Regulation of Cl$^-$/$\text{HCO}_3^-$ Exchange by Cystic Fibrosis Transmembrane Conductance Regulator Expressed in NIH 3T3 and HEK 293 Cells*

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A central function of cystic fibrosis transmembrane conductance regulator (CFTR)-expressing tissues is the secretion of fluid containing 100–140 mM HCO$_3^-$, High levels of HCO$_3^-$ maintain secreted proteins such as mucins (all tissues) and digestive enzymes (pancreas) in a soluble and/or inactive state. HCO$_3^-$ secretion is impaired in CF in all CFTR-expressing, HCO$_3^-$-secreting tissues examined. The mechanism responsible for this critical problem in CF is unknown. Since a major component of HCO$_3^-$ secretion in CFTR-expressing cells is mediated by the action of a Cl$^-$/HCO$_3^-$ exchanger (AE), in the present work we examined the regulation of AE activity by CFTR. In NIH 3T3 cells stably transfected with wild type CFTR and in HEK 293 cells expressing WT and several mutant CFTR, activation of CFTR by cAMP stimulated AE activity. Pharmacological and mutagenesis studies indicated that expression of CFTR in the plasma membrane, but not the Cl$^-$ conductive function of CFTR was required for activation of AE. Furthermore, mutations in NBD2 altered regulation of AE activity by CFTR independent of their effect on Cl$^-$ channel activity. At very high expression levels CFTR modified the sensitivity of AE to 4,4$'$-diisothiocyanatostilbene-2,2$'$-disulfonate. The novel finding of regulation of Cl$^-$/HCO$_3^-$ exchange by CFTR reported here may have important physiological implications and explain, at least in part, the impaired HCO$_3^-$ secretion in CF.

Except for the sweat gland (1–3), all CFTR$^+$-expressing cells of various ductal systems absorb Cl$^-$ and secrete HCO$_3^-$ (4–6). Since the discovery that Cl$^-$ transport is defective in CF (7) and that CFTR functions as a Cl$^-$ channel (8), Cl$^-$ transport by CFTR-expressing tissues has been extensively studied (9). By contrast, transepithelial HCO$_3^-$ secretion is poorly understood (5), and little studied, even though HCO$_3^-$ secretion is impaired in CF (10).

In most CFTR-expressing tissues, HCO$_3^-$ secretion has electrogenic and electroneutral components (4–6). The electrogenic component is assumed to be mediated by an unknown HCO$_3^-$ channel or due to HCO$_3^-$ transport through CFTR itself (11, 12). The electroneutral component is assumed to be mediated by a Cl$^-$/HCO$_3^-$ exchange activity. However, direct evidence for a Cl$^-$/HCO$_3^-$ exchange activity in the luminal membrane is limited to the perfused pancreatic (13) and submandibular ducts (14).

Most models of HCO$_3^-$ secretion assume that CFTR and the luminal Cl$^-$/HCO$_3^-$ anion exchanger (AE) are indirectly coupled. In these models Cl$^-$ absorbed by the AE across the luminal membrane is secreted into the lumen by CFTR to support further HCO$_3^-$ secretion (4, 15). However, if Cl$^-$/HCO$_3^-$ exchange is unaltered in CF, such a mechanism cannot adequately explain the concomitant acidity of the secreted fluid and the impaired Cl$^-$ absorption observed in CF (16–18). If Cl$^-$/HCO$_3^-$ exchange is responsible for the bulk of Cl$^-$ absorption and HCO$_3^-$ secretion and CFTR function is required only for return of Cl$^-$ to the lumen, then in CF Cl$^-$ absorption should be normal (normal Cl$^-$/HCO$_3^-$ exchange) and the secreted fluid should be acidic due to the limited supply of luminal Cl$^-$. This is not the case (16–18). Alternatively, if Cl$^-$ reabsorption is singularly impaired in CF, the model predicts that the high Cl$^-$ concentration in the luminal fluid should increase HCO$_3^-$ secretion by AE to produce an alkaline fluid with high Cl$^-$ concentration. Again, this is not observed.

The observation that the luminal fluid is acidic with high Cl$^-$ concentration in CF (16–18) suggests that CFTR regulates HCO$_3^-$ secretion in CFTR-expressing tissues. CFTR could regulate the electrogenic, electroneutral, or both components of HCO$_3^-$ secretion. In the present work, we explored the existence of these regulatory mechanisms in cells stably or transiently expressing wild type (WT) or several mutated CFTR constructs. We report that a cAMP-activated CFTR regulates Cl$^-$/HCO$_3^-$ exchange activity in several experimental systems. Expression of CFTR in the plasma membrane was required for regulation of Cl$^-$/HCO$_3^-$ exchange, as expression of several folding mutants, including ΔF508, had no effect on Cl$^-$/HCO$_3^-$ exchange activity. Surprisingly, the Cl$^-$ conductive function of CFTR was not required for activation of Cl$^-$/HCO$_3^-$ exchange. Furthermore, mutations in NBD2 altered regulation of AE activity by CFTR independent of their effect on Cl$^-$ channel activity. At very high expression levels, CFTR modified the sensitivity of Cl$^-$/HCO$_3^-$ exchange to DIDS. The novel finding of regulation of Cl$^-$/HCO$_3^-$ exchange by CFTR reported here...
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**EXPERIMENTAL PROCEDURES**

**Culture of NIH 3T3 Cells**—Mock-transfected NIH 3T3 cells or NIH 3T3 cells stably transfected with WT or ΔF508 CFTR were kindly provided by Dr. Michael J. Welch (University of Iowa, Iowa City, IA). The cells were maintained in Dulbecco’s modified Eagle’s medium containing 10 mM glucose (DMEM-HG) and 10% fetal calf serum and plated on a sterile 22 × 40-mm coverslip at a density of 2.5 × 10$^5$ cells/cm$^2$ for intracellular pH (pH$_i$) measurements.

**Site-directed Mutagenesis**—The pCMVNo6.2 plasmids containing human WT or ΔF508 CFTR CDNA were a generous gift from Dr. Johanna Rommens (Hospital for Sick Children, Toronto, Canada). Oligonucleotide-directed mutagenesis using the GeneEditor mutagenesis kit (Promega, Madison, WI) was performed to generate the mutant CFTR in the expression vector pCMVNo6.2. Briefly, mutants were selected based upon the incorporation of a second-site mutation in β-lactamase, which alters its substrate specificity allowing resistance of transformed bacteria to cefotaxime and ceftriaxone in addition to ampicillin. Incorporation of the mutation was verified by DNA sequencing. The mutagenesis primers were as follows: P205S primer, 5′-CGT GTG GAC TAG GTC TCT CAC GCA ATC ATT CAG GA-3′; P846term, 5′-GAG ATT CCC GAC AGC AGT GAC TAT TTA ATG GAA CAG-3′. The PCR products were subcloned into the vector pCMVNo6.2, and multiple experiments were analyzed using paired or non-paired Student’s t-test as appropriate.

**Expression of WT and Mutant CFTR in HEK 293 Cells**—HEK 293 cells were maintained in DMEM-HG supplemented with 10% fetal calf serum, and plated on coverslips. On the following day, WT or mutant CFTR plasmids and green fluorescent protein (GFP)-expressing plasmids (Life Technologies, Inc.) were transfected into 293 cells using the FuGene mammalian transfection kit (Boehringer Mannheim) according to instructions provided by the manufacturer. Briefly, the mixture of plasmids and FuGene solution (pCMVNto6.2, 1.5 μg; pCMVGF, 1.5 μg; FuGene, 12 μl) was incubated in 100 μl of DMEM for 30 min before addition to the culture media. The cells were used for immunocytochemistry or pH$_i$ measurements 48–72 h after transfection.

**Immunocytochemistry**—HEK 293 cells transfected with expressing vectors were stained with a rat polyclonal anti-C-terminal CFTR antibody (StressGen Biotechnologies, Victoria, BC, Canada) and/or mouse monoclonal anti-rat Gluconolactone-1 (GIP) antibody (StressGen Biotechnologies, Victoria, BC, Canada) to determine their expression patterns using a published procedure (20). For double-labeling, primary and secondary incubations were repeated with antibodies against the second protein of interest. Images were obtained using a Bio-Rad MRC 1024 confocal microscope.

**RESULTS AND DISCUSSION**

**AE in Cells Stably Transfected with CFTR**—The first set of experiments to study regulation of AE activity by CFTR was performed in NIH 3T3 cells stably transfected and expressing high levels of CFTR protein. This particular model system has been used to extensively characterize the properties of CFTR Cl$^-$ channel activity (17). Mock-transfected cells of the same parental line were used as controls. Significantly, results identical to mock-transfected cells were obtained in cells stably transfected with ΔF508 CFTR (data not shown). A standard protocol of removal and addition of Cl$^-$ to the incubation medium buffered with HCO$_3^-$ was used to follow Cl$^-$/HCO$_3^-$ exchange activity. All the changes in pH$_i$ reported here were dependent on the presence of HCO$_3^-$ in the incubation media (data not shown). Fig. 1 illustrates the basic observation that CFTR-expressing cells exhibited a forskolin-dependent activation of the AE. Fig. 2 summarizes the results of 5–17 experiments under each condition. Removal of Cl$^-$ from the incubation medium of mock-transfected cells resulted in a slow and modest increase in pH$_i$, which was completely reversed on addition of Cl$^-$ to the medium. Stimulation of control cells with 5 μM forskolin had no effect on basal level of pH$_i$, or the pH$_i$ changes observed upon removal and readdition of Cl$^-$ (15). Finally, treating the cells with 0.5 mM DIDS, a blocker of Cl$^-$/HCO$_3^-$ exchange activity (24), nearly abolished pH$_i$ changes resulting from changes in transcellular Cl$^-$ concentration. These properties are commonly used to demonstrate Cl$^-$/HCO$_3^-$ exchange activity in cells (13, 14, 24). CFTR-expressing cells showed marginal statistical difference in Cl$^-$/HCO$_3^-$ exchange activity under resting conditions when compared with mock-transfected cells (p = 0.11), or cells stably transfected with ΔF508 CFTR (data not shown). Interestingly, Cl$^-$/HCO$_3^-$ exchange activity observed in resting cells expressing CFTR was inhibited by DIDS to the same extent as that measured in control cells (Fig. 2).

**Stimulation of CFTR-expressing 3T3 cells with forskolin caused a time-dependent intracellular acidification that was complete after 3 min of incubation at 37°C. This acidification was observed only in cells expressing CFTR in all experiments tested (n = 17) and was not inhibited by DIDS (n = 9). Furthermore, removal of Cl$^-$ from the incubation medium of forskolin-stimulated, CFTR-expressing cells caused a rapid and a large increase in pH$_i$ that was reversed upon readdition of Cl$^-$ to the medium (Fig. 1b). Fig. 2 shows that after forskolin stimulation the rate of pH$_i$ change due to changes in transcellular Cl$^-$ gradient in CFTR-expressing cells is 5-fold faster than that before forskolin stimulation in the same cells, or before and after forskolin stimulation in control cells. Thus, the increased rate of pH$_i$ changes required both expression of CFTR and activation of the protein by cAMP-dependent mechanisms. It is well established that CFTR-mediated Cl$^-$ channel activity is regulated by a CAMP-dependent phosphorylation (2, 7, 8). The finding that after forskolin stimulation, the pH$_i$ changes
due to changes in transcellular Cl\(^-\) gradient are resistant to inhibition by DIDS (Figs. 1 and 2) was unexpected. Since the same NIH 3T3 cell line was used to suggest that CFTR may function as a Cl\(^-\) and a HCO\(_3\)\(^-\)-permeable channel (25), we considered the possibility that the pH\(_i\) changes illustrated in Figs. 1 and 2 are due to CFTR functioning as a HCO\(_3\)\(^-\) channel. Several lines of evidence indicate that this is not the case. In contrast to Poulsen \textit{et al.} (25), in more than 10 experiments, we did not see any effect of HCO\(_3\)\(^-\) addition on pH\(_i\) of forskolin-stimulated acidified NIH 3T3 cells (using the protocol of Fig. 1 in Ref. 25). Furthermore, depolarization of the plasma membrane with 5 mM Ba\(^{2+}\) (data not shown), 100 mM K\(^+\) (see Fig. 10) or 125 mM K\(^+\) (data not shown) had no effect on the changes in pH\(_i\) observed on removal and addition of Cl\(^-\) as would be expected if CFTR was functioning as a HCO\(_3\)\(^-\) channel. Additional evidence that CFTR conductance was not responsible for the pH\(_i\) changes in Figs. 1 and 2 is provided by testing the effect of inhibitors of CFTR Cl\(^-\) channel activity. Fig. 3 shows the effect of 100 \(\mu\)M DPC and 100 \(\mu\)M glibenclamide on Cl\(^-\) channel activity of CFTR-expressing cells. At 100 \(\mu\)M these blockers inhibited CFTR-dependent Cl\(^-\) current by at least 90\% (Fig. 3c). Notably, these blockers had no effect on the ability of CFTR to stimulate pH\(_i\) changes upon Cl\(^-\) removal or addition in a forskolin-dependent manner (Fig. 4).

The lack of effect imparted by changes in membrane potential and inhibitors of CFTR Cl\(^-\) channel activity strongly suggested that the pH\(_i\) changes observed on removal and addition of Cl\(^-\) are not mediated by an electrogenic pathway. Rather, it appears that expression and stimulation of CFTR by cAMP activated an electroneutral HCO\(_3\)\(^-\) transport mechanism. If this pathway transports Cl\(^-\) in exchange for HCO\(_3\)\(^-\), then the pH\(_i\) changes should be a function of intracellular Cl\(^-\) content. Fig. 5 shows the protocol used to test this prediction. The cells were first treated with DIDS to prevent the initial changes in pH\(_i\).
due to Cl⁻ removal. Then the cells were incubated in a HCO₃⁻-buffered, Cl⁻-free medium for 1 min (Fig. 5a), 60 min (Fig. 5b), or various times between 5 and 30 min (data not shown) to deplete intracellular Cl⁻. The cells were then stimulated with forskolin to activate CFTR and, thus, Cl⁻/HCO₃⁻ exchange. Progressive depletion of intracellular Cl⁻ resulted in a graded inhibition of forskolin-activated pHᵢ increase (Fig. 5). Readdition of Cl⁻ to the incubation medium resulted in a pronounced acidification, as expected from HCO₃⁻ᵢ/Cl⁻ᵢ₀ exchange. Removal and readdition of Cl⁻ in these cells showed the expected changes in pHᵢ (Fig. 5). In additional experiments we incubated the cells in a HEPES-buffered, Cl⁻-free medium for 30–60 min to deplete intracellular Cl⁻. Such incubations were as effective in inhibiting the effect of forskolin on pHᵢ in the presence of HCO₃⁻ as the incubation in HCO₃⁻-buffered, Cl⁻-free medium shown in Fig. 5b (data not shown).

Expression and Localization of WT CFTR and CFTR Mutants in HEK 293 Cells—All the experimental protocols used to identify the HCO₃⁻ transporter activated by forskolin in CFTR-expressing 3T3 cells except for the lack of inhibition by DIDS point to a Cl⁻/HCO₃⁻ exchanger. These include (a) requirement for a HCO₃⁻ gradient, (b) requirement for a Cl⁻ gradient, (c) independence from Na⁺ᵢ₀, (d) electroneutrality, and (e) insensitivity to Cl⁻ channel blockers. A possible explanation for these observations is that high level expression of CFTR in 3T3 cells activated the anion exchanger and modified its sensitivity to DIDS. To test this hypothesis and provide additional evidence for regulation of anion exchange by CFTR, we examined the effect of transient expression of WT and mutant CFTR on anion exchange in HEK 293 cells. To identify the transfected cells and evaluate the extent of protein expression, the cells were co-transfected with GFP and the various CFTR plasmids.

Many CFTR mutants, including some used in the present work, are known folding mutants (26, 27) that are rapidly degraded by the ubiquitin-dependent proteasome system (28) before substantial amount of the protein reaches the plasma membrane. Therefore, we first determined the expression and localization of the CFTR mutants used in the present work. To
Fig. 5. Dependence of pH_{i} changes on intracellular Cl^{-} content. NIH 3T3 cells stably transfected with WT CFTR and incubated in HCO_{3}^{-}-buffered media were treated with 0.5 mM DIDS before incubation in Cl^{-}-free medium (a and b). After 1 min (a) or 1 h (b) of incubation in Cl^{-}-free medium, the cells were stimulated with 5 μM forskolin while still in Cl^{-}-free medium. Approximately 3 min after forskolin stimulation, the cells were incubated in Cl^{-}-containing medium, which caused a rapid reduction in pH_{i}. Subsequently the cells were subjected to another round of incubation in Cl^{-}-free and Cl^{-}-containing media while still incubated with 0.5 mM DIDS and stimulated with 5 μM forskolin. Similar results were observed in at least three experiments under each experimental condition.

WT CFTR and Anion Exchange Activity in HEK 293 Cells—

The WT CFTR and GFP constructs were used to determine whether expression of CFTR in 293 cells affected AE activity as observed in stably transfected NIH 3T3 cells (Figs. 1–5). Fig. 7 shows representative traces, and Figs. 8 and 9 summarize the results of multiple experiments. In these experiments GFP fluorescence was measured prior to loading with BCECF.

Expression of CFTR in 293 cells was sufficient to increase the DIDS-inhibitable pH_{i} increase upon removal of external Cl^{-} and prior to stimulation with forskolin. The increase in AE activity in unstimulated cells was statistically significant only at high levels of WT CFTR expression (Fig. 8; p = 0.018). Stimulation of cells expressing moderate or high levels of WT CFTR with forskolin caused an initial acidification, as was observed in NIH 3T3 cells (Fig. 1). Stimulation with forskolin resulted in a rapid rise in pH_{i} that was significantly greater than the unstimulated control (Fig. 8).

DIDS influx caused a rapid reduction in pH_{i} (Fig. 8). Subsequently the cells were subjected to another round of incubation in Cl^{-}-free and Cl^{-}-containing media while still incubated with 0.5 mM DIDS and stimulated with 5 μM forskolin. Similar results were observed in at least three experiments under each experimental condition.
dramatically increased AE activity and the increased activity (n = 16) correlated with the extent of WT CFTR expression (Fig. 8). Figs. 7d and 9 show that inhibitors of CFTR Cl$^{-}$ current, DPC and glibenclamide, had no measurable effect on AE activity after stimulation with forskolin. Again, these results are similar to those found in NIH 3T3 cells stably expressing WT CFTR (Fig. 4).

Of all known HCO$_3$ transport pathways (including HCO$_3$ conductance and the Na$^{+}$-HCO$_3$ cotransporters), only the AE is electroneutral and its activity is independent of Na$^{+}$ (24). Hence, as a further test for the HCO$_3$ transport activity stimulated by CFTR we determined the effect of membrane potential and external Na$^{+}$ on this activity using two experimental conditions. In the first set of experiments, cells incubated in HCO$_3$-buffered solutions in which all NaCl was replaced with KCl and all NaHCO$_3$ was replaced with choline-HCO$_3$. In these solutions, as needed, Cl$^{-}$ was replaced with gluconate using K$^{+}$-gluconate. To prevent intracellular acidification due to incubation of the cells in these Na$^{+}$-free solutions, all solutions also contained 5 mM Na$^{+}$-H$^+$ exchange inhibitor, ethylisopropyl-amiloride. Under these conditions removal of external Na$^{+}$ still caused substantial intracellular acidification, probably due to the activity of a Na$^{+}$-HCO$_3$ cotransporter (data not shown). However, the effect of removal and addition of Cl$^{-}$ was identical to those illustrated in Fig. 10 using the second experimental protocol. In these experiments external Na$^{+}$ was reduced from 140 to 40 mM, which removed the need to include ethylisopropyl-amiloride. The complete (Fig. 10a) or partial (Fig. 10b) resistance of the Cl$^{-}$-dependent pH$_i$ changes to 0.5 mM DIDS was preserved under high K$^{+}$ conditions. However, a detailed examination of the result in 293 cells show that the sensitivity of AE activity to inhibition by DIDS was a function of the level of WT CFTR expression. At moderate expression levels of WT CFTR, DIDS inhibited the activity by approximately 60%.

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**Fig. 7.** Effect of WT CFTR on AE activity of HEK 293 cells. HEK 293 cells were transiently transfected with WT CFTR and the effect of forskolin stimulation, 0.5 mM DIDS, or 0.1 mM DPC on AE activity was measured using the protocol of Fig. 1. Panel a shows the results obtained in cells transfected with pCMVGFP and the empty vector for CFTR and expressed high levels of GFP. Panel b shows the results obtained with cells expressing modest levels of WT CFTR as judged from the intensity of GFP fluorescence. Panels c and d show results obtained with cells expressing high levels of WT CFTR.

**Fig. 8.** Relationship between CFTR expression and AE activity. HEK 293 cells were transfected with empty vector and GFP (mock, open bars) or WT CFTR and GFP. Based on intensity of GFP fluorescence excited at 475 nm and measured at emission wavelengths of 530 nm, the cells were divided into two groups: medium (four experiments, hatched bars) and high (eight experiments, closed bars) expressors of WT CFTR (Fig. 4).

**Fig. 9.** Pharmacological characterization of CFTR-regulated AE activity. HEK 293 cells transiently transfected with high levels of WT CFTR were stimulated with 5 μM forskolin, and the protocol of Fig. 7 was used to evaluate the effect of 0.1 mM DPC, 0.1 mM glibenclamide (Glib), or 0.5 mM DIDS on pH$_i$ changes induced by removal and addition of Cl$^{-}$. The figure shows the mean ± S.E. of the number of experiments performed. DIDS inhibited the activity by approximately 60%.

AE activity after stimulation with forskolin. Again, these results are similar to those found in NIH 3T3 cells stably expressing WT CFTR (Fig. 4).
In conclusion, we believe that the combined results in NIH 3T3 and HEK 293 cells provide strong evidence for regulation of AE activity by CFTR. It is important to reiterate that such regulation required the activation of CFTR by cAMP but did not require Cl− transport by CFTR. The increased activity observed in non-stimulated cells expressing high levels of CFTR probably reflects tonic activation of CFTR in resting cells as a result of routine cell handling during an experiment.

**CFTR Mutants and AE Activity**—To begin to elucidate the mechanism by which CFTR domains regulate AE activity, the effect of several mutations in CFTR that have been previously characterized in terms of CFTR Cl− channel activity were assessed. Fig. 11 shows the results obtained with CFTR mutants that did not affect AE activity. ΔF508 and P205S CFTR are known maturation mutants (26, 27) that do not reach the plasma membrane of 293 cells (Fig. 6). Hence, it was not surprising that they had no effect on AE activity. CFTR truncated at Asp-836 (between the R domain and NBD2) was reported to maintain Cl− channel activity when expressed in HeLa cells (31). However, expression of a similar construct truncated at Trp-846 in 293 cells was insufficient to activate AE (Fig. 11c).

Another series of mutations in NBD2 that are known to affect channel activity (Fig. 12) indicate that there is no correlation between Cl− channel activity and activation of AE, as predicted from the lack of effect of Cl− channel blockers. For example, the G1247D/G1249E CFTR double mutant was reported to have no Cl− channel activity (32), was expressed in the plasma membrane (Fig. 6i), and had no effect on AE activity (Fig. 12a). The K1250M CFTR mutant had increased channel activity (32), was expressed in the plasma membrane (Fig. 6j) and activated AE similar to WT CFTR (Fig. 12b). However, D1370N CFTR had nearly normal Cl− channel activity (32) and was expressed in the plasma membrane (Fig. 6j), but was unable to activate AE (Fig. 12c).

Taken together, the results presented here show that CFTR regulates Cl−/HCO3− exchange activity in stably transfected NIH 3T3 cells and transiently transfected HEK 293 cells. The anion exchange activity stimulated by CFTR has all the kinetic properties associated with anion exchange reported in many cell types (24). The only deviation was the relative insensitivity of the exchange activity to DIDS. However, expression of CFTR at high level was apparently responsible for this behavior. This finding highlights the need for caution when using cell lines and overexpression of CFTR to reach conclusions as to its function in native tissues.
Kinetic, pharmacological, and molecular data indicate that it is highly unlikely that the Cl−/HCO3− exchange activated by CFTR was mediated by CFTR itself. This is concluded from the findings that inhibitors and mutants of CFTR Cl− channel activity had no effect on Cl−/HCO3− exchange activity stimulated by CFTR. More importantly, this lack of correlation demonstrates that transport of Cl− by CFTR was not needed to observe increased exchange activity, although CFTR had to be activated by a cAMP-dependent mechanism to exert its effect on the AE. Thus, an activated conformation of the protein was needed for activation of AE. This is further supported by the findings with the Δ508 and P205S CFTR maturation mutants, which showed that expression of CFTR in the plasma membrane, rather than mere expression of CFTR in the cells, was required for activation of AE. In this respect the results obtained with D1370N CFTR are of particular interest since this mutation in NBD2 did not ablate channel activity (32) but eliminated regulation of AE activity by CFTR. Recently, it was reported that the D1506A mutation in NBD2 did not ablate AE exchange activity by CFTR in the intestinal cell line T84 and, more importantly, in duct cells of the mouse submandibular gland and pancreas.2 In these studies, we also discuss the physiological significance of regulation of AE by CFTR.

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