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CFTR Chloride Channel in the Apical Compartments: Spatiotemporal Coupling to its Interacting Partners

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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride channel located primarily at the apical or luminal surfaces of epithelial cells in the airway, intestine, pancreas, kidney, sweat gland, as well as male reproductive tract, where it plays a crucial role in transepithelial fluid homeostasis. CFTR dysfunction can be detrimental and may result in life-threatening disorders. CFTR hypofunctioning because of genetic defects leads to cystic fibrosis, the most common lethal genetic disease in Caucasians, whereas CFTR hyperfunctioning resulting from various infections evokes secretory diarrhea, the leading cause of mortality in early childhood. Therefore, maintaining a dynamic balance between CFTR up-regulating processes and CFTR down-regulating processes is essential for maintaining fluid and body homeostasis. Accumulating evidence suggests that protein-protein interactions play a critical role in the fine-tuned regulation of CFTR function. A growing number of proteins have been reported to interact directly or indirectly with CFTR chloride channel, suggesting that CFTR might be coupled spatially and temporally to a wide variety of interacting partners including ion channels, receptors, transporters, scaffolding proteins, enzyme molecules, signaling molecules, and effectors. Most interactions occur primarily between the opposing terminal tails (amino or carboxyl) of CFTR protein and its binding partners, either directly or mediated through various PDZ scaffolding proteins. These dynamic interactions impact the channel function, as well as localization and processing of CFTR protein within cells. This article reviews the most recent progress and findings about the interactions between CFTR and its binding partners through PDZ scaffolding proteins, as well as the spatiotemporal regulation of CFTR-containing macromolecular signaling complexes in the apical compartments of polarized cells lining the secretory epithelia.

Introduction

CFTR is an apical plasma membrane chloride channel

Cystic fibrosis (CF) is the most common genetic disease in Caucasians and affects approximately 1 in every 2,500 newborns.¹ CF is caused by dysfunction of a single gene encoding the CF transmembrane conductance regulator (CFTR), which is a member of the ATP-binding cassette (ABC) transporter superfamily. All ABC transporters bind ATP and use the energy to drive the transport of a wide variety of substrates across cellular membranes.² CFTR is composed of two repeated motifs, each of which consists of a hydrophobic membrane-spanning domain (MSD) containing six helices and a cytosolic

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hydrophilic region for binding with ATP (*i.e.*, nucleotide binding domain, NBD)³ (Fig. 1). These two motifs are linked by a cytoplasmic regulatory (R) domain that contains many charged residues and multiple consensus phosphorylation sites (substrates for various protein kinases, such as PKA, PKC, and cGMP-dependent protein kinase II). Both the amino (N) and carboxyl (C) terminal tails of this membrane protein are inside the cytoplasm and mediate the interaction between CFTR and a growing number of binding proteins,^{4–7} as will be discussed in the following sections.

CFTR is a plasma membrane cAMP-regulated Cl[−] channel that is responsible for transepithelial salt and fluid transport.^{8–10} It is primarily localized to the luminal, or apical, membranes of epithelial cells in several functionally diverse tissues including the airway, intestine, pancreas, kidney, vas deferens, and sweat duct. As its name implies, in addition to functioning as a secretory Cl[−] channel, CFTR also acts as a regulator exerting modulatory influences over a wide variety of other ion channels, transporters, and processes, such as the epithelial Na⁺ channel (ENaC),^{11–13} the outwardly rectifying chloride channel,^{14–16} apical K⁺ channels from renal epithelial cell ROMKs,^{17–21} Ca²⁺-activated Cl[−] channels,^{22,23} aquaporin water channels,^{24–26} Cl[−]/HCO₃[−] exchangers,^{27–31} sodium-bicarbonate transporters,^{32–34} Na⁺/H⁺ exchangers,^{34,35} and ATP release mechanisms.^{36,37}

CFTR is involved in two major diseases: cystic fibrosis and secretory diarrhea

Several human diseases result from altered function of CFTR chloride channel, among which cystic fibrosis and secretory diarrhea are the two major disorders.^{1,4,5} CF is a lethal autosomal recessive inherited disease that is caused by the loss or dysfunction of the CFTR Cl[−] channel activity resulting from the mutations that decrease either the biosynthesis or the ion channel function of the protein.^{38,39} The absence or dysfunction of CFTR chloride channel leads to aberrant ion and fluid homeostasis at epithelial surfaces where it is normally expressed. Clinically, CF is dominated by chronic lung disease, which is the main cause of morbidity and mortality for CF patients.⁴⁰ 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 causes bronchiectasis and progressive airway destruction, eventually leading to the loss of pulmonary function. Other symptoms include, but are not limited to, exocrine pancreatic insufficiency and the resultant meconium ileus, elevated sweat electrolytes, and male infertility.^{1,40} To date, around 1600 CFTR mutations have been described, among which F508del-CFTR is the most common CF mutation.⁴⁰ F508del-CFTR is the deletion of three nucleotides resulting in the deletion of a single phenylalanine (F) residue at position 508 on the protein molecule,^{41,42} and it is responsible for ~70% of CF alleles.⁴³ F508del-CFTR mutant is associated with a severe form of the disease, and more than 90% of CF patients have at least one F508del-CFTR allele. It is estimated that approximately half of the CF patients are homozygous for the mutation F508del-CFTR. This allele encodes an unstable and inefficiently folded CFTR protein, which fails to be correctly processed and delivered to its proper cellular location in the plasma membrane.^{38,39,44} Subsequently, the mutant protein is trapped in the endoplasmic reticulum (ER) and is rapidly targeted for degradation in lysosomal compartments.^{38,45} The investigations about the mechanisms underlying the biosynthesis, trafficking, and degradation of F508del-CFTR are providing unique opportunities to understand the pathogenesis of this inherited disorder at the molecular level.⁴⁶

Secretory diarrhea is another major dysfunction involving CFTR and is caused by excessive activation of this chloride channel in the gastrointestinal tract.^{47,48} The pioneer work by Gabriel et al. implied an important role of CFTR in secretory diarrhea by showing that bacterial toxins failed to induce secretory diarrhea in CF mice.⁴⁸ A major cause for acquired secretory and inflammatory diarrhea is the intestinal colonization by pathogenic

microorganisms, including *E. coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Shigella flexneri*, and *Salmonella typhimurium*.^{49,50} CFTR is expressed at the apical membranes of secretory epithelial cells lining the lumen of the gut where it is normally inactive.⁵¹ When the gut lumen is exposed to the various enterotoxins, excessive intracellular second messengers (cAMP and/or cGMP) are generated, which, by activating luminal CFTR, lead to overstimulation of the secretory pathway.^{52,53} Excessive cyclic nucleotides (cAMP and/or cGMP) activate PKA and/or PKG, which subsequently excessively phosphorylate the CFTR channel and lead to Cl⁻ secretion across the epithelium that consequently 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^{54,55} (Fig. 2). In parallel, electroneutral absorption by Na⁺/H⁺ exchanger and electrogenic absorption by ENaC are inhibited.^{56,57} Cholera toxin and heat-labile *E. coli* toxin induce intestinal fluid secretion by excessive increase in intracellular cAMP, because of irreversible activation of the adenylate cyclase resulting from the ADP-ribosylation of the α -subunit of a stimulatory G protein, Gs α , by the toxins.^{58,59} Moreover, cholera toxin increases intracellular cAMP in both crypt and villus epithelial cells, and thus both of the epithelial cells are likely to contribute to generating secretory diarrhea⁶⁰ (Fig. 2). Other toxins, such as heat-stable *E. coli* toxin or *Y. enterocolitica* toxin, enhance intracellular cGMP and lead to stimulation of cGMP-dependent protein kinase II (cGKII), an apical membrane-targeted kinase that efficiently phosphorylates CFTR and results in the activation of Cl⁻ secretion in crypts and apical membranes of the intestine.^{61–65}

Because CFTR plays the central role in certain forms of secretory diarrhea as described above, 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 by high throughput screening promising specific blockers of CFTR.^{66,67} 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.⁶⁸ These blockers could also be useful for treating secretory diarrhea, because they inhibit the equivalent short-circuit current induced by prostaglandin E₂, vasoactive intestinal polypeptide, adenosine, cholera toxin, and cAMP in distal rabbit colon from both the mucosal and the serosal sides of the epithelium and show fairly low IC₅₀ values.⁶⁸ Gabriel and his colleagues⁶⁹ 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. They demonstrated that SP-303 is effective against *in vivo* cholera toxin-induced fluid secretion and *in vitro* cAMP-mediated Cl⁻ secretion.⁶⁹ A potent and selective small-molecule CFTR inhibitor (CFTR_{inh}-172) was identified recently by Verkman's group by high-throughput screening.^{70,71} 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. Schuier et al. investigated the effects of cocoa flavonols on CFTR-mediated Cl⁻ secretion in intestinal cells and found that cocoa flavonols act as mild CFTR blockers.⁷² 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, suggesting that LPA-rich foods may present an alternative method of treating certain forms of diarrhea.⁷³

CFTR interacts directly with multiple PDZ scaffolding proteins

A fast-growing body of evidence has been documented to suggest the existence of various physical and functional interactions between CFTR and an ever-increasing number of

proteins, including transporters, ion channels, receptors, kinases, phosphatases, signaling molecules, and cytoskeletal elements, and these interactions between CFTR and its binding proteins have been shown to play an important role in regulating CFTR-mediated transepithelial ion transport *in vitro* and most probably *in vivo*.^{11–35} Among these reported interactions, many are mediated through a physical interaction between these binding proteins with both the amino terminal and carboxyl terminal tails of the CFTR chloride channel. In this review article, we will focus only on the recent progress and findings about the interactions between CFTR carboxyl terminal tail, which possesses a protein-binding motif, and a group of scaffoldings proteins that contain a specific binding module referred to as PDZ domains.

PDZ domains and PDZ scaffolding proteins

PDZ domains, the most abundant protein-interaction modules in the human genome, are composed of about 80–90 amino acid residues that form peptide-binding clefts and mediate interactions, usually with the carboxyl termini of target proteins, which terminate in consensus PDZ-binding sequences (also referred to as PDZ-motif; most often in the cytoplasmic tails of transmembrane receptors, transporters, and channels).^{74–77} Their name derives from the first three proteins in which PDZ domains were first identified: the postsynaptic density protein PSD-95/SAP-90, the *Drosophila* junctional protein Disc-large DLG, and the epithelial tight junction protein zonula occludens (ZO)-1. The building principle of the PDZ domain is a sandwich structure of six β -strands and two α -helices that form a hydrophobic cleft into which a short peptide (*i.e.*, PDZ-motif) can be accommodated. Proteins that possess PDZ domains (PDZ domain-containing proteins, PDZ scaffolding proteins, or PDZ proteins) 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 described below. Many studies have reported the association of these PDZ proteins with functionally related groups of proteins including ion channels, receptors, transporters, and other signaling proteins in the apical surfaces of cells, suggesting that apical membrane PDZ proteins can facilitate the formation of multiprotein complexes clustered within microdomains that modulate trafficking, transport, and signaling in polarized epithelial cells.^{20,26,73,78–86}

PDZ proteins that are primarily localized to the apical surfaces of epithelial cells include, but are not limited to, 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), PDZK1 (PDZ domain-containing protein in kidney 1; also called CFTR-associated protein 70, Cap70; and NHERF3), PDZK2 (also called intestinal and kidney-enriched PDZ protein, IKEPP; and NHERF4), and Shank2 (SH3 and Ankyrin repeats containing protein 2; also called Cortactin-binding protein 1, CortBP1) (Fig. 3).^{4,87–89} NHERF1 and NHERF2 are highly homologous, with about 52% amino acid identity.^{90,91} NHERF1 and NHERF2 both contain two PDZ domains and a C-terminal ERM domain that mediates association with MERM (merlin-ezrin-radixin-moesin) proteins through which to link CFTR to the cytoskeleton. In addition, both NHERF1 and NHERF2 have been shown to form homodimers and heterodimers.^{92,93} PDZK1 and PDZK2 are also highly homologous, and they both contain four tandem PDZ domains, but no other protein interaction domains have been identified.^{74–76} PDZK1 has been shown to form hetero-oligomers with NHERF1 *in vitro*.⁹⁴ This suggests that dimerization or oligomerization between various PDZ scaffolding proteins may allow the formation of larger clusters of PDZ adapter proteins together with other structurally/functionally related proteins underneath the apical plasma membrane.

Interactions between CFTR and PDZ scaffolding proteins

So far, six different PDZ scaffolding proteins have been reported to bind to the carboxyl terminal tail of the CFTR channel with various affinities: NHERF1, NHERF2, PDZK1, PDZK2, CAL (CFTR-associated ligand), and Shank2 (Fig. 3).^{78,79,81,95–100} Among these PDZ proteins, four of them (NHERF1, NHERF2, PDZK1, and PDZK2) possess multiple PDZ domains, whereas CAL and Shank2 have only one PDZ domain. As stated above, NHERF1, NHERF2, PDZK1, PDZK2, and Shank2 also have been reported to be localized to the apical membranes of epithelial cells where CFTR also resides, while CAL is primarily localized to Golgi. These CFTR-interacting scaffold proteins demonstrate different tissue distributions, suggesting that different PDZ proteins might interact with CFTR in different tissues.

The PDZ-motif within the CFTR chloride channel that is recognized by PDZ domains in various PDZ proteins is the last four amino acids at the C-terminus of the CFTR protein (*i.e.*, 1477-DTRL-1480 in human CFTR).^{78,79,81,95–100} There are three major classes of PDZ domains based on target sequence specificity, and these CFTR-interacting PDZ domains belong to class I with consensus target sequence of -X-(S/T)-X-Φ (referred to as PDZ-motif, where X = any amino acid, and Φ = hydrophobic amino acid).^{76,77} Interestingly, CFTR can bind to more than one PDZ domain of both NHERFs and PDZK1, albeit with varying affinities.^{81,101} This multivalency with respect to CFTR binding has been shown to be functionally significant, suggesting that PDZ proteins may facilitate formation of macromolecular signaling complex, as will be discussed in detail in the following sections.

It has been reported that the ERM domain within the C-terminal tails of NHERF1 and NHERF2 tether NHERF1 and NHERF2 to the cortical cytoskeletal elements via binding to ezrin.⁹¹ Based on these observations, it has been proposed that interactions between CFTR and NHERF1 could anchor CFTR chloride channel to the cytoskeleton associated with the apical membrane.⁷⁹ In addition, it has been reported that 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,¹⁰² suggesting that an intact PDZ recognition sequence (PDZ-motif) is essential for the proper and efficient localization of CFTR to the apical surfaces of epithelial cells. However, it is also argued that this PDZ motif is not sufficient, and other C-terminal sequences in addition to a PDZ-binding motif are also required for localizing CFTR to the apical plasma membrane.^{103,104} Interestingly, the clinical phenotype of individuals harboring CFTR deletion mutant lacking the last 26 amino acid residues exhibits moderately elevated sweat chloride concentration without obvious pancreatic and pulmonary dysfunction,¹⁰⁵ suggesting the involvement of other factors in addition to NHERF1 and the C terminus of CFTR in the apical targeting of CFTR. Recently, Lukacs's group reported that disruption of the complex formation between CFTR and NHERF1 by C-terminal deletions, C-terminal epitope tag attachments, or overexpression of a dominant negative NHERF1 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.¹⁰⁶ In addition, Welsh's group 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.¹⁰⁷ Therefore, it is possible that apical localization of CFTR involves sorting signals other than the C-terminal 26 amino acid residues and the PDZ-binding motif in differentiated epithelia and may help explain the relative rarity of CF-associated mutations in the C terminus.

As a member of the A-kinase anchoring proteins (AKAPs), ezrin is responsible for the subcellular sequestration of A-kinase (*e.g.*, cAMP-dependent protein kinase, PKA). It was

reported that ezrin binds the catalytic and regulatory subunit of PKA,¹⁰⁸ which 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 was demonstrated by Huang et al., 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.¹⁰⁹ 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.^{95,110} CFTR was demonstrated to not only colocalize with NHERF2 and ezrin at or near the apical surfaces of Calu-3 airway epithelial cells, but also co-immunoprecipitate with each of these proteins (NHERF2, ezrin, and PKA). In addition, the binding of PKA to ezrin, as well as the activation of CFTR channels by cAMP agonists, was inhibited by a synthetic peptide that blocks the binding of PKA regulatory subunits to AKAPs. These observations strongly suggest the existence of macromolecular complexes of CFTR with these proteins that play an essential role in scaffolding, signaling, trafficking, etc., which would be important in regulating channel property and function.

As stated above, some PDZ proteins contain multiple PDZ domains that can bind to the PDZ motif at the CFTR C-terminal tail (Fig. 3), and CFTR can bind to more than one PDZ domain of these PDZ proteins. These multivalent PDZ proteins (*i.e.*, PDZK1 and NHERF1) have been proposed to regulate the gating of CFTR channels at the plasma membrane.^{81,101} Two groups reported that PDZK1 and NHERF1 can enhance CFTR channel activity in excised inside-out membrane patches.^{81,101} A recombinant fragment of NHERF1 containing the two 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 .¹⁰¹ The multivalent PDZK1 potentiates CFTR channel activity probably through its dimeric binding to the C terminus of CFTR.⁸¹ 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.¹¹¹ These interactions could increase the dynamic range of CFTR channel activity beyond that provided by cAMP stimulation alone, especially because NHERF1 and PDZK1 were observed to enhance CFTR activity in the presence of normally saturating doses of PKA and ATP.^{81,101}

CAL (also called Golgi-associated PDZ and coiled-coil domain containing, GOPC), a PDZ scaffolding protein with only one PDZ domain, was first reported by Guggino's group to bind directly to CFTR via PDZ-mediated interaction⁹⁶ (Fig. 3). CAL was shown to locate primarily in the perinuclear region of the cell and to be associated with the Golgi apparatus, primarily at the *trans*-Golgi network. CAL also contains two amino terminal coiled-coil domains that facilitate homomultimerization and probably also membrane association. The interaction of the second coiled-coil domain with TC10,¹¹² a small GTPase, and Syntaxin 6,¹¹³ a SNARE protein, suggests that CAL is involved in vesicle trafficking. Cheng et al. reported that deletion of the last four amino acid residues of CFTR abolished the interaction between CAL and CFTR. Cotransfection of CAL with CFTR reduced CFTR currents and surface expression of CFTR.⁹⁶ This inhibitory effect of CAL could be reversed by co-expression with NHERF1, which blocks the binding of CAL to the CFTR C-terminal tail. CAL was also reported to regulate the expression of mature CFTR, because co-expression of CAL with CFTR in COS-7 cells causes a dose-dependent reduction in mature CFTR.¹¹⁴ In

addition, expression of the dominant-negative dynamin 2 K44A, a GTPase, which 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.^{114,115} Based on these findings, it was possible that the observed increase in cell surface CFTR could be related to a decrease or inhibition of endocytosis. Guerra et al.¹¹⁶ studied the role of NHERF in regulating CFTR in polarized airway cells by monitoring the activity and trafficking of CFTR in two human bronchial cell lines: 16HBE14o-cells expressing wild-type CFTR and CFBE41o-cells homozygous for the F508del-CFTR mutant. They found that both normal and CF cells express both NHERF1 and NHERF2, while NHERF1 follows CFTR expression patterns. Both of the PDZ1 and PDZ2 domains and the ERM domain of NHERF1 are involved in the polarized expression of CFTR and in the regulation of CFTR-dependent chloride efflux. Further, targeted NHERF1 overexpression stimulates CFTR-dependent chloride efflux by increasing apical CFTR expression in normal 16HBE14o-cells. Importantly, in the CF cell lines (CFBE41o- and CFT1-C2), targeted NHERF1 overexpression induced the redistribution of CFTR from the cytoplasm to the plasma membrane and rescued CFTR activity. A possible mechanism for this NHERF1-dependent redistribution of F508del-CFTR to the apical membrane may be that NHERF favors surface expression by competing with CAL for CFTR binding.⁹⁶ If CAL is indeed a negative regulator for plasma membrane CFTR level, then suppression of endogenous CAL expression levels will increase the cell-surface expression of functional F508del-CFTR. Recently, Wolde et al.¹¹⁷ showed that RNA interference targeting endogenous CAL specifically increased cell-surface expression of the disease-associated F508del-CFTR mutant and thus enhanced transepithelial chloride currents in a polarized human patient bronchial epithelial cell line, suggesting a role for CAL PDZ binding domain as a candidate therapeutic target for correction of postmaturation trafficking defects in cystic fibrosis.¹¹⁸

NHERF1 and NHERF2 functionally stabilize cell-surface CFTR, while CAL not only limits cell-surface levels of mutant F508del-CFTR but also mediates degradation of WT-CFTR, targeting it to lysosomes following endocytosis. How exactly CAL, NHERF1, and NHERF2 compete to regulate CFTR endocytic processing remains elusive. Recently, using fluorescence polarization, isothermal titration calorimetry, and surface-plasmon resonance analysis, Cushing et al.¹¹⁹ quantitatively compared the relative binding affinities of CFTR with these functionally antagonistic PDZ scaffolding proteins. They found that the affinity of the CAL PDZ domain for the CFTR C terminus is much weaker than those of NHERF1 and NHERF2, enabling wild type CFTR to avoid premature entrapment in the lysosomal pathway. In the meanwhile, CAL's affinity is evidently sufficient to capture and degrade more rapidly cycling mutants, such as F508del-CFTR. As a result, the CAL:CFTR complex may be susceptible to selective pharmacological targeting as an approach to retaining rescued Δ F508-CFTR at the apical membrane. Furthermore, the weak affinity of CFTR for CAL compared to NHERF1 provides a biochemical explanation for the efficient postendocytic recycling of WT CFTR.

Shank2, an isoform of the recently identified family of multimodular adaptors,¹²⁰ is also a PDZ domain scaffolding protein that is reported to interact with CFTR.^{99,100} In addition to a PDZ domain, Shank2 also contains other sites for protein-protein interaction, including an SH3 domain, a long proline-rich region, and a sterile alpha motif (SAM) domain (Fig. 3). Shank2 is expressed abundantly in brain as well as in kidney, liver, intestine, and pancreas^{99,100,121,122} and is localized to the luminal pole in pancreatic duct cells and luminal area of colonic epithelia.⁹⁹ Shank2 has been shown to be associated with CFTR through its PDZ domain in the yeast two-hybrid system and in mammalian cells.⁹⁹ Measurements in CFTR-expressing NIH 3T3 cells revealed that Shank2 overexpression suppressed the cAMP-induced phosphorylation and activation of CFTR. In addition,

antisense-Shank2 treatment augmented the CFTR-dependent Cl^- transport in T84 epithelial cells, in which CFTR and Shank2 are endogenously expressed. Because aberrant CFTR activity, especially uncontrolled hyperfunctioning of CFTR, evokes life-threatening conditions such as diarrhea in cholera infection, the fine regulation of CFTR activity by Shank2 may play an important role in maintaining epithelial and body homeostasis. To further define the mechanisms for negative regulation of the CFTR by Shank2, using the surface plasmon resonance assays and consecutive patch clamping, this same group went on to demonstrate a physical and physiological competition between NHERF1-CFTR and Shank2-CFTR associations.¹⁰⁰ They found that the dissociation constant of CFTR-Shank2 binding was similar to that of CFTR-NHERF1 binding and that both proteins compete for binding at the same site. CFTR Cl^- channel activity was dynamically regulated by the competition of Shank2 and NHERF1 binding. In contrast to the PKA/AKAP recruitment by NHERF1, Shank2 was found to tether PDE4D to the CFTR complex, thus attenuating cAMP/PKA signals¹⁰⁰ and suggesting that the competitive balance between Shank2 and NHERF1 binding to the CFTR channel may maintain homeostatic regulation of epithelial ion and fluid transport.

The studies and observations described above raise a possibility that CFTR activity can be optimized through interactions with various PDZ scaffolding proteins in several complementary/anticomplementary ways. Such interactions may regulate either the numbers of functional CFTR channels at the apical membranes of epithelial cells, the functional activities of those channels within this membrane, or both. However, unresolved issues still remain, such as 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). It was reported that individuals whose CFTR channels lack the PDZ recognition sequence do not have cystic fibrosis.¹⁰⁵ However, these individuals have been reported to exhibit elevated sweat chloride concentrations, which could be caused by reduced CFTR function in the reabsorptive sweat duct.⁸ In addition, it has been argued that the major clinical manifestations of CF (pathology of the lung and pancreas) occur only when CFTR expression or function has been reduced by more than 90%.¹²³ In short, the occurrence of classical CF may not be a sufficiently sensitive indicator of the physiologic importance of protein-protein interactions involving CFTR that are regulatory in nature. As described above, there are at least six different PDZ scaffolding proteins that were reported to be associated with CFTR (Fig. 3). Some of these proteins have distinct intracellular locations and tissue distributions (*e.g.*, CAL, which localizes to the Golgi).^{96,114,115} Based on these considerations, it seems reasonable to propose that CFTR channels could be recruited into distinct macromolecular signaling complexes (*i.e.*, complexes with distinct protein components) in different cell compartments and tissues by combinatorial interactions with various PDZ proteins *in vitro* and probably *in vivo*, which will be discussed in detail in the following section.

Spatiotemporal coupling of CFTR and its interacting partners in apical plasma membranes

It is now well documented and accepted that the formation of multiprotein macromolecular signaling 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 surface do not always lead to detectable changes in the concentration of specific second messengers (cAMP, cGMP, Ca^{2+} , etc.), although the cellular response is significantly altered. This notion is supported by studies by Huang et al.,¹⁰⁹ 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

finding implies that receptors (or ion channels or transporters), signaling intermediates, and effectors are compartmentalized into regulatory complexes that increase the efficiency of signaling and that this compartmentalization 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.^{4–7,124} 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.^{20,26,73,83,84} A well-documented example of such macromolecular complex of signaling molecules is the INAD complex in *Drosophila* photoreceptor cells.¹²⁵ This multivalent PDZ protein (INAD) serves as a scaffolding molecule to assemble different components of the phototransduction pathway, which includes at least two distinct ion channels that are coupled to multiple signaling molecules by INAD that contains five PDZ domains.¹²⁶ 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 below.

A macromolecular signaling complex of β_2 adrenergic receptor, NHERF1, and CFTR couples β_2 adrenergic signaling to CFTR function

CFTR Cl^- channel can be either absorptive or secretory and plays a major role in water and salt transport in these epithelial cells. Normal airway epithelial cells consist of an absorptive surface epithelium and a secretory submucosal gland.¹²⁷ The secretory function of the submucosal gland is essential for the maintenance of the airway surface liquid and for the mucociliary clearance.¹²⁸ The secretory function is also essential for the clearance of mucus from the submucosal gland.¹²⁸ In individuals harboring mutant forms of CFTR, the secretory function of the submucosal gland is impaired and leads to the accumulation of mucus in the airway surfaces, in turn leading to serious respiratory complications. As the major adrenergic receptor isoform expressed in airway epithelial cells, β_2 adrenergic receptor ($\beta_2\text{AR}$) stimulated with β receptor agonist (isoproterenol) leads to activation of CFTR-dependent chloride transport *in vivo*.¹²⁹ We recently demonstrated that both $\beta_2\text{AR}$ and CFTR bind NHERF1/EBP50 through their PDZ motifs, which form a complex at the apical surfaces of airway epithelial cells.⁸³ Deletion of the PDZ motif from CFTR was shown to uncouple the channel from $\beta_2\text{AR}$ receptor both physically and functionally, and this uncoupling is specific to the $\beta_2\text{AR}$ receptor and does not affect CFTR coupling to other receptors (*e.g.*, adenosine receptor pathway). We demonstrated the existence of a macromolecular complex involving CFTR-NHERF1- $\beta_2\text{AR}$ in a PDZ-dependent manner, which is regulated by PKA-dependent phosphorylation. Interestingly, PKA phosphorylation of CFTR inhibited 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.⁸³ A model was proposed to depict the coupling of $\beta_2\text{AR}$ signaling to CFTR chloride channel function (Fig. 4). The macromolecular complex assembly is essential for full activation of CFTR channel by $\beta_2\text{AR}$ pathway, which can be circumvented by using other pathways (*e.g.*, adenosine or forskolin). This interaction is critical for a rapid and specific signal transduction from the $\beta_2\text{AR}$ to the CFTR channel in a compartmentalized fashion. The interactions of this sort may also be important to CFTR regulation of other ion

channels, receptors, and transporter proteins, which will be demonstrated in the following sections. 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 F508del-CFTR and other mutations in which 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.

Most recently, Singh et al.⁸⁵ reported the same complex of CFTR-NHERF1- β_2 AR in the apical membrane of duodenal enterocytes in mice. They showed that CFTR was localized to the apical membranes in the duodenocytes predominantly in the cryptal region and Brunner's glands; β_2 -AR was also localized to the apical membrane of the villi, as well as crypt duodenocytes and the epithelial lining of the Brunner's glands. In wild type mice, there is a colocalization of CFTR and β_2 -AR in the duodenum where apical localization of NHERF1 in the crypt and villous region has been established,¹³⁰ while in the absence of NHERF1 (*Nherf1*-deficient mice), the colocalization staining appeared more diffuse, although a substantial part of both proteins was still localized in the brush border membrane.⁸⁵ They also observed that, in WT mice, but not in *Nherf1*^{-/-} mice, a specific β_2 -AR antagonist, ICI-118551, significantly inhibited CFTR-dependent basal HCO_3^- secretion in the duodenum, indicative of an involvement of β_2 -AR activation in the basal HCO_3^- secretory tone which is NHERF1 dependent. A selective β_2 -AR agonist, clenbuterol, added in the luminal perfusate caused a significant stimulation of CFTR-dependent duodenal HCO_3^- secretion in the WT mice that was completely absent in the *Nherf1*^{-/-} mice, and this demonstrates the necessity of NHERF1-mediated interaction for the β_2 -AR-mediated stimulation of CFTR and strengthens the concept of NHERF1-mediated complex formation between the β_2 -AR and CFTR. More interestingly, both the β_2 -AR antagonist, ICI-118551, and agonist, clenbuterol, still had inhibitory and activating effects in *Nherf2*^{-/-} and *Pdzk1*^{-/-} mice, which indicates that the β_2 -AR-dependent regulation of CFTR is specific for NHERF1.⁸⁵

A macromolecular signaling complex of LPA2, NHERF2, and CFTR mediates LPA-elicited inhibition of CFTR function

Secretory diarrhea is one of the leading causes of death in children in both developing and industrialized countries. Two major organisms causing infectious diarrhea in humans are *Escherichia coli* and *Vibrio cholera*, whose secreted toxins (heat-stable or heat-labile toxin and cholera toxin (CTX), respectively) influence gastrointestinal epithelial cell function and induce diarrhea by numerous mechanisms.⁵² It is well established that CFTR plays a pivotal role in cholera toxin-induced secretory diarrhea in model cell systems and experimental animals (Fig. 2). Recently, we demonstrated that lysophosphatidic acid (LPA), a naturally occurring phospholipid in blood and foods, can significantly inhibit cholera toxin-induced CFTR-mediated secretory diarrhea.⁷³ And this inhibition is mediated through a type 2 LPA receptor (LPA2), which is localized to the luminal membrane of colonic epithelial cells and gut mucosal epithelia where CFTR and NHERF2 also reside. LPA2, but not LPA1 or LPA3, forms a macromolecular signaling complex with CFTR mediated by NHERF2 in a PDZ-motif dependent manner. LPA inhibited CFTR-dependent iodide efflux, short-circuit currents, and single-channel activity in a compartmentalized fashion. CFTR-dependent intestinal fluid secretion induced by CTX in mice was reduced substantially by LPA administration, and disruption of this complex using a cell-permeant LPA₂-specific peptide reversed LPA₂-mediated inhibition. This was the first confirmation that a macromolecular complex (CFTR-NHERF2-LPA₂) formed *in vitro* was also physiologically and functionally relevant *in vivo*. In our study, we observed that cAMP accumulation inside polarized epithelial cells (Calu-3), upon stimulation with a low concentration of adenosine (2 μM),

was almost indistinguishable from that of unstimulated cells, while CFTR was active under these conditions and LPA could significantly inhibit channel activity. This is a very important observation, which is consistent with the results from Huang et al.,¹⁰⁹ who demonstrated compartmentalized signaling from the receptor (A2b) to the channel (CFTR) by using electrophysiological methods. They reported that CFTR chloride channel function was observed upon stimulation of the cells by a low concentration of adenosine (1 μ M), wherein the accumulation of cAMP inside the cell was not significantly higher than that from unstimulated control cells.¹⁰⁹ Interestingly, in our study, we found that LPA efficiently inhibited CFTR function (in response to 2 μ M adenosine) without causing a decrease in the global cAMP accumulation in the cell.⁷³ Our results support the notion that cAMP is generated in a compartmentalized pocket upon stimulation by receptor-mediated agonists (adenosine). However, when the adenosine level is increased to 20 μ M, we observed that CFTR function increases slightly compared to 2 μ M adenosine, but there was a significant increase in cAMP accumulation inside the cell (global cAMP accumulation). Although there was a significant decrease in cAMP accumulation with LPA treatment, the CFTR-mediated short circuit current was not significantly different (in the presence or absence of LPA), suggesting that a global increase in cAMP may offset CFTR inhibition elicited by LPA.

Diarrhea is the most common gastrointestinal disorder, with CFTR playing a central role in this process.^{48,53,55} Any reagent or factor that inhibits CFTR channel could be beneficial in ameliorating diarrhea symptoms in cases of CFTR-mediated diarrhea. Given that LPA₂ is expressed in the gut, localized to the apical surface, physically interacts with CFTR via NHERF2 PDZ scaffolding protein, and activates the G_i pathway leading to decreased cAMP accumulation, a model was proposed to depict the signaling from LPA₂ activation to CFTR inhibition (Fig. 5).⁷³ LPA₂ and CFTR are physically associated with NHERF2, which clusters LPA₂ and CFTR into a macromolecular signaling complex at the apical plasma membranes of epithelial cells. This macromolecular complex is the foundation of functional coupling between LPA signaling and CFTR-mediated Cl⁻ transport. If the physical association is disrupted, functional coupling will be compromised. Upon LPA stimulation of the receptor, adenylate cyclase is inhibited through the G_i pathway, leading to a decrease in cAMP level. This decreased local or compartmentalized accumulation of cAMP results in the reduced activation of Cl⁻ channel in the vicinity by CFTR agonists (*e.g.*, adenosine). Our findings suggest an alternative method of treating such forms of diarrhea by using LPA-rich foods, a cost-effective therapeutic possibility that could be implemented in Third World countries where the cost of drug treatment is often limiting.

Most recently, it was reported that NHERF2 is also essential for lysophosphatidic acid inhibition of CFTR-mediated duodenal HCO₃⁻ secretion in mice.⁸⁵ LPA₂ and NHERF2 proteins were found to be enriched in the apical membrane of isolated duodenal brush border membrane. Quantitative PCR demonstrated that mRNAs of LPA₂, NHERF2, and CFTR were strongly enriched in the crypt region, whereas proteins typically predominant in the villous (such as NHE3) were appropriately enriched in the villous region. In WT mice, LPA elicited a significantly reduced response of FSK-stimulated duodenal HCO₃⁻ secretion, while in *Nherf2*^{-/-} mice, no significant difference was seen in the HCO₃⁻ stimulatory response to FSK with and without LPA. However, the loss of *Nherf1* or *Pdzk1* did not abolish the inhibitory effect of LPA on duodenal HCO₃⁻ secretion *in vivo*. These results demonstrate an absolute requirement of NHERF2 for LPA₂-mediated inhibition of CFTR *in vivo*.⁸⁵

A macromolecular signaling complex of MRP4, PDZK1, and CFTR spatiotemporally couples MRP4 cAMP transporter activity to CFTR function

The cyclic nucleotides (*e.g.*, cAMP) are important second messengers involved in the cellular responses to extracellular and intracellular signals in every living cell. In epithelial

cells lining the gut, kidney, and lung, cAMP also plays key roles in extracellular regulation of fluid homeostasis.¹³¹ Tight regulation of intracellular cAMP levels is critical, because excessive cAMP production within cells leads to over-stimulation of certain secretory events, dysregulation of cell function, or even cell toxicity.¹³¹ Most recently, by taking a series of biochemical, physiological, electrophysiological, and microscopic approaches, we identified a novel means of regulating cAMP levels in a compartmentalized microdomain underneath the surface membrane, an efflux path for cAMP via MRP4 transporter in the close vicinity of CFTR-containing signaling complex.⁸⁴ We showed that the multidrug resistance protein 4 (MRP4), a cAMP transporter, functionally and physically associates with CFTR. MRP4 inhibition potentiated adenosine-stimulated CFTR-mediated chloride currents, and this potentiation is directly coupled to attenuated cAMP efflux through the apical cAMP transporter. CFTR single-channel recordings and intracellular cAMP dynamics suggest a compartmentalized coupling of cAMP transporter and CFTR via the PDZ scaffolding protein (PDZK1), forming a macromolecular signaling complex at apical surfaces of gut epithelia. Disrupting this complex abrogates the functional coupling of cAMP transporter activity to CFTR function. In addition, *Mrp4*-deficient mice are more prone to CFTR-mediated secretory diarrhea. In this study, we observed that inhibition of a cAMP transporter MRP4 by a potent MRP4 inhibitor, MK571, potentiated CFTR-mediated Cl^- currents. We also observed that potentiation of CFTR function by MK571 was most prominent when CFTR was activated by lower concentrations of adenosine ($< 20 \mu\text{M}$) but not with higher doses of agonists ($> 20 \mu\text{M}$). This suggests that lower doses of adenosine may lead to compartmentalized cAMP accumulation, which is further enhanced by the reduced or inhibited cAMP efflux through MRP4 by MK571. This finding is consistent with the observation described above that cAMP is generated in a compartmentalized pocket upon stimulation by $2 \mu\text{M}$ adenosine, which is enough to activate CFTR function in proximity, and that LPA, a cAMP-lowering agent, significantly inhibits CFTR function.⁷³ Here the concept of cAMP compartmentalization was recapitulated with the treatment of MK571, which significantly potentiated CFTR function when it was stimulated by lower doses of adenosine. It has been reported that inhibiting MRP4 transporter does not lead to substantial increases in intracellular cyclic nucleotide levels (globally); neither does overexpression of MRP4 lead to a substantial decrease in intracellular cAMP levels, even when these levels are high.¹³² This is probably because the inhibition of MRP4 resulted only in localized fluctuations of cyclic nucleotide levels but not global levels.

The specificity of spatially restricted and temporally modulated compartmentalization of cAMP signaling has been well documented to rely substantially on the organization of macromolecular signaling complexes that effectively assemble multiple proteins (from receptors/channels/transporters to targets) into three-dimensional arrays at subcellular locations, and that proximity of these signaling proteins to their ultimate targets guarantees the velocity of response and the specificity of signaling. Based on previous findings and the data obtained from one of our studies, a model was proposed to depict the spatiotemporal coupling of cAMP transporter to CFTR Cl^- channel function in the gut epithelia (Fig. 6).⁸⁴ Underneath the apical plasma membrane exist highly localized compartments that are composed of a series of signaling molecules such as adenosine receptor (AR); G protein (Gs); AC; PKA and its anchoring proteins AKAPs; CFTR; cAMP transporter (MRP4); and PDZ scaffolding proteins (in this case, PDZK1), which functions to physically connect CFTR to MRP4. This macromolecular signaling complex provides an anatomical basis for generating and modulating local cAMP compartments. When an agonist (in this case, adenosine) binds AR, a series of G-protein-mediated reactions leads to activation of AC present in the apical membrane. Sufficient cAMP is locally generated in a diffusionally restricted apical microdomain (but not in other cellular compartments). cAMP activates PKA, which is anchored also to the apical membrane by AKAP (*i.e.*, ezrin), and phosphorylates CFTR Cl^- channel in close vicinity, resulting in an increase of Cl^- currents.

The CFTR-mediated Cl^- currents can be further increased (potentiated) by increasing local cAMP resulting from the reduced or blocked efflux via a neighboring apical membrane cAMP transporter (MRP4) in the same subcellular compartment. In general, it is believed that phosphodiesterases provide the sole means for degrading cAMP in cells and play a vital role in shaping intracellular gradients of the second messenger.^{131,133} Here, we identified additional means of regulating cAMP levels in a microdomain underneath the surface membrane, an efflux path for cAMP via MRP4 transporter in the close vicinity of CFTR-containing signaling complex.⁸⁴ The interaction between CFTR and MRP4 provides an additional layer of mechanism to regulate CFTR function, which is important in maintaining epithelial and body homeostasis.

Recently, it was demonstrated that a lack of PDZK1 in murine small intestine leads to a mild reduction in maximal CFTR activation.¹³³ In *pdzk1*-deficient murine duodenal mucosa, the basal CFTR-mediated short-circuit current and HCO_3^- secretory rate were not changed, but a significant, yet mild, reduction of forskolin-stimulated short-circuit current and HCO_3^- secretory rate was observed compared to wild type tissue.¹³³

A macromolecular signaling complex of ROMK, NHERFs, and CFTR

The regulation of salt and fluid excretion in the kidney is essential for volume and osmotic homeostasis of the body, which requires the dynamic and delicate coordination of a variety of transport proteins for different ions, water, and solutes that are differentially distributed along the nephron. CFTR is highly expressed in all segments of the mammalian nephron^{134–136} and not only functions as a Cl^- channel but also as a regulator for other ion channels, including the renal secretory renal outer medullar potassium (ROMK) channel.^{124,127,137} The ROMK (also known as Kir 1.1 or KCNJ1) subtypes of weakly inward rectifying potassium channels are primarily localized on the apical membrane of epithelial cells in the thick ascending limb (TAL) of Henle's loop, distal tubule, and collecting duct in the kidney and play critical roles in salt and water homeostasis in the kidney.^{138,139} Loss-of-function mutations in the human ROMK gene (*KCNJ1*) cause type II Bartter syndrome, a familial renal salt wasting nephropathy.^{140,141} Mice lacking the ROMK gene manifest a similar disorder.¹⁴²

ROMK1-3 channels also contain 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.¹⁴³ 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,^{17,144} raising the possibility that trafficking of ROMK and interaction with CFTR are linked by a common PDZ domain-based scaffold. Recently, Welling and colleagues²⁰ provided evidence that ROMK associates directly with NHERF1 and NHERF2 through a PDZ binding interaction to facilitate 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 binds to PDZ1 within NHERF1 and to PDZ2 within NHERF2. 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.²⁰ These observations raise the possibility that PDZ-based interactions may underscore physiological regulation and membrane targeting of ROMK in the kidney. More recently, Lu et al.²¹ reported that both ATP and glibenclamide sensitivities of ROMK in TAL cells were absent in mice lacking CFTR and in mice homozygous for the ΔF508 mutation. They also demonstrated that the effect of CFTR on ATP sensitivity was abrogated by increasing PKA activity, suggesting that CFTR regulates the renal K secretory channel by providing a PKA-regulated functional

switch that determines the distribution of open and ATP-inhibited K channels in apical membranes.

A macromolecular signaling complex of AQP9, NHERF1, and CFTR

Ion transport and water transport represent crucial events in establishing and maintaining male fertility. The epididymal epithelial cells transport electrolytes and water to create an intraluminal fluid environment that is optimal for sperm maturation and storage.^{145,146} These transport processes are under tight control by nerves and hormones. Various neurohumoral agents influence fluid secretion via G-protein-coupled receptors linked to various second-message cascades culminating in the activation of ion channels; of particular importance is the CFTR channel.^{145,146}

In the more distal regions of the epididymis and in the vas deferens, water secretion, driven by CFTR-dependent chloride transport, occurs and controls the fluidity of the luminal content.^{26,147} In a variety of epithelia, water channels (also known as aquaporins, AQPs) are involved in transepithelial bulk water flow driven by an osmotic gradient that results from NaCl transport secondary to active chloride transported by CFTR.¹⁴⁸ One aquaporin isoform, aquaporin-9 (AQP9; also belongs to the aquaglyceroporin subgroup), is reported to be the major apical aquaporin in the male reproductive system that is constitutively expressed in the apical stereocilia of principal cells along the entire length of the epididymis and vas deferens, as well as in the apical membrane of nonciliated cells of the efferent ducts.^{26,149} AQP9 allows passage of a wide range of solutes, including glycerol, urea, mannitol, and sorbitol, in addition to water.¹⁵⁰

With similar tissue localization, CFTR and AQP9 may cooperate to regulate epididymal luminal fluid composition and ion strength. Cheung et al.²⁵ demonstrated that rat epididymis CFTR and AQP9 expressed in *Xenopus* oocytes interact to regulate water permeability of the oocytes. They showed that AQP9 alone caused an increase in oocyte water permeability, which could be further potentiated by CFTR, and this potentiation was markedly reduced by inhibitors of AQP9 and CFTR. They also demonstrated the regulation of water permeability by CFTR in intact rat epididymis luminally perfused with a hypo-osmotic solution. These results suggest a role for CFTR in controlling water permeability in the epididymis *in vivo*. AQP9 also contains a putative PDZ binding motif, SVIM, in its carboxyl terminus, suggesting the potential intervention of PDZ proteins in its regulation. Most recently, it was reported that NHERF1, CFTR, and AQP9 colocalized in the apical membrane of principal cells of the rat epididymis and the vas deferens and that both NHERF1 and CFTR were coimmunoprecipitated with AQP9.²⁶ They showed that AQP9 bound to both the PDZ1 and PDZ2 domains of NHERF1, with an apparently higher affinity for PDZ1 *versus* PDZ2, and this interaction between AQP9 and NHERF1 is AQP9 PDZ (*i.e.*, SVIM) motif-dependent. Using functional assays on isolated tubules perfused *in vitro*, they also found that inhibition of CFTR significantly impaired apical AQP9-dependent cAMP-activated glycerol permeability. These observations suggest that CFTR is an important regulator of AQP9 and that the interaction among AQP9, NHERF1, and CFTR may facilitate the activation of AQP9 by cAMP. In the distal portion of the epididymis, water secretion, driven by CFTR-dependent chloride secretion, is an important step that helps control the final fluidity of the luminal content. Therefore, the coordinated regulation of AQP9 by CFTR would facilitate the fine control of water and solute transport in the male reproductive tract. It is important to note that out of all organs affected by cystic fibrosis, the epididymis and vas deferens are among the most seriously affected.^{147,151} Defective water transport might be the leading cause of the obstructive pathologies, followed by atrophy and infertility, which are observed in the epididymis and vas deferens of men with cystic fibrosis. It is possible that disruption of a functional complex involving AQP9, NHERF1, and CFTR might contribute to the pathogenesis of male infertility in cystic fibrosis. However, it was reported that AQP

deficient mice showed no signs of epididymal tubule dilatation or other histological abnormalities and the weight of the whole epididymis did not differ statistically from wild type mice.¹⁵² AQP9 deficient male mice were fertile, and the spermatozoa showed normal motility, although they demonstrated defective glycerol metabolism (markedly increased plasma glycerol and triglyceride levels).¹⁵²

Regulatory interactions between CFTR and HCO_3^- salvage transporters (NHE3, NBC3) and between CFTR and HCO_3^- secretory transporters (SLC26A3/DRA, SLC26A6/PAT1)

HCO_3^- is an important constituent of secreted fluids by many secretory epithelia in the respiratory, digestive, and reproductive systems, as it is not only the biological pH buffer but it also affects the solubility of macromolecules and ions in biological fluids.¹⁵³ The main function of the pancreatic duct is the secretion of fluid rich in HCO_3^- in response to feeding stimuli such as vasoactive intestinal peptide and secretin. Ductal HCO_3^- homeostasis and regulation is tightly controlled directly or indirectly through several luminal and basolateral transporters, such as Na^+/H^+ exchanger isoform 3 (NHE3), $\text{Na}^+-\text{HCO}_3^-$ cotransporter (NBC3), $\text{Cl}^-/\text{HCO}_3^-$ exchangers (SLC26 anion transporters, SLC26A3 also known as DRA, and SLC26A6 also known as PAT1).^{34,153} The importance of HCO_3^- homeostasis is evident from the marked reduction in Cl^- and HCO_3^- -dependent fluid secretion in the pancreatic juice of cystic fibrosis patients, which suggests that HCO_3^- secretion by the epithelial cells lining the pancreatic ducts appears to be critically dependent on CFTR activity.^{28,154}

CFTR regulates both HCO_3^- secretion and HCO_3^- salvage in secretory epithelia. At least two luminal transporters mediate HCO_3^- salvage, NHE3 and NBC3. When stimulated by protein kinase A, CFTR can activate HCO_3^- secretion and at the same time it can inhibit HCO_3^- salvage that takes place in the resting state.^{28,155,156} It was reported that stimulation of CFTR expressed in heterologous systems²⁷ and in the native submandibular gland and the pancreatic ducts²⁸ activates $\text{Cl}^-/\text{HCO}_3^-$ exchange. Activation of CFTR also inhibited NHE3 activity both in the native pancreatic duct and in heterologous systems.³⁵

Ahn et al. reported that CFTR physically and functionally interacts with NHE3 in both transfected PS120 fibroblasts and pancreatic duct.³⁵ They observed that CFTR, NHERF1, and NHE3 were colocalized in the luminal membrane of the mouse pancreatic duct. Coimmunoprecipitation demonstrated that NHE3 and CFTR existed in the same multiprotein complex from PS120 cells expressing both proteins and the pancreatic duct of wild type mice but not from PS120 cells lacking CFTR or the pancreas of F508del-CFTR mice. The interaction between CFTR and NHE3 required the COOH-terminal PDZ binding motif of CFTR, and mutant CFTR proteins lacking the C terminus were not coimmunoprecipitated with NHE3. Functionally, CFTR was shown to regulate NHE3 activity via both acute and chronic mechanisms: 1) CFTR acutely augments the cAMP-dependent inhibition of NHE3, and 2) CFTR increases expression of NHE3 in the luminal membrane of the pancreatic duct. However, NHERF1 was required for cAMP-induced inhibition of NHE3 in PS120 fibroblasts.⁵⁶ Interestingly, wild type CFTR, but not CFTR mutants lacking the C-terminal PDZ binding motif, when coexpressed with NHE3 in PS120 cells, further augmented cAMP-dependent inhibition of NHE3 activity by 31%, beyond the inhibition of NHE3 activity observed in cells expressing NHE3 and NHERF1 alone. Such a regulatory interaction may be mediated by binding of CFTR and NHE3 to NHERF1 or other related PDZ scaffolding proteins. Formation of the CFTR-NHE3 complex was dependent on an intact PDZ binding motif of CFTR, NHERF1, as well as CFTR, which were all colocalized at the luminal pole of pancreatic ducts. NHERF1 has two PDZ domains and an ERM-binding domain. Separate reports showed that the first PDZ domain of NHERF1 preferentially binds the C terminus PDZ-motif of CFTR⁷⁹ and that NHE3 interacts with NHERF1 via a nontraditional association that requires the second PDZ domain and the C-

terminal ERM binding domain of NHERF1.¹⁵⁷ The NHERF1-associated protein, ezrin, possesses an amphipathic helix that can associate *in vitro* with the regulatory subunit of protein kinase A.¹⁰⁸ Therefore, it is very possible that protein kinase A may also be contained within CFTR-NHERF1-NHE3 protein complexes by association with ezrin or other cellular A kinase anchoring proteins. CFTR was also shown to increase NHE3 expression in the luminal membrane of the pancreatic duct. Luminal NHE3 expression was reduced by 53% in ducts of homozygous F508del-CFTR mice, with a corresponding decrease by 60% in the luminal Na⁺-dependent recovery from an acid load in ducts of F508del-CFTR mice. Of most importance is the observation that the pancreatic duct of F508del-CFTR mice of all ages showed reduced NHE3 activity, suggesting that reduction in NHE3 expression in the F508del-CFTR mice occurs early in development rather than being an adaptive response to the lack of function of CFTR.³⁵ The age-independent reduction in NHE3 expression in ducts of F508del-CFTR mice suggests that the most plausible mechanism for control of NHE3 expression by CFTR is stabilization of NHE3 protein expression. CFTR may increase transcription of NHE3 mRNA or the half-life of NHE3 mRNA to increase expression of the protein. Alternatively, by forming a complex including CFTR, NHERF1, and NHE3, CFTR may enhance the stability of the expressed NHE3 by either preventing its degradation or by enhancing its delivery to the luminal membrane of the pancreatic duct.

The same group extended their above-described observations by demonstrating that another HCO₃⁻ salvage transporter, human Na⁺-HCO₃⁻ cotransporter isoform 3 (NBC3), might also be included in this same or similar complex in the HCO₃⁻ salvage mechanism.³³ They found another interaction between NBC3 and CFTR *in vivo* and in heterologous systems, suggesting that CFTR regulates NBC3 activity in a manner similar to the regulation of NHE3 activity by CFTR. In their study, they observed that CFTR stimulation with forskolin substantially inhibited NBC3 activity, and this inhibition could be prevented by the inhibition of protein kinase A. NBC3 and CFTR, together with NHERF1, were coimmunoprecipitated from transfected HEK cells and from the native pancreas and submandibular and parotid glands. In addition, the deletion of the C-terminal PDZ binding motifs of CFTR or hNBC3 prevented coimmunoprecipitation of the proteins and inhibition of hNBC3 activity by CFTR. They concluded that CFTR and NBC3 reside in the same HCO₃⁻-transporting complex with the aid of PDZ domain-containing scaffolds, and this interaction is essential for regulating NBC3 activity by CFTR. Importantly, not only are CFTR and NBC3 expressed in the luminal membrane of secretory cells,^{155,158,159} they could be coimmunoprecipitated, indicating that the two proteins also interact *in vivo*.³³

Recently, many studies have demonstrated that CFTR also functionally and physically interacts with members of the SLC26 Cl⁻/HCO₃⁻ exchangers, such as SLC26A3/DRA and SLC26A6/PAT1, which are expressed in the luminal membrane together with CFTR.^{30,160} The activation of apical Cl⁻/HCO₃⁻ exchange by cAMP was initially shown to depend on the presence of functional CFTR in pancreatic duct cells. Stimulation of CFTR with cAMP increased Cl⁻/HCO₃⁻ exchange activity both in model systems of cells stably or transiently transfected with CFTR and in cells naturally expressing CFTR (T84 cells).^{27,28} More importantly, stimulation of CFTR by cAMP resulted in selective activation of luminal Cl⁻/HCO₃⁻ exchange activity in submandibular gland and pancreatic ducts of WT mice, tissues that express high levels of CFTR in the luminal membrane, which was absent in ducts prepared from $\Delta F/\Delta F$ mice. CFTR regulates luminal Cl⁻/HCO₃⁻ exchange activity of submandibular gland and pancreatic ducts and probably other CFTR-expressing cells.²⁸ Recently, Ko et al.³⁰ reported a reciprocal regulatory interaction between the SLC26A3/DRA, SLC26A6, and CFTR. DRA dramatically activated CFTR by increasing its overall open probability 6-fold. Like CFTR, the carboxyl terminus of SLC26A3 and SLC26A6 bind to PDZ domains of NHERF1 and NHERF2.^{161,162} These PDZ-binding proteins help

maintain an assembly of SLC26 transporters and CFTR channels, which functions as an HCO_3^- -transporting complex in the apical membrane.¹⁶³ Within the microdomain, SLC26 transporters and CFTR were shown to interact through the STAS domain (a conserved domain in the C-terminal tail of all SLC26 transporters¹⁶⁴) of the SLC26 transporters and the R domain of CFTR, thereby mutually enhancing each other's transport activity.^{29,30} Recombinant STAS domain has been shown to increase the CFTR current not only in CFTR-expressing HEK293 cells but also in mouse parotid duct cells.³⁰ The regulatory interactions between the R domain of CFTR and the STAS domain of the SLC26 transporters that is facilitated by their PDZ ligands substantially activates both transporters to increase CFTR channel open probability and DRA activity. The findings provide a new mechanism for regulating CFTR activity, epithelial chloride absorption, and bicarbonate secretion and have important implications for both cystic fibrosis and diseases associated with SLC26 transporters (such as congenital chloride diarrhea with DRA/SLC26A3 mutation). Recently, this same group reported that SLC26A6 controls CFTR activity and ductal fluid and HCO_3^- secretion. However, deletion of SLC26A6 enhanced spontaneous and decreased stimulated fluid and HCO_3^- secretion into sealed intralobular pancreatic ducts, as well as resulted in dysregulation of CFTR activity by removing tonic inhibition of CFTR by SLC26A6.³¹ These findings reveal the intricate regulation of CFTR activity by SLC26A6 in both the resting and stimulated states and the essential role of SLC26A6 in pancreatic HCO_3^- secretion *in vivo*.

Most recently, Singh et al.⁸⁵ observed significantly reduced basal and FSK-stimulated duodenal HCO_3^- secretory rates in *Nherf1*^{-/-} mice *in vivo*. They demonstrated that the basal HCO_3^- secretory rate in the duodenum of anesthetized *Nherf1*^{-/-}, as well as *Nherf1*^{-/-} *Cftr*^{-/-} double-deficient mice, was dramatically reduced compared with wild type littermates, and the response to a maximally stimulatory concentration of FSK was strongly reduced, but still significant, in the *Nherf1*^{-/-} duodenum and virtually abolished in the *Nherf1*^{-/-} *Cftr*^{-/-} mice. This suggests a presence of NHERF1, which is essential for maximal stimulation of CFTR by an increase in intracellular cAMP in the murine duodenum. They also observed that *Nherf1*^{-/-} *Cftr*^{-/-} double-deficient mice displayed no significant stimulation of duodenal HCO_3^- secretion similar to that in *Cftr*^{-/-} mice upon FSK treatment, which shows that the NHERF1-dependent reduction of duodenal HCO_3^- secretion was caused by reduced CFTR activation.⁸⁵

In summary, the findings described above suggest that CFTR regulates the overall transcellular HCO_3^- transport by regulating the activity of all luminal HCO_3^- secretion and salvage mechanisms of secretory epithelial cells, at least partially facilitated/mediated through the PDZ adaptor proteins. A model was suggested to depict the physiological significance of regulating the overall Na^+ -dependent HCO_3^- salvage and secretory mechanisms by CFTR. Pancreatic ductal fluid and HCO_3^- secretion is stimulated by the G_s -coupled secretin or VIP receptors. Under basal conditions, CFTR is not phosphorylated and does not inhibit the Na^+ -dependent HCO_3^- salvage mechanisms. Therefore, HCO_3^- salvage is maximal and produces the small volume of the acidic pancreatic juice. When cells are stimulated, cellular cAMP is increased, and the CFTR-NHERF1-NHE3 complex and CFTR-NHERF1-NBC3 are formed, modified by other protein associations, or undergo a conformational change to allow regulatory inhibition of Na^+ -dependent H^+/OH^- fluxes by CFTR. At the same time, CFTR stimulates HCO_3^- secretion by activating a $\text{Cl}^-/\text{HCO}_3^-$ exchange process mediated by SLC26 transporters SLC26A3/DRA and/or SLC26A6/PAT1^{27,154} in the luminal membrane of the pancreatic duct.²⁸ The overall result is production of an alkaline pancreatic juice. These findings reveal that CFTR controls overall HCO_3^- homeostasis by regulating both pancreatic ductal HCO_3^- secretory and salvage mechanisms.

However, at least two outstanding issues remain: How can CFTR regulate so many transporters and transport functions? How can NHERFs, which have only two PDZ domains, and CAL and Shank2, each of which has only one PDZ domains, mediate so many interactions? CFTR is probably a central member of a protein complex in the luminal membrane of secretory epithelial cells. There might be more than one scaffolding protein involved in assembling the complex, as secretory cells express more than one scaffolding protein in their luminal pole that can bind CFTR. Furthermore, the complex probably contains more than one scaffolding protein, similar to other multiprotein complexes as found in the postsynaptic density and in caveolae.^{165,166} Thus, at present, it is not clear how the HCO_3^- transport complex is assembled and whether CFTR and NBC3 and/or NHE3 bind to the same scaffold. Nonetheless, their ability to bind to PDZ binding domains is one mechanism by which CFTR can function as an HCO_3^- sensor governing the activity of the HCO_3^- transporting complex.

Concluding remarks

In summary, CFTR interacts both functionally and physically with a wide variety of proteins that function as transporters and channels in many other important biological functions and processes. Protein-protein interactions that influence the expression or functional activity of CFTR channel at the plasma membrane have significant physiological importance, as this channel not only transports Cl^- and HCO_3^- but also regulates the activities of many other transporters and channels. Studies suggest that CFTR protein-protein interactions not only play a critical role in certain signaling cascades, but also would provide additional layers of regulation for the channel activity beyond those provided by the cAMP pathway *per se*.^{73,83,84} 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 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 epithelial cells.⁵

Although there is an explosion of knowledge regarding identifying and elucidating the macromolecular signaling complexes that regulate CFTR activity, it is clear that we have only scratched the surface of CFTR regulation. To understand completely the functional regulation of CFTR and to identify novel and effective targets for cystic fibrosis therapy, we must unveil all of the CFTR-interacting proteins and understand how these proteins are dynamically regulated by protein-protein interactions and by posttranslational mechanisms, including phosphorylation, dephosphorylation, and ubiquitylation. It will be even more physiologically significant if we can fully understand how CFTR mutations affect the formation and localization of macromolecular signaling complexes and whether alterations in signaling-complex formation account for the numerous phenotypic changes that are observed in patients with cystic fibrosis.⁷ Toward these ends, mass spectroscopy will be particularly useful, especially in identifying the CFTR interactome for wild type and mutant forms of CFTR protein. The identification of the CFTR-binding partners will show how CFTR functions as an ion channel, as well as a regulator for other transporters and channels, which will not only help to identify new drug targets for cystic fibrosis and other diseases resulting from CFTR dysfunction, but also provide insights into the etiology of many diseases that are linked to other ABC transporters.⁷

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Fig. 1. Putative CFTR topology and its interactions with various binding proteins

CFTR is a member of the ABC transporter superfamily and consists of two repeated motifs, each composed of a membrane-spanning domain (MSD) containing six helices and a cytosolic nucleotide binding domain (NBD), which can bind and hydrolyze ATP. These two identical motifs are linked by a cytoplasmic regulatory (R) domain that 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 phosphorylation of the R domain by various protein kinases and by ATP binding to, and hydrolysis by, the NBD domain. Both the amino (NH₂) and carboxyl (COOH) terminal tails of this membrane protein are cytoplasmically oriented and mediate the interaction between CFTR and a wide variety of binding proteins.

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). 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 (cGK II), which in turn activates the CFTR chloride channel, resulting in Cl^- secretion into the lumen. As a consequence, Na^+ and water are effluxed 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 $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransporter (NKCC). K^+ recycles through basolateral K^+ channels, and Na^+ is pumped out of the cell by $\text{Na}^+-\text{K}^+-\text{ATPase}$ (adapted from ref. 4).

Fig. 3. PDZ domain-containing scaffolding proteins that can bind CFTR protein

Six different PDZ domain-containing scaffolding proteins have been reported to bind to the C-terminal tail of the CFTR protein mediated through their PDZ domains: NHERF1/EBP50, NHERF2/E3KARP, PDZK1/CAP70, PDZK2/IKEPP, CAL, and Shank2. CAL is primarily localized to 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 two 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 PDZK1 and PDZK2 contain four tandem PDZ domains, and PDZ3 and PDZ4 of PDZK1 are reported to bind two CFTR molecules simultaneously. CAL possesses only one PDZ domain and two coiled-coil (CC) domains that associate CAL to the membrane. In addition to one PDZ domain, Shank2 also contains other sites for protein-protein interaction, including an SH3 domain, a long proline-rich region, and a sterile alpha motif (SAM) domain. Shank2 is expressed abundantly in brain, as well as in kidney, liver, intestine, and pancreas, and is localized to the luminal pole in pancreatic duct cells and luminal area of colonic epithelia. 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 ref. ⁴).

Fig. 4. The spatiotemporal coupling of β_2 -adrenergic receptor signaling to CFTR channel function in the airway

CFTR, NHERF1, and β_2 AR form a macromolecular complex together with signaling molecules at the apical surfaces of airway epithelia. G proteins can be associated with β_2 AR 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 G_s 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_2 AR to the CFTR channel. Phosphorylation disrupts the complex, leading to the receptor-based activation of CFTR.

Fig. 5. The spatiotemporal coupling of LPA inhibition to CFTR-dependent Cl⁻ transport in the gut

LPA₂ and CFTR are physically associated with NHERF2, which clusters LPA₂ and CFTR into a macromolecular signaling complex at the apical plasma membranes of gut epithelia. This macromolecular complex is the foundation of functional coupling between LPA signaling and CFTR-mediated Cl⁻ transport. Upon LPA stimulation of the receptor, adenylate cyclase is inhibited through the G_i pathway, leading to a decrease in cAMP level. This decreased local or compartmentalized accumulation of cAMP results in the reduced activation of Cl⁻ channel in the vicinity by CFTR agonists (*e.g.*, adenosine) (adapted from ref. ⁷³).

Fig. 6. The spatiotemporal coupling of MRP4 cAMP transporter to CFTR Cl^- channel function in the gut

Underneath the apical plasma membrane in the gut epithelia, there exist highly localized compartments that are composed of a series of signaling molecules such as adenosine receptor (AR); G protein (G_s); AC; PKA and its anchoring proteins, AKAPs (not shown in this figure); CFTR; cAMP transporter (MRP4); and PDZ scaffolding protein (PDZK1), which functions to physically connect CFTR to MRP4. This macromolecular signaling complex provides an anatomical basis for the generating and modulating local cAMP compartments. When an agonist (such as adenosine) binds AR, a series of G-protein-mediated reactions leads to activation of AC present in the apical membrane. Sufficient cAMP is locally generated in a diffusionally restricted apical microdomain (but not in other cellular compartments). cAMP activates PKA, which is anchored also to the apical membrane by AKAP (*i.e.*, ezrin), and phosphorylates CFTR Cl^- channel in close vicinity, resulting in an increase of Cl^- currents. The CFTR-mediated Cl^- currents can be further increased (potentiated) through the additional increase of local cAMP resulting from the reduced or blocked efflux via a neighboring apical membrane cAMP transporter (MRP4) in the same subcellular compartment. The interaction between CFTR and MRP4 provides an additional layer of mechanism to regulate CFTR function, which is important in maintaining epithelial and body homeostasis (adapted from ref. ⁸⁴).