotype (Mickle et al., 1998), suggesting the involvement of other factors in addition to NHERF and the C terminus of CFTR in the apical targeting of CFTR. Recently, Lukacs's group reported that the disruption of the complex formation between CFTR and NHERF by C-terminal deletions, C-terminal epitope tag attachments, or overexpression of a dominant negative NHERF mutant had no discernible effect on the apical localization of CFTR in epithelia derived from the trachea, pancreatic duct, intestine, as well as the distal tubule of the kidney (Benharouga

et al., 2003). In addition, Welsh's group also demonstrated that neither the C-terminal PDZ-interacting motif nor other C-terminal sequences were absolutely required for apical expression in airway epithelia, because constructs containing deletions in the C-terminal tail expressed in well-differentiated CF airway epithelia were still localized predominantly to the apical membrane and generated transepithelial chloride current (Ostedgaard et al., 2003). Therefore, it is possible that the apical localization of CFTR involves sorting signals other than the C-terminal 26 amino acid residues and the PDZ-binding motif in differentiated epithelia, which may help explain the relative paucity of CF-associated mutations in the C terminus.

Ezrin is a member of the A-kinase anchoring proteins (AKAPs), which are responsible for the subcellular sequestration of the A-kinase (e.g., cAMP-dependent protein kinase). Ezrin has been reported to bind the catalytic and regulatory subunit of PKA (Dransfield et al., 1997). PKA is a critical regulator for CFTR activity in epithelial cells. PKA-mediated regulation of CFTR requires that PKA be compartmentalized with CFTR at the apical cell surfaces of epithelial cells. Functional evidence arguing for this compartmentalization is demonstrated by Huang et al. (2001), who, using electrophysiological techniques, showed that A_{2b} adenosine receptor couples to G protein, adenylyl cyclase, and PKA, at the inner apical membrane surface of epithelial cells to activate colocalized CFTR. It is therefore likely that the apical epithelial AKAP (in this case, ezrin) may be a component of such signaling complexes, and it has been argued that CFTR forms a multiprotein complex with NHERF2, ezrin, and PKA (Sun et al., 2000a, 2000b). NHERF2, ezrin, and CFTR colocalize at or near the apical surfaces of Calu-3 airway epithelial cells. CFTR co-immunoprecipates with each of these proteins (NHERF2, ezrin, and PKA). In addition, a synthetic peptide that blocks the binding of PKA regulatory subunits to AKAPs inhibits the binding of PKA to ezrin as well as the activation of CFTR channels by cAMP agonists. These observations strongly suggest that CFTR is likely to be in a macromolecular complex with these proteins that play a role in scaffolding, signaling, etc., which would be important in defining the channel property and function.

Guggino's group reported a novel CFTR interacting protein, CFTR-associated ligand (CAL), a PDZ protein that can bind directly to CFTR via PDZ-mediated interaction (Fig. 4; Cheng et al., 2002). CAL is located primarily in the perinuclear region of the cell and associated with the Golgi apparatus, primarily at the *trans*-Golgi network. The deletion of the last 4 amino acid residues of CFTR abolished the interaction between CAL and CFTR. Cotransfection of CAL with CFTR reduces CFTR currents and the surface expression of CFTR (Cheng et al., 2002). This inhibitory effect of CAL could be reversed by coexpression with NHERF1, which blocks the binding of CAL to the CFTR C-terminal tail. However, it is not clear if the inhibitory effect of overexpressed CAL on the surface localization of CFTR reflects a normal physiologic function of this protein to regulate CFTR trafficking. More recently, this group reported that CAL also regulates the expression of mature CFTR because the coexpression of CAL with CFTR in COS-7 cells causes a dose-dependent reduction in mature CFTR (Cheng et al., 2004). In addition, the expression of the dominant-negative dynamin 2 K44A, a GTPase that is known to inhibit clathrin-mediated endocytosis and vesicle formation in the Golgi, increases cell surface CFTR and restores cell surface CFTR in CAL-overexpressing cells, suggesting that CAL retains CFTR in the cell and targets CFTR for degradation (Cheng et al., 2004). One could speculate that the observed increase in cell surface CFTR could be related to a decrease or inhibition of endocytosis.

As stated above, some PDZ proteins contain multiple PDZ domains that can bind to the PDZ-motif at the CFTR C-terminal tail (Fig. 4), and CFTR can bind to more than 1 PDZ domain of these PDZ proteins. These multivalent PDZ proteins (i.e., CAP70 and NHERF1) have been proposed to regulate the gating of CFTR channels at the plasma membrane (Wang et al., 2000; Raghuram et al., 2001). Two groups reported that CAP70 and NHERF1 can enhance CFTR channel activity in excised inside–out membrane patches (Wang et al., 2000; Raghuram et al., 2001). A recombinant fragment of NHERF1 containing the 2 PDZ domains (PDZ1-2) enhances the open probability (P_o) of single CFTR channels from a lung submucosal gland cell line (Calu-3), and this functional enhancement requires both PDZ domains because monovalent PDZ domains, either alone or together, abolish the bivalent PDZ domain (PDZ1-2)-mediated stimulation of channel P_o (Raghuram et al., 2001). The multivalent CAP70 potentiates CFTR channel activity probably through its dimeric binding to the C terminus of CFTR (Wang et al., 2000). This potentiation of channel activity could be mimicked by linking CFTR into dimer through bivalent binding by a monoclonal antibody that recognizes the DTRL sequence at the CFTR C terminus. These findings support a model in which CFTR polypeptides can be cross-linked into dimers by multivalent PDZ domain-containing proteins with a subsequent enhancement of channel activity, which is supported by a study suggesting a cooperative CFTR channel gating in Calu-3 cells (Krouse & Wine, 2001).

It has also been reported that CFTR channels can physically associate with the adaptor protein complexes 2 (AP-2; Weixel & Bradbury, 2000, 2001), the major proteins that drive clathrin coat formation (Kirchhausen et al., 1997). This interaction occurs through the C-terminal region of the CFTR polypeptide and μ2 subunit of the AP-2 heterotetramer (Weixel & Bradbury, 2001). It has been reported that the efficient, constitutive internalization of surface CFTR occurs predominantly by clathrin-dependent endocytosis (Lukacs et al., 1997; Bradbury et al., 1999) and probably determines the number of functional channels present at the apical cell surface. Given that AP-2 is an important component of clathrin-coated vesicle formation (Kirchhausen et al., 1997),

such interactions between CFTR and AP-2 appear to be capable of influencing the amount of CFTR proteins at the plasma membrane (Weixel & Bradbury, 2001).

Another direct interaction was also observed between CFTR and the AMP-activated kinase (AMPK; Hallows et al., 2000, 2003a, 2003b). AMPK is a member of the metabolite-sensing ubiquitous serine/threonine kinase family found in all eukaryotes (Kemp et al., 1999) and exists as a heterotrimer with a catalytic α subunit and regulatory β and γ subunits (Hardie & Carling, 1997). The interaction between CFTR and AMPK was mapped to the residues 1420–1457 in CFTR and the C-terminal regulatory domain of α1-AMPK (Hallows et al., 2000). AMPK phosphorylated CFTR and inhibited CFTR channel activity in both heterologous expression systems and polarized epithelial cells (Hallows et al., 2000, 2003a, 2003b). More interestingly, CFTR and AMPK share an overlapping apical distribution in a number of epithelial tissues (including nasopharynx, submandibular gland, pancreas, and ileum; Hallows et al., 2003a), raising the possibility that the 2 proteins could interact with each other at the apical membrane in vivo. The interaction between AMPK and CFTR is of potential intrigue because it might couple CFTR channel activity to the cellular metabolic status in epithelial tissues, fine tuning CFTR transport activity on a short time scale in response to metabolic conditions. Therefore, the AMPK-dependent regulation of CFTR may present a novel general mechanism to couple epithelial ion transport to cellular metabolism.

Based on all these studies and observations, it appears reasonable to propose that CFTR activity can be optimized through interactions with these aforementioned PDZ proteins in a number of complementary ways. As suggested above, such interactions may enhance either the numbers of functional CFTR channels at the apical membranes of epithelial cells or the functional activities of those channels within this membrane, or both. However, there still remain unresolved issues, such as the specific identities of the PDZ proteins that perform these tasks, the detailed mechanisms that underlie these effects, and the extent to which these interactions might regulate CFTR function in vivo (i.e., in animal models; Cormet-Boyaka et al., 2003). Another important question is whether there exist PDZ proteins that can recruit CFTR channels into multiprotein complexes with signaling molecules and/or other ion channels, or transporters, which will be discussed in detail in Section 4.

4. Macromolecular complex assembly of cystic fibrosis transmembrane conductance regulator and its interacting partners

It is now well documented and accepted that the formation of multiprotein macromolecular complexes at specialized subcellular microdomains increases the specificity and efficiency of signaling in cells. One interesting feature of epithelial cells is that signals originating at either the apical or basolateral cell surfaces do not always lead to detectable changes in the concentration of specific second messengers (cAMP, cGMP, or Ca^{2+}, etc.), although the cellular response is significantly altered. This notion is supported by studies from Huang et al. (2001), who demonstrated an efficient stimulation of chloride secretion by adenosine in Calu-3 cells, but with little change in intracellular cAMP level, suggesting a highly localized regulation of CFTR by adenosine receptor in the apical cell membrane. This implies that receptors or ion channels or transporters, signaling intermediates, and effectors are compartmentalized into regulatory complexes that increase the efficiency of signaling, and this compartmentation ensures that the right components localize at the right place at the right time.

PDZ domain-mediated interactions are also proposed to coordinate and promote cross-talk between CFTR chloride channels and parallel ion transport pathways. As mentioned above, in addition to its role as a chloride channel, CFTR also acts as a conductance regulator, coordinating an ensemble of transmembrane ion fluxes in polarized epithelia (for review, see Schwiebert et al., 1999). The underlying mechanisms as to how this single ion channel can regulate the activities of so many other transporters are still not well defined.

A growing number of studies and observations suggest that CFTR might directly or indirectly interact with other transport molecules in macromolecular complexes mediated through various PDZ proteins. A well-documented example of such macromolecular complex of signaling molecules is the INAD complex in *Drosophila* photoreceptor cells (Xu et al., 1998). This multivalent PDZ protein (INAD) serves as a scaffolding molecule to assemble different components of the phototransduction pathway, which includes at least 2 distinct ion channels that are coupled to multiple signaling molecules by INAD that contains 5 PDZ domains (Tsunoda & Zuker, 1999). This macromolecular organization endows photoreceptors with high sensitivity, fast activation and deactivation kinetics, exquisite feedback regulation, as well as specificity of signaling. Imaginably, as a component of similar macromolecular complexes in epithelial cells, CFTR can also both functionally and physically interact with a wide variety of other transporters and signaling molecules, as will be discussed in Sections 4.1 to 4.2.

4.1. A macromolecular complex of β_2 adrenergic receptor, Na/H exchanger regulatory factor 1, and cystic fibrosis transmembrane conductance regulator

The β_2 adrenergic receptor (β_2AR) is the major adrenergic receptor isoform expressed in airway epithelial cells, and its stimulation with β receptor agonist (iso-

proterenol) activates CFTR-dependent chloride transport in vivo (Walker et al., 1997). We recently demonstrated that both β_2AR and CFTR bind NHERF1/EBP50 through their PDZ motif. This complex is likely to be present at the apical surfaces of airway epithelial cells (Naren et al., 2003). The deletion of the PDZ motif from CFTR uncouples the channel from β_2AR receptor both physically and functionally, and this uncoupling is specific to the β_2AR receptor and does not affect CFTR coupling to other receptors (e.g., adenosine receptor pathway). Biochemical studies have demonstrated the existence of a macromolecular complex involving CFTR–NHERF1– β_2AR through PDZ-based interactions (Naren et al., 2003). We further demonstrated that the assembly of the complex is regulated by PKA-dependent phosphorylation. Interestingly, PKA phosphorylation of CFTR inhibited the formation of the macromolecular complex in a dose-dependent manner, while deleting the regulatory R-domain of CFTR abolishes PKA regulation of the complex assembly (Naren et al., 2003).

Based on these observations, we proposed a model depicting the coupling of β_2AR signaling to CFTR chloride channel function (Fig. 5). Our results suggest that protein–protein interactions (macromolecular complex assembly) may be essential for full activation of the channel by β_2AR pathway, which can be circumvented by using other pathways (e.g., adenosine or forskolin). This interaction may be critical for a rapid and specific signal transduction

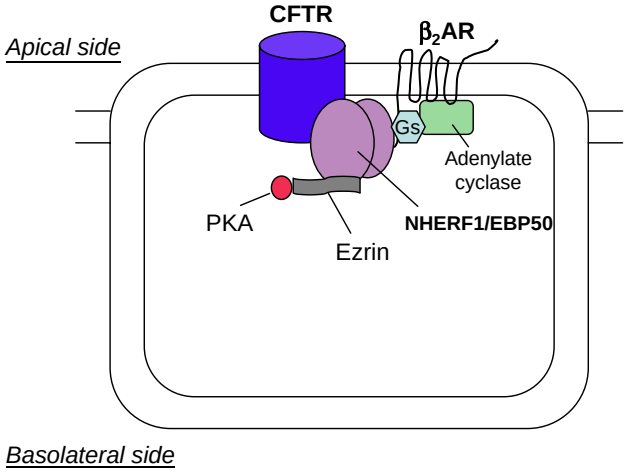

Fig. 5. The coupling of β_2-adrenergic receptor signaling to CFTR chloride channel function. CFTR, EBP50/NHERF1, and β_2AR can exist as a macromolecular complex at the apical surfaces of epithelial cells. G proteins can be associated with β_2AR and protein kinase A (PKA) anchored to AKAP (ezrin) and is likely to be in the complex. Upon agonist activation of the receptor, adenylate cyclase is stimulated through the Gs pathway, leading to an increase in highly compartmentalized cAMP. This increased local concentration of cAMP leads to the activation of PKA, which is in close proximity to CFTR, resulting in a compartmentalized and specific signaling from β_2AR to the CFTR channel. Phosphorylation disrupts the complex, leading to the receptor-based activation of CFTR (adapted from Naren et al., 2003).

from the receptor to the channel in a compartmentalized fashion. Our report demonstrates cross-talk between receptor and the channel through PDZ-mediated physical interaction. We speculate that interactions of this sort may also be important to CFTR regulation of other ion channels, receptors, and transporter proteins. Our results also suggest how certain defective forms of CFTR may lead to abnormal CFTR function in the context of receptor-based signaling and signal compartmentalization. For instance, in patients with ΔF508 CFTR and other mutations that the mutant CFTR channel is either degraded or mislocalized, disruption in signal transduction may have effects broader than those predicted simply from the absence of a functioning CFTR chloride channel.

4.2. A macromolecular complex of renal outer medullary potassium channel, Na/H exchanger regulatory factors, and cystic fibrosis transmembrane conductance regulator

The ROMK (Kir 1.1) subtypes of inward rectifying potassium channels play critical roles in salt and water homeostasis in the kidney (Ho et al., 1993). The C-terminal domain of ROMK1-3 channels also contains a canonical type I PDZ binding motif (-TQM>), and this PDZ binding sequence is reported to be a determinant of ROMK channel function, being required for efficient expression of active ROMK channels on the plasma membrane (Flagg et al., 2002). It was also reported that the coexpression of CFTR with ROMK in *Xenopus* oocytes leads to the formation of weakly inward rectifying channels that have acquired sensitivity to sulfonylurea agents and ATP-dependent gating properties like the native channel (McNicholas et al., 1996; Ruknudin et al., 1998), raising the possibility that the trafficking of ROMK and interaction with CFTR are linked by a common PDZ domain-based scaffold. Recently, Yoo et al. (2004) provided evidence that ROMK associates directly with NHERF1 and NHERF2 through a PDZ binding interaction to facilitate the expression of ROMK on the plasmalemma and to coordinate the assembly of ROMK and CFTR into a ternary complex. This group found that ROMK preferentially associates with the second PDZ domain of NHERF1 and with the first PDZ domain of NHERF2. The coexpression of NHERF2 with ROMK and CFTR dramatically increases the amount of ROMK proteins that both physically and functionally interact with CFTR, implying a role of NHERFs in facilitating assembly of a ternary complex containing ROMK and CFTR. These observations raise the possibility that PDZ-based interactions may underscore physiological regulation and membrane targeting of ROMK in the kidney.

Based on these findings, it seems reasonable to propose that CFTR channels could be clustered with the interacting partners into distinct macromolecular complexes, and these complexes may be compartmentalized into distinct sub-

cellular locations and tissues through their specific interactions with various PDZ proteins.

5. Currents limitations and future directions

In summary, CFTR interacts with a wide variety of proteins, physically and functionally. The unique properties of CFTR proteins, being a chloride channel, a regulator of other channels, and important in the pathogenesis of many serious human diseases, make it an important target for structural and functional studies. It is suggested that protein–protein interactions that influence the expression or functional activity of the CFTR channel at plasma membrane would provide additional layers of regulation beyond those provided by the cAMP pathway per se (Cormet-Boyaka et al., 2003). The activity range of CFTR chloride channel could be altered dynamically by the inputs from multiple regulatory networks that can be integrated or cross-talked with the channel. The physiological significance of these interactions is most probably that they not only provide a means to link CFTR activity to various epithelial functions and processes, but also coordinate the CFTR chloride channel function with the overall physiologic demands of an epithelial cell (Cormet-Boyaka et al., 2003).

However, there are still some outstanding issues to be considered. The biochemical techniques (such as co-immunoprecipitation) used to study the assembly of protein complexes are limited by steric hindrance from bulky fusion proteins or antibodies, the use of detergents to solubilize complexes from cells, and unsatisfactory yield of interacting partners through intermediate proteins (i.e., poor detection limits). Even when biochemical methods work ideally, they still do not provide information about the existence or localization of the complex in intact living cells. Thus, precipitation methods cannot provide a complete, accurate depiction of the in vivo condition. Techniques such as FRET (Selvin, 2000) or BRET (Xu et al., 2003), which would allow the study of macromolecular complexes in living cells in real time holds promise in elucidating these mechanisms and pathways.

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References

Aguilar-Bryan, L., Clement 4th, J. P., Gonzalez, G., Kunjilwar, K., Babenko, A., & Bryan, J. (1998). Toward understanding the assembly and structure of KATP channels. *Physiol Rev 78*, 227–245.

Altschuler, Y., Hodson, C., & Milgram, S. L. (2003). The apical compartment: trafficking pathways, regulators and scaffolding proteins. *Curr Opin Cell Biol 15*, 423–429.

Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., et al. (1991). Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science 253*, 202–205.

Barrett, K. E., & Keely, S. J. (2000). Chloride secretion by the intestinal epithelium: molecular basis and regulatory aspects. *Annu Rev Physiol 62*, 535–572.

Baxter, P. S., Goldhill, J., Hardcastle, J., Hardcastle, P. T., & Taylor, C. J. (1988). Accounting for cystic fibrosis. *Nature 335*, 211.

Bear, C. E., Li, C., Kartner, N., Bridges, R. J., Jensen, T. J., Ramjeesingh, M., et al. (1992). Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell 68*, 809–818.

Benharouga, M., Sharma, M., So, J., Haardt, M., Drzymala, L., Popov, M., et al. (2003). The role of the C terminus and Na+/H+ exchanger regulatory factor in the functional expression of cystic fibrosis transmembrane conductance regulator in nonpolarized cells and epithelia. *J Biol Chem 278*, 22079–22089.

Bezprozvanny, I., & Maximov, A. (2001). PDZ domains: more than just a glue. *Proc Natl Acad Sci U S A 98*, 787–789.

Boucher, R. C., Stutts, M. J., Knowles, M. R., Cantley, L., & Gatzy, J. T. (1986). Sodium transport in cystic fibrosis epithelia. Abnormal basal rate and response to adenylate cyclase activation. *J Clin Invest 78*, 1245–1252.

Bradbury, N. A., Clark, J. A., Watkins, S. C., Widnell, C. C., Smith 4th, H. S., & Bridges, R. J. (1999). Characterization of the internalization pathways for the cystic fibrosis transmembrane conductance regulator. *Am J Physiol 276*, L659–L668.

Cahill, P., Nason Jr., M. W., Ambrose, C., Yao, T. Y., Thomas, P., & Egan, M. E. (2000). Identification of the cystic fibrosis transmembrane conductance regulator domains that are important for interactions with ROMK2. *J Biol Chem 75*, 16697–16701.

Chang, S. Y., Di, A., Naren, A. P., Kirk, K. L., Palfery, H. C., & Nelson, D. J. (2002). Mechanism of CFTR regulation by syntaxin 1A and PKA. *J Cell Sci 115*, 783–791.

Chen, J. H., Chang, X. B., Aleksandrov, A. A., & Riordan, J. R. (2002). CFTR is a monomer: biochemical and functional evidence. *J Membr Biol 188*, 55–71.

Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., et al. (1990). Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell 63*, 827–834.

Cheng, J., Moyer, B. D., Milewski, M., Loffing, J., Ikeda, M., Mickle, J. E., et al. (2002). A Golgi-associated PDZ domain protein modulates cystic fibrosis transmembrane regulator plasma membrane expression. *J Biol Chem 277*, 3520–3529.

Cheng, J., Wang, H., & Guggino, W. B. (2004). Modulation of mature cystic fibrosis transmembrane regulator protein by the PDZ domain protein CAL. *J Biol Chem 279*, 1892–1898.

Clarke, L. L., Grubb, B. R., Gabriel, S. E., Smithies, O., Koller, B. H., & Boucher, R. C. (1992). Defective epithelial chloride transport in a gene targeted mouse model of cystic fibrosis. *Science 257*, 1125–1128.

Cormet-Boyaka, E., Di, A., Chang, S. Y., Naren, A. P., Tousson, A., Nelson, D. J., et al. (2002). CFTR chloride channels are regulated by

a SNAP-23/syntaxin 1A complex. *Proc Natl Acad Sci U S A 99*, 12477–12482.

Cormet-Boyaka, E., Naren, A. P., & Kirk, K. L. (2003). CFTR interacting proteins. In K. L. Kirk, & D. C. Dawson (Eds.), *The cystic fibrosis transmembrane conductance regulator* (pp. 94–118). New York: Kluwer Academic Publishing/Plenum.

Cormet-Boyaka, E., Jablonsky, M., Naren, A. P., Jackson, P. L., Muccio, D. D., & Kirk, K. L. (2004). Rescuing cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by transcomplementation. *Proc Natl Acad Sci U S A 101*, 8221–8226.

Cuthbert, A. W., Hickman, M. E., MacVinish, L. J., Evans, M. J., Colledge, W. H., Ratcliff, R., et al. (1994). Chloride secretion in response to guanylin in colonic epithelial from normal and transgenic cystic fibrosis mice. *Br J Pharmacol 112*, 31–36.

Cuthbert, A. W., Halstead, J., Ratcliff, R., Colledge, W. H., & Evans, M. J. (1995). The genetic advantage hypothesis in cystic fibrosis heterozygotes: a murine study. *J Physiol (Lond) 482*, 449–454.

Davis, P. B., Drumm, M., & Konstan, M. W. (1996). Cystic fibrosis. *Am J Respir Crit Care Med 154*, 1229–1256.

Dean, M., Rzhetsky, A., & Allikmets, R. (2001). The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res 11*, 1156–1166.

De Jonge, H. R. (1975). The response of small intestinal villous and crypt epithelium to cholera toxin in rat and guinea pig. Evidence against a specific role of the crypt cells in choleragen-induced secretion. *Biochim Biophys Acta 381*, 128–143.

Dransfield, D. T., Bradford, A. J., Smith, J., Martin, M., Roy, C., Mangeat, P. H., et al. (1997). Ezrin is a cyclic AMP-dependent protein kinase anchoring protein. *EMBO J 16*, 35–43.

Egan, M. E., Glockner-Pagel, J., Ambrose, C., Cahill, P. A., Pappoe, L., Balamuth, N., et al. (2002). Calcium-pump inhibitors induce functional surface expression of Delta F508-CFTR protein in cystic fibrosis epithelial cells. *Nat Med 8*, 485–492.

Eskandari, S., Wright, E. M., Kreman, M., Starace, D. M., & Zampighi, G. A. (1998). Structural analysis of cloned plasma membrane proteins by freeze-fracture electron microscopy. *Proc Natl Acad Sci U S A 95*, 11235–11240.

Fanning, A. S., & Anderson, J. M. (1999). Protein modules as organizers of membrane structure. *Curr Opin Cell Biol 11*, 432–439.

Field, M. (1971). Intestinal secretion: effect of cyclicAMP and its role in cholera. *N Engl J Med 284*, 1137–1144.

Field, M., Fromm, D., Al-Awqati, Q., & Greenough, W. B. (1972). Effect of cholera enterotoxin on ion transport across isolated ileal mucosa. *J Clin Invest 51*, 796–804.

Flagg, T. P., Yoo, D., Sciortino, C. M., Tate, M., Romero, M. F., & Welling, P. A. (2002). Molecular mechanism of a COOH-terminal gating determinant in the ROMK channel revealed by a Bartter's disease mutation. *J Physiol (Lond) 544*, 351–362.

French, P. J., Bijman, J., Edixhoven, M., Vaandrager, A. B., Scholte, B. J., Lohmann, S. M., et al. (1995). Isotype-specific activation of cystic fibrosis transmembrane conductance regulator-chloride channels by cGMP-dependent protein kinase II. *J Biol Chem 270*, 26626–26631.

Frizzell, R. A., & Halm, D. R. (1990). Chloride channels in epithelial cells. F. Bronner, S. I. Helman, & W. Van Driessche (Eds.), *Current Topics in Membranes and Transport vol. 37*, (pp. 247–282). New York, NY: Academic Press.

Gabriel, S. E., Clarke, L. L., Boucher, R. C., & Stutts, M. J. (1993). CFTR and outwardly rectifying chloride channels are distinct proteins with a regulatory relationship. *Nature 363*, 263–268.

Gabriel, S. E., Brigman, K. N., Koller, B. H., Boucher, R. C., & Stutts, M. J. (1994). Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. *Science 266*, 107–109.

Gabriel, S. E., Davenport, S. E., Steagall, R. J., Vimal, V., Carlson, T., & Rozhon, E. J. (1999). A novel plant-derived inhibitor of cAMP-mediated fluid and chloride secretion. *Am J Physiol 276*, G58–G63.

Galietta, L. V., Springsteel, M. F., Eda, M., Niedzinski, E. J., By, K., Haddadin, M. J., et al. (2001). Novel CFTR chloride channel activators identified by screening of combinatorial libraries based on flavone and benzoquinolizinium lead compounds. *J Biol Chem 276*, 19723–19728.

Grubb, B. R., & Boucher, R. C. (1999). Pathophysiology of gene-targeted mouse models for cystic fibrosis. *Physiol Rev 79*(Suppl), S193–S214.

Hall, R. A., Ostedgaard, L. S., Premont, R. T., Blitzer, J. T., Rahman, N., Welsh, M. J., et al. (1998). A C-terminal motif found in the beta2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na+/H+ exchanger regulatory factor family of PDZ proteins. *Proc Natl Acad Sci U S A 95*, 8496–8501.

Hallows, K. R., Raghuram, V., Kemp, B. E., Witters, L. A., & Foskett, J. K. (2000). Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase. *J Clin Invest 105*, 1711–1721.

Hallows, K. R., Kobinger, G. P., Wilson, J. M., Witters, L. A., & Foskett, J. K. (2003a). Physiological modulation of CFTR activity by AMP-activated protein kinase in polarized T84 cells. *Am J Physiol Cell Physiol 284*, C1297–C1308.

Hallows, K. R., McCane, J. E., Kemp, B. E., Witters, L. A., & Foskett, J. K. (2003b). Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis transmembrane conductance regulator activity in lung submucosal cells. *J Biol Chem 278*, 998–1004.

Hardie, D. G., & Carling, D. (1997). The AMP-activated protein kinase-fuel gauge of the mammalian cell? *Eur J Biochem 246*, 259–273.

Harris, B. Z., & Lim, W. A. (2001). Mechanism and role of PDZ domains in signaling complex assembly. *J Cell Science 114*, 3219–3231.

Hegedus, T., Sessler, T., Scott, R., Thelin, W., Bakos, E., Varadi, A., et al. (2003). C-terminal phosphorylation of MRP2 modulates its interaction with PDZ proteins. *Biochem Biophys Res Commun 302*, 454–461.

Ho, K., Nichols, C. G., Lederer, W. J., Lytton, J., Vassilev, P. M., Kanazirska, M. V., et al. (1993). Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. *Nature 362*, 31–38.

Huang, P., Lazarowski, E. R., Tarran, R., Milgram, S. L., Boucher, R. C., & Stutts, M. J. (2001). Compartmentalized autocrine signaling to cystic fibrosis transmembrane conductance regulator at the apical membrane of airway epithelial cells. *Proc Natl Acad Sci U S A 98*, 14120–14125.

Hung, A. Y., & Sheng, M. (2002). PDZ domains: structural modules for protein complex assembly. *J Biol Chem 277*, 5699–5702.

Hyams, J. S. (2000). Inflammatory bowel disease. *Pediatr Rev 21*, 291–295.

Jovov, B., Ismailov, I. I., & Benos, D. J. (1995). Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. *J Biol Chem 270*, 1521–1528.

Kalin, N., Claass, A., Sommer, M., Puchelle, E., & Tummler, B. (1999). ΔF508 CFTR protein expression in tissues from patients with cystic fibrosis. *J Clin Invest 103*, 1379–1389.

Kantor, H. S. (1975). Enterotoxins of *Escherichia coli* and *Vibrio cholerae*: tools for the molecular biologist. *J Infect Dis 131*, S22–S32.

Kemp, B. E., Mitchelhill, K. L., Stapleton, D., Michell, B. J., Chen, Z. P., & Witters, L. A. (1999). Dealing with energy demand: the AMP-activated protein kinase. *Trends Biochem Sci 24*, 22–25.

Kerem, B., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., et al. (1989). Identification of the cystic fibrosis gene: genetic analysis. *Science 245*, 1073–1080.

Kimberg, D. V., Field, M., Johnson, J., Henderson, A., & Gershon, E. (1971). Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. *J Clin Invest 50*, 1218–1230.

Kirchhausen, T., Bonifacino, J. S., & Riezman, H. (1997). Linking cargo to vesicle formation: receptor tail interactions with coat proteins. *Current Opin Cell Biol 9*, 488–495.

Knowles, M. R., Stutts, M. J., Spock, A., Fischer, N., Gatzy, J. T., & Boucher, R. C. (1983). Abnormal ion permeation through cystic fibrosis respiratory epithelium. *Science 221*, 1067–1070.

Kocher, O., Comella, N., Gilchrist, A., Pal, R., Tognazzi, K., Brown, L. F., et al. (1999). PDZK1, a novel PDZ domain-containing protein up-

regulated in carcinomas and mapped to chromosome 1q21, interacts with cMOAT (MRP2), the multidrug resistance-associated protein. *Lab Invest 79*, 1161–1170.

Kopito, R. R. (1999). Biosynthesis and degradation of CFTR. *Physiol Rev 79*, S167–S173.

Krouse, M. E., & Wine, J. J. (2001). Evidence that CFTR channels can regulate the open duration of other CFTR channels: cooperativity. *J Membr Biol 182*, 223–232.

Kunzelmann, K., & Schreiber, R. (1999). CFTR, a regulator of channels. *J Membr Biol 168*, 1–8.

Lee, M. G., Wigley, W. C., Zeng, W., Noel, L. E., Marino, C. E., Thomas, P. J., et al. (1999). Regulation of Cl^-:$HCO3^-$ exchange by cystic fibrosis transmembrane conductance regulator expressed in NIH 3T3 and HEK 293 cells. *J Biol Chem 274*, 3414–3421.

Lewis, H. A., Buchanan, S. G., Burley, S. K., Conners, K., Dickey, M., Dorwart, M., et al. (2004). Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator. *EMBO J 23*, 282–293.

Li, C., Roy, K., Dandridge, K., & Naren, A. P. (2004). Molecular assembly of cystic fibrosis transmembrane conductance regulator in plasma membrane. *J Biol Chem 279*, 24673–24684.

Li, C., Dandridge, K. S., Harris, E. L., Roy, K., Marrs, K. L., Jackson, J. S., Makarova, N. V., Fujiwara, Y., Farrar, P. L., Tigyi, G. J., & Naren, A. P. (submitted for publication). Lysophosphatidic acid inhibits cholera toxin-induced secretory diarrhea through CFTR-dependent protein interactions.

Lohmann, S. M., Vaandrager, A. B., Smolenski, A., Walter, U., & DeJonge, H. R. (1997). Distinct and specific functions of cGMP-dependent protein kinases. *Trends Biochem Sci 22*, 307–312.

Lohrmann, E., Burhoff, I., Nitschke, R. B., Lang, H.-J., Mania, D., Englert, H. C., et al. (1995). A new class of inhibitors of cAMP-mediated Cl^- secretion in rabbit colon, acting by the reduction of cAMP-activated K^+ conductance. *Pflügers Arch 429*, 517–530.

Lukacs, G. L., Segal, G., Kartner, N., Grinstein, S., & Zhang, F. (1997). Constitutive internalization of cystic fibrosis transmembrane conductance regulator occurs via clathrin-dependent endocytosis and is regulated by protein phosphorylation. *Biochem J 328*, 353–361.

Ma, J., Tasch, J. E., Tao, T., Zhao, J., Xie, J., & Drumm, M. L., et al. (1996). Phosphorylation-dependent block of cystic fibrosis transmembrane conductance regulator chloride channel by exogenous R domain protein. *J Biol Chem 271*, 7351–7356.

Ma, T., Thiagarajah, J. R., Yang, H., Sonawane, N. D., Folli, C., Galietta, L. J., et al. (2002). Thiazolidinone CFTR inhibitor identified by high-throughput screening blocks cholera toxin-induced intestinal fluid secretion. *J Clin Invest 110*, 1651–1658.

Marshall, J., Fang, S., Ostedgaard, L. S., O'Riordan, C. R., Ferrara, D., Amara, J. F., et al. (1994). Stoichiometry of recombinant cystic fibrosis transmembrane conductance regulator in epithelial cells and its functional reconstitution into cells in vitro. *J Biol Chem 269*, 2987–2995.

McNicholas, C. M., Guggino, W. B., Schwiebert, E. M., Hebert, S. C., Giebisch, G., & Egan, M. E. (1996). Sensitivity of a renal K+ channel (Romk2) to the inhibitory sulfonylurea compound glibenclamide is enhanced by coexpression with the ATP-binding cassette transporter cystic fibrosis transmembrane regulator. *Proc Natl Acad Sci U S A 93*, 8083–8088.

McNicholas, C. M., Nason Jr., M. W., Guggino, W. B., Schwiebert, E. M., Hebert, S. C., Giebisch, G., et al. (1997). A functional CFTR-NBF1 is required for ROMK2–CFTR interaction. *Am J Physiol Renal Physiol 273*, F843–F848.

Mickle, J., Macek, M., Fulmer-Smentek, S., Egan, M., Schwiebert, E., Guggino, W., et al. (1998). A mutation in the cystic fibrosis transmembrane conductance regulator gene associated with elevated sweat chloride concentrations in the absence of cystic fibrosis. *Hum Mol Genet 7*, 729–735.

Milewski, M. I., Mickle, J. E., Forrest, J. K., Stafford, D. M., Moyer, B. D., Cheng, J., et al. (2001). A PDZ-binding motif is essential but not sufficient to localize the C terminus of CFTR to the apical membrane. *J Cell Sci 114*, 719–726.

Milewski, M. I., Lopez, A., Jurkowska, M., Larusch, J., & Cutting, G. R. (2005). PDZ-binding motifs are unable to ensure correct polarized protein distribution in the absence of additional localization signals. *FEBS Lett 579*, 483–487.

Moyer, B. D., Denton, J., Karlson, K. H., Reynolds, D., Wang, S., Mickle, J. E. (1999). A PDZ-interacting domain in CFTR is an apical membrane polarization signal. *J Clin Invest 104*, 1353–1361.

Naren, A. P., & Kirk, K. L. (2000). CFTR chloride channels: binding partners and regulatory networks. *News Physiol Sci 15*, 57–61.

Naren, A. P., Nelson, D. J., Xie, W., Jovov, B., Pevsner, J., Bennett, M. K., et al. (1997). Regulation of CFTR chloride channels by syntaxin and Munc18 isoforms. *Nature 390*, 302–305.

Naren, A. P., Quick, M. W., Collawn, J. F., Nelson, D. J., & Kirk, K. L. (1998). Syntaxin 1A inhibits CFTR chloride channels by means of domain-specific protein–protein interactions. *Proc Natl Acad Sci U S A 95*, 10972–10977.

Naren, A. P., Di, A., Cormet-Boyaka, E., Boyaka, P. N., McGhee, J. R., Zhou, W. (2000). Syntaxin 1A is expressed in airway epithelial cells, where it modulates CFTR Cl^- currents. *J Clin Invest 105*, 377–386.

Naren, A. P., Cobb, B., Li, C., Roy, K., Nelson, D., Heda, G. D., et al. (2003). A macromolecular complex of beta 2 adrenergic receptor, CFTR, and ezrin/radixin/moesin-binding phosphoprotein 50 is regulated by PKA. *Proc Natl Acad Sci U S A 100*, 342–346.

Ostedgaard, L. S., Randak, C., Rokhlina, T., Karp, P., Vermeer, D., Ashbourne Excoffon, K. J., et al. (2003). Effects of C-terminal deletions on cystic fibrosis transmembrane conductance regulator function in cystic fibrosis airway epithelia. *Proc Natl Acad Sci U S A 100*, 1937–1942.

Paton, A. W., Morona, R., & Paton, J. C. (2000). A new biological agent for treatment of Shiga toxigenic *Escherichia coli* infections and dysentery in humans. *Nat Med 6*, 265–270.

Peters, K. W., Qi, J., Watkins, S. C., & Frizzell, R. A. (1999). Syntaxin 1A inhibits regulated CFTR trafficking in *Xenopus* oocytes. *Am J Physiol 277*, C174–C180.

Poruchynsky, M. S., & Ling, V. (1994). Detection of oligomeric and monomeric forms of P-glycoprotein in multidrug resistant cells. *Biochemistry 33*, 4163–4174.

Quinton, P. M. (1983). Chloride impermeability in cystic fibrosis. *Nature 301*, 421–422.

Quinton, P. M. (1994). Human genetics. What is good about cystic fibrosis? *Curr Biol 4*, 742–743.

Raghuram, V., Mak, D. -O. D., & Foskett, J. K. (2001). Regulation of cystic fibrosis transmembrane conductance regulator single-channel gating by bivalent PDZ-domain-mediated interaction. *Proc Natl Acad Sci U S A 98*, 1300–1305.

Ramjeesingh, M., Li, C., Kogan, I., Wang, Y., Huan, L. J., & Bear, C. E. (2001). A monomer is the minimum functional unit required for channel and ATPase activity of the cystic fibrosis transmembrane conductance regulator. *Biochemistry 40*, 10700–10706.

Reczek, D., & Bretscher, A. (1998). The carboxyl-terminal region of EBP50 binds to a site in the amino-terminal domain of ezrin that is masked in the dormant molecule. *J Biol Chem 273*, 18452–18458.

Reisin, I. L., Prat, A. G., Abraham, E. H., Amara, J. F., Gregory, R. J., Ausiello, D. A., et al. (1994). The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J Biol Chem 269*, 20581–20584.

Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., et al. (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science 245*, 1066–1073.

Rosenberg, M. F., Kamis, A. B., Aleksandrov, L. A., Ford, R. C., & Riordan, J. R. (2004). Purification and crystallization of the cystic fibrosis transmembrane conductance regulator (CFTR). *J Biol Chem 279*, 39051–39057.

Ruknudin, A., Schulze, D. H., Sullivan, S. K., Lederer, W. J., & Welling, P. A. (1998). Novel subunit composition of a renal epithelial K_{ATP} channel. *J Biol Chem 273*, 14165–14171.

Schillers, H., Shahin, V., Albermann, L., Schafer, C., & Oberleithner, H. (2004). Imaging CFTR: a tail to tail dimer with a central pore. *Cell Physiol Biochem 14*, 1–10.

Schreiber, R., Nitschke, R., Greger, R., & Kunzelmann, K. (1999). The cystic fibrosis transmembrane conductance regulator activates aquaporin 3 in airway epithelial cells. *J Biol Chem 274*, 11811–11816.

Schultz, B. D., Singh, A. K., Devor, D. C., & Bridges, R. J. (1999). Pharmacology of CFTR chloride channel activity. *Physiol Rev 79*, S109–S144.

Schwiebert, E. M., Egan, M. E., Hwang, T. H., Fulmer, S. B., Allen, S. S., Cutting, G. R., et al. (1995). CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell 81*, 1063–1073.

Schwiebert, E. M., Benos, D. J., Egan, M. E., Stutts, M. J., & Guggino, W. B. (1999). CFTR is a conductance regulator as well as a chloride channel. *Physiol Rev 79*, S145–S166.

Scott, R. O., Thelin, W. R., & Milgram, S. L. (2002). A novel PDZ protein regulates the activity of guanylyl cyclase C, the heat-stable enterotoxin receptor. *J Biol Chem 277*, 22934–22941.

Sears, C. L., & Kaper, J. B. (1996). Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. *Microbiol Rev 60*, 167–215.

Selvin, P. R. (2000). The renaissance of fluorescence resonance energy transfer. *Nat Struct Biol 7*, 730–734.

Shenolikar, S., Minkoff, C. M., Steplock, D. A., Evangelista, C., Liu, M., & Weinman, E. J. (2001). N-terminal PDZ domain is required for NHERF dimerization. *FEBS Lett 489*, 233–236.

Short, D. B., Trotter, K. W., Reczek, D., Kreda, S. M., Bretscher, A., & Boucher, R. C., et al. (1998). An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. *J Biol Chem 273*, 19797–19801.

Stutts, M. J., Canessa, C. M., Olsen, J. C., Hamrick, M., Cohn, J. A., Rossier, B. C., et al. (1995). CFTR as a cAMP-dependent regulator of sodium channels. *Science 269*, 847–850.

Sugita, M., Yue, Y., & Foskett, J. K. (1998). CFTR Cl^- channel and CFTR-associated ATP channel: distinct pores regulated by common gates. *EMBO J 17*, 808–898.

Sun, F., Hug, M. J., Bradbury, N. A., & Frizzell, R. A. (2000a). Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. *J Biol Chem 275*, 14360–14366.

Sun, F., Hug, M. J., Lewarchik, C. M., Yun, C. H., Bradbury, N. A., & Frizzell, R. A. (2000b). E3KARP mediates the association of ezrin and protein kinase A with the cystic fibrosis transmembrane conductance regulator in airway cells. *J Biol Chem 275*, 29539–29546.

Swenson, E. S., Mann, E. A., Jump, M. L., Witte, D. P., & Giannella, R. A. (1996). The guanylin/STa receptor is expressed in crypts and apical epithelium throughout the mouse intestine. *Biochem Biophys Res Commun 225*, 1009–1014.

Taylor, C. J., Baxter, P. S., Hardcastle, J., & Hardcastle, P. T. (1988). Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. *Gut 29*, 957–962.

Thiagarajah, J. R., Broadbent, T., Hsieh, E., & Verkman, A. S. (2004). Prevention of toxin-induced intestinal ion and fluid secretion by a small-molecule CFTR inhibitor. *Gastroenterology 126*, 511–519.

Thomas, P. J., Shenbagamurthi, P., Sondek, J., Hullihen, J. M., & Pedersen, P. L. (1992). The cystic fibrosis transmembrane conductance regulator: effects of the most common cystic fibrosis-causing mutation on the secondary structure and the stability of a synthetic peptide. *J Biol Chem 267*, 5727–5730.

Tsui, L. C. (1995). The cystic fibrosis transmembrane conductance regulator gene. *Am J Respir Crit Care Med 151*, S47–S53.

Tsunoda, S., & Zuker, C. S. (1999). The organization of INAD-signaling complexes by a multivalent PDZ domain protein in *Drosophila* photoreceptor cells ensures sensitivity and speed of signaling. *Cell Calcium 26*, 165–171.

Vaandrager, A. B., Smolenski, A., Tilly, B. C., Houtsmuller, A. B., Ehlert, E. M., Bot, A. G., et al. (1998). Membrane targeting of cGMP-dependent protein kinase is required for cystic fibrosis transmembrane conductance regulator Cl^- channel activation. *Proc Natl Acad Sci U S A 95*, 1466–14671.

Vaandrager, A. B., Bot, A. G., Ruth, P., Pfeifer, A., Hofmann, F., & De Jonge, H. R. (2000). Differential role of cyclic GMP-dependent protein kinase II in ion transport in murine small intestine and colon. *Gastroenterology 118*, 108–114.

Walker, L. C., Venglarik, C. J., Aubin, G., Weatherly, M. R., McCarty, N. A., Lesnick, B., et al. (1997). Relationship between airway ion transport and a mild pulmonary disease mutation in CFTR. *Am J Respir Crit Care Med 155*, 1684–1689.

Wang, S., Yue, H., Derin, R. B., Guggino, W. B., & Li, M. (2000). Accessory protein facilitated CFTR–CFTR interaction, a molecular mechanism to potentiate the chloride channel activity. *Cell 103*, 169–179.

Ward, C. L., Omura, S., & Kopito, R. R. (1995). Degradation of CFTR by the ubiquitin–proteasome pathway. *Cell 83*, 122–127.

Weinman, E. J., Steplock, D., Wang, Y., & Shenolikar, S. (1995). Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na(+)–H+ exchanger. *J Clin Invest 95*, 2143–2149.

Weixel, K. M., & Bradbury, N. A. (2000). The carboxyl terminus of the cystic fibrosis transmembrane conductance regulator binds to AP-2 clathrin adaptors. *J Biol Chem 275*, 3655–3660.

Weixel, K. M., & Bradbury, N. A. (2001). μ2 binding directs the cystic fibrosis transmembrane conductance regulator to clathrin-mediated endocytic pathway. *J Biol Chem 276*, 46251–46259.

Welsh, M. J., & Smith, A. E. (1993). Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell 73*, 1251–1254.

Welsh, M. J., Tsui, L. -C., Boat, T. F., & Beaudet, A. L. (1995). Cystic fibrosis. In C. Scriver, A. L. Beaudet, W. S. Sly, & D. Valle (Eds.), *The Metabolic and Molecular Basis of Inherited Diseases: Membrane Transport Systems* (pp. 3799–3876). New York, NY: McGraw-Hill.

Xu, X. Z., Choudhury, A., Li, X., & Montell, C. (1998). Coordination of an array of signaling proteins through homo and heteromeric interactions between PDZ domains and target proteins. *J Cell Biol 142*, 545–555.

Xu, Y., Kanauchi, A., von Arnim, A. G., Piston, D. W., & Johnson, C. H. (2003). Bioluminescence resonance energy transfer: monitoring protein–protein interactions in living cells. *Methods Enzymol 360*, 289–301.

Yoo, D., Flagg, T. P., Olsen, O., Raghuram, V., Foskett, J. K., & Welling, P. A. (2004). Assembly and trafficking of a multiprotein ROMK (Kir 1.1) channel complex by PDZ interactions. *J Biol Chem 279*, 6863–6873.

Yun, C. H., Oh, S., Zizak, M., Steplock, D., Tsao, S., Tse, C. M., et al. (1997). cAMP-mediated inhibition of the epithelial brush border Na+/H+ exchanger, NHE3, requires an associated regulatory protein. *Proc Natl Acad Sci U S A 94*, 3010–3015.

Zerhusen, B., Zhao, J., Xie, J., Davis, P. B., & Ma, J. (1999). A single conductance pore for chloride ions formed by two cystic fibrosis transmembrane conductance regulator molecules. *J Biol Chem 274*, 7627–7630.

Zielenski, J., & Tsui, L. -C. (1995). Cystic fibrosis: genotypic and phenotypic variations. *Annu Rev Genet 29*, 771–807.