Cystic Fibrosis Transmembrane Conductance Regulator Facilitates ATP Release by Stimulating a Separate ATP Release Channel for Autocrine Control of Cell Volume Regulation*

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These studies provide evidence that cystic fibrosis transmembrane conductance regulator (CFTR) potentiates and accelerates regulatory volume decrease (RVD) following hypotonic challenge by an autocrine mechanism involving ATP release and signaling. In wild-type CFTR-expressing cells, CFTR augments constitutive ATP release and enhances ATP release stimulated by hypotonic challenge. CFTR itself does not appear to conduct ATP. Instead, ATP is released by a separate channel, whose activity is potentiated by CFTR. Blockade of ATP release by ion channel blocking drugs, gadolinium chloride (Gd3+), and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), attenuated the effects of CFTR on acceleration and potentiation of RVD. These results support a key role for extracellular ATP and autocrine and paracrine purinergic signaling in the regulation of membrane ion permeability and suggest that CFTR potentiates ATP release by stimulating a separate ATP channel to strengthen autocrine control of cell volume regulation.

ATP and its metabolites function as potent autocrine and paracrine agonists that act within tissues to control cell function through activation of P2 purinergic receptors (1–3). ATP and a related triphosphate nucleotide, UTP, stimulate epithelial chloride (Cl−) channels alternative to CFTR via purinergic receptors (11–16). Supraphysiologically concentrations of ATP also stimulate CFTR (17). Metabolites of ATP can also act as Cl− secretagogues (15, 16, 18). Despite the diverse roles of purinergic signaling, the cellular mechanisms that govern ATP release are not fully defined. CFTR and related ATP-binding cassette (ABC) transporters such as mdr-1 or P-glycoprotein have been implicated as facilitators of ATP release in some cell models (14, 19–24), while other laboratories have failed to show evidence of CFTR-facilitated ATP conduction or release (25–30).

Release of ATP via a conductive pathway has been implicated as an essential autocrine regulator of cell volume in rat hepatoma cells (5). Moreover, ABC transporters have been shown to modulate volume-sensitive Cl− channels and cell volume (31–34). As such, we tested the hypotheses that CFTR facilitates ATP release under constitutive and hypotonic conditions for autocrine control of cell volume regulation. These hypotheses were also based on the fact that airway surface liquid on CF epithelia is hypertonic with respect to NaCl (35) and/or reduced in volume (36) or both (37, 38) when compared with non-CF epithelia. These airway surface liquid composition abnormalities may reflect an inability of CF epithelial cells to sense changes in external mucus environment and/or an inability of CF cells to regulate their own cell volume.

To this end, complimentary observations using a variety of techniques suggest that expression of CFTR enhances ATP release and modulates the dynamic relationship between cell volume, purinergic signaling, and membrane ion permeability. Given the cellular challenges related to changes in solute transport and maintenance of airway surface liquid, defective cell volume regulation in CF epithelia may underlie the pathogenesis of CF in the lung and airways.

MATERIALS AND METHODS

Cell Culture—The 16HBE14o− non-CF human bronchial epithelial cell line, the 9HTEo− non-CF human tracheal epithelial cell line, and the CBFBE41o− CF human bronchial epithelial cell line (homozygous for the AF508-CFTR mutation) were grown as described previously (39–42). COS-7 cells were grown in similar medium. Parental CFPA1-CF human pancreatic epithelial cells and CFPA1-1 cells complemented stably with the wild-type CFTR gene (CFPA1-PLJ-WT clone 20) were grown in similar medium without and with G418 (1 mg/ml; Cellgro/ Mediatech) (43). 3T3-CFTR fibroblasts expressing wild-type CFTR boxylic acid; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; ORCC, outwardly rectifying Cl− channel; ANOVA, analysis of variance.
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(3T3-WT-H7), expressing ΔF508-CFTR (3T3-ΔF508), or mock-transfected (3T3-Mock) were a generous gift from Michael Welsh (University of Iowa, Iowa City, Iowa, Howard Hughes Medical Institute) (44) and were grown in a similar medium except that a Dulbecco’s minimal essential medium with high glucose (Life Technologies, Inc.) was used as the basal medium. CFT-1 cells were grown in LHC-8 medium (Biofluids; Rockville, MD) without serum. For consistency, all CFTR cell models were grown on similar substrates and in similar media.

**Transient Transfection of COS-7 Cells with LipofectAMINE PLUS—**

COS-7 cells were seeded to 33% confluence 1 day prior to transfection with LipofectAMINE PLUS™ reagent (Life Technologies, Inc.) (45–47). To control for transfection efficiency and to correlate relative CFTR protein expression from culture to culture, triple transfections were performed with different CFTR mammalian expression vectors bearing wild-type or mutant CFTR together with green fluorescence protein- and luc-bearing mammalian expression vectors. Green fluorescence protein expression was assessed in each culture as was luc protein expression to standardize ATP release assays in the transient transfection cultures.

**Transient Infection of COS-7 Cells with Vaccinia Virus—**

These methods have been published previously (48).

**Bioluminescent Detection of Released ATP from Heterologous Cell Cultures and Epithelial Monolayers—**

The specifics of this assay have been published previously (42). Epithelial cells were seeded to near confluence on collagen-coated dishes. Epithelial cells were seeded at high density (at least 10⁵ cells per filter) onto collagen-coated permeable supports (Millicell).

**Analysis of Chloride and ATP Channel Activity in a Panel of CFTR Protein Preparations in Planar Lipid Bilayers—**

These methods have been published previously (49, 50).

**Coulter Counter Channelizer Analysis of Cell Volume—**

Mean cell volume was measured in cell suspensions by electronic cell sizing (Coulter Multisizer II, AccuComp software version 1.19, Hialeah, FL) using an 100-μm aperture. Cells in subconfluent culture were harvested with minimal trypsin (0.05%), suspended in cell culture medium, centrifuged for 1 min at 1,000 × g, resuspended in 3 ml of isotonic buffer, and incubated with gentle agitation for 30–45 min. Aliquots of cell suspension were added to 20 ml of isotonic or hypotonic buffer. Measurements of 20,000 cells on average at specified time points after exposure to isotonic or hypotonic buffer were compared with basal values in isotonic buffer (time 0). Changes in volumes were expressed as relative volume normalized to the basal period (5).

**SPQ Halide Permeability Assay—**

SPQ (Molecular Probes) was solubilized in cell culture medium at a concentration of 2 mg/ml and was loaded into cell by an overnight incubation. The protocol was 2 min in NaI Ringer followed by 2–3 min in NaNO₃ Ringer and then several minutes in NaNO₃ Ringer containing forskolin or diluted by 25% with NaI Ringer followed by 2–3 min in NaNO₃ Ringer and then several minutes in NaNO₃ Ringer containing forskolin or diluted by 25% with NaI Ringer. Responses were reversed fully in NaI Ringer. Specific concerning the SPQ assay and system have been published previously (48).

**Immunoprecipitation of CFTR Protein—**

Freshly grown epithelial cells or fibroblasts (a single 35-mm dish or 12-mm diameter filter confluent and/or tight to fluid) were lysed for 30 min at 4 °C in a buffer containing 20 mM HEPES, 150 mM NaCl, 1 mM EDTA, and 1% Nonidet P-40 (pH 7.4) supplemented with 1 mg/ml aprotinin, 1 mg/ml leupeptin, and 1 mg/ml 4-(2-aminoethyl)-benzenesulfonyl fluoride. These samples were immunoprecipitated with anti-CFTR antibody as described previously (47).

**Cellular Cyclic AMP Bioassay—**

These methods have been published previously (48).

**Data Analysis and Statistics—**

Data values were compiled into Microsoft Excel spreadsheets in which the mean ± S.E. was calculated for each time point in each set of experimental time courses. Data were then plotted in SigmaPlot for Windows using the same arbitrary light unit values. Statistics were performed using SigmaStat for Windows; paired Student’s t test or unpaired ANOVA with a Bonferroni ad-hoc test were performed as appropriate. A p value of <0.05 was considered significant.

**RESULTS**

**Wild-type (WT) CFTR Potentiates and Accelerates Regulatory Volume Decrease (RVD)—**

To evaluate whether CFTR expression plays a role in cell volume regulation, cell volume recovery from swelling induced by hypertonic challenge (e.g. RVD) was assessed in cells stably expressing wild-type CFTR (WT-CFTR) versus ΔF508-CFTR (Fig. 1A). In all cells, hypertonic exposure

**FIG. 1.** Wild-type, but not ΔF508-CFTR, accelerates and potentiates RVD dynamics: dependence of RVD on CFTR, ATP release, and extracellular ATP signaling. **A.** Raw cell volume data illustrating the difference in RVD dynamics between wild-type CFTR- and ΔF508-CFTR-expressing fibroblasts (n = 3 for each cell type for this representative data set; n = 6 overall). Using a confidence interval of 0.99 for a linear regression, the slope of the line fit to the cell volume values measured from the peak of hypertonicity-induced cell swelling to the end of the RVD time course was measured to estimate the rate (* reflects p < 0.05 significance in slope of RVD dynamics by ANOVA). The slope of the wild-type CFTR RVD time course was −4.77 ± 10⁻², while the ΔF508-CFTR RVD slope was −2.93 ± 10⁻². B, percent RVD from the peak of swelling (2 min) to the 30-min time point following RVD is plotted (* reflects p < 0.05 significance by ANOVA). C, apyrase (1 unit/ml) blocks RVD in wild-type CFTR-expressing fibroblasts; this effect is rescued fully by ATPγS (25 μM) (n = 3 for each fibroblast clone). Note different expanded scale on the graph in C versus A.
caused an initial increase in cell volume to a similar degree that peaked at 2 min following challenge (Fig. 1A). However, expression of WT-CFTR accelerated the rate of RVD 2-fold when compared with fibroblasts devoid of CFTR (not shown) or expressing the ΔF508-CFTR mutation (Fig. 1, A and B). When expressed as percent recovery from peak swelling, expression of WT-CFTR increased the rate of volume recovery 2-fold (Fig. 1B). Taken together, these results show that wild-type CFTR accelerated and potentiated cell volume regulation.

**WT-CFTR-facilitated RVD Depends upon Extracellular ATP Signaling**—To assess whether the effects of CFTR expression on cell volume regulation are related to release of ATP, the effects of the ATP scavenger apyrase were determined. In fibroblasts expressing WT-CFTR, depletion of extracellular ATP by apyrase blocked RVD completely (Fig. 1C). Addition of ATPγS, a poorly hydrolyzable ATP analog resistant to apyrase, reversed this inhibition (Fig. 1C). These results suggest that extracellular ATP is required for RVD and for maintenance of cell volume.

**WT-CFTR Potentiates ATP Release under Basal Conditions**—To determine whether these effects of CFTR on volume regulation are related to ATP release, additional studies were performed using a luciferin-luciferase assay to quantify ATP release into the medium (Fig. 2A). Under basal conditions, WT-CFTR-expressing fibroblasts release 3-fold greater ATP than mock-transfected fibroblasts and 2-fold greater ATP than ΔF508-CFTR-expressing fibroblasts (Fig. 2A). Interestingly, ATP release was always detectable (above background), even in the absence of CFTR or in the presence of ΔF508-CFTR, suggesting that the ATP release mechanism is an entity separate from the ABC transporter itself.

Additional studies were performed to assess the effects of transient transfection of CFTR on ATP release using COS-7 cells. Mock-transfected cells had no detectable CFTR. WT-CFTR-transfected cells expressed both partially (band B) and fully glycosylated (band C) forms of CFTR, while ΔF508-CFTR-transfected cells had only the partially glycosylated band B form of CFTR (47, 51). To more carefully monitor the amount of transduction of a given heterologous cell with CFTR, COS-7 cells were transfected transiently with two different WT-CFTR constructs or a ΔF508-CFTR constructs (Fig. 2B). Similar results were also found in the fibroblast clones stably expressing WT-CFTR and ΔF508-CFTR (Fig. 2B). As in the fibroblast clones studied above, wild-type CFTR potentiated ATP release...
2.9 ± 0.5-fold when cultures were studied 48 h post-transfection, while ΔF508-CFTR failed to potentiate ATP release (1.2 ± 0.5-fold; mock-transfected and parental data normalized to 1-fold; n = 6–9). Taken together, these data show that wild-type CFTR potentiates ATP release from cells under basal conditions.

**WT-CFTR Potentiates and Sensitizes Hypotonicity-induced ATP Release**—Because WT-CFTR potentiates RVD and ATP release, we tested the hypothesis that CFTR may also potentiate ATP release triggered by an increase in cell volume after a hypotonic challenge. Hypotonic challenge to increase cell volume triggered an immediate and robust increase in ATP release from non-CF epithelia expressing WT-CFTR (Fig. 3A). As little as 4% dilution triggered a change in luminescence of Δ+10.9 ALU from non-CF epithelia (Fig. 3A). In sharp contrast, 33% dilution was required to stimulate any ATP release (only a Δ+0.7 ALU) from CFBE41o⁻ monolayers. These results were confirmed by another model of CF. In CFPAC-1 monolayers lacking CFTR, a large dilution of 24% was again required to elicit a small change (only a Δ+1.6 ALU) in ATP release (Fig. 3A). When CFPAC-1 monolayers are stably complemented with WT-CFTR, as little as 4% dilution is enough to trigger ATP release (Δ+6.8 ALU) (Fig. 3A). When challenged with more robust dilutions of the medium osmolality, 51% hypotonicity triggered a robust ATP release in WT-CFTR-expressing non-CF epithelia (Δ+48.7 ALU) and in WT-CFTR-complemented CFPAC-1 monolayers (Δ+33.0 ALU) (Fig. 3A). In contrast, 51% dilution with distilled water only stimulated small increases in luminescence in CFBE41o⁻ monolayers (Δ+3.0 ALU) and in CFPAC-1 monolayers (Δ+3.9 ALU) (Fig. 3A). Taken together, these results show that WT-CFTR potentiates...
ATP release into the apical medium and heightens the sensitivity of the apical membrane of the epithelium to hypotonic challenge and subsequent increases in cell volume.

To better define CFTR protein expression in these non-CF and CF airway epithelial cell lines, immunoprecipitation of CFTR protein was performed in parallel. Expression of the mature band C form of CFTR protein was demonstrated in the non-CF 16HBE140- cell line with a trace amount of the band B form (Fig. 3B). In sharp contrast, the CF human airway epithelial cell line (CFBE41o-) lacked any detectable CFTR protein (Fig. 3B). Characterization of the parental CFPAC-1 cells and the stably complemented CFPAC-1 clone have been published previously (43). Thus, these dynamic changes in ATP release correlate with expression of CFTR protein.

**WT-CFTR Potentiates Volume-sensitive ATP Release: Further Potentiation by Cyclic AMP in WT-CFTR-Expressing Fibroblasts**—Because the cyclic AMP-dependent protein kinase signaling cascade stimulates CFTR chloride channel (51) and because cyclic AMP stimulates ATP release in WT-CFTR-expressing cells (14, 23), we tested the hypothesis that protein kinase A activity modulates hypotonicity-induced ATP release.

A typical time course of the response to forskolin (2 μM) and to 33% dilution of the medium osmolality is shown in Fig. 4A. Forskolin stimulated ATP release transiently and only in fibroblasts expressing WT-CFTR (Fig. 4A). Hypotonicity stimulated ATP release in all three fibroblast clones, but most robustly in WT-CFTR-expressing fibroblasts (Fig. 4A). More intriguingly, pretreatment with forskolin potentiated volume-sensitive ATP release, but, again, only in WT-CFTR-expressing fibroblasts (Fig. 4B). More intriguingly, pretreatment with forskolin potentiated volume-sensitive ATP release, but, again, only in WT-CFTR-expressing fibroblasts (Fig. 4B), where values increased 3-fold above those in the absence of cyclic AMP (Fig. 4B). No such enhancement was observed in mock or ΔF508-CFTR-expressing fibroblasts (Fig. 4B). These results suggest that cyclic AMP and hypotonicity function in a synergistic manner to stimulate ATP release, but only in cells expressing WT-CFTR.

**CFTR Is Closely Associated with an ATP Release Channel That Appears Separate from the CFTR Channel Protein**—To further define whether CFTR conducts ATP or whether CFTR is closely associated with a separate ATP release channel, protein preparations containing or lacking WT-CFTR were analyzed for both Cl− and ATP− single channel conductance in planar lipid bilayers. The protein preparation used in these bilayer studies represents an epithelial cell protein extract from membrane vesicles derived from epithelial cells (49, 50). Consistent with the studies of Kopito and co-workers and of Bear and colleagues (28, 30), CFTR immunoprecipitated and highly purified from this epithelial vesicle protein and fused with planar lipid bilayers formed channels that conducted Cl− in 18 out of 18 preparations (Cl− conductance = 11 ± 2 picosiemens) in which channels were observed. DPC (200 μM) blocked CFTR Cl− channel activity, while DIDS or gadolinium chloride (200 μM each) had no effect on CFTR Cl− channel activity (data not shown). Conversely, an ATP conductance was observed in only 1 out of 18 preparations (ATP conductance = 22 picosiemens). We hypothesized that this one example of ATP channel activity may represent the fusion and activity of a contaminating protein that conducts ATP. In support of this latter hypothesis, less pure vesicle protein preparations containing CFTR and other proteins were also examined (Fig. 5A).

In this preparation, CFTR Cl− channels, outwardly rectifying Cl− channels (ORCCs), and ATP channels were observed frequently and in similar incidence (see example in Fig. 5A). In these recordings, ATP channel activity was recorded first in symmetrical 100 mM NaATP, so as not to have possible contamination by Cl− channel activity or by trace amounts of Cl− in the recording chamber. Subsequently, ORCC and CFTR Cl− channel activity were observed when ATP-containing solutions were replaced with Cl−-containing solutions (Fig. 5A). Addition of DIDS (300 μM) inhibited ORCCs, while DPC blocked residual CFTR Cl− channel activity (Fig. 5A). These results show that two different Cl− conductances and an ATP conductance are present in this bovine tracheal epithelial vesicle protein material that contains CFTR. However, because highly purified CFTR does not appear to reliably conduct ATP, this ATP conductance is likely not conferred by CFTR, but may be conferred by another anion channel protein.

We next asked the question: what if CFTR is removed from this material? Are CFTR Cl− channels and ATP channels lost or can this activity be dissociated? To answer these questions, CFTR was immunodepleted out of this material, and this preparation was fused with planar lipid bilayers. Importantly, an ATP conductance was still observed, even in the nominal absence of CFTR protein (Fig. 5B). In these experiments, a pres-
sure gradient applied to the membrane increased the open probability ($P_o$) of the ATP channel from 0.50 ± 0.10 to 0.80 ± 0.10, while GdCl$_3$, a mechanosensitive ion channel blocker, decreased markedly the $P_o$ of ATP channels to 0.13 ± 0.05 (Fig. 5; $n = 6$). DPC and DIDS, broad specificity Cl$^-$ channel blockers, also inhibit this ATP conductance (data not shown). Taken together, the simplest interpretation of these data is that an anion channel distinct from CFTR plays a primary role in conferring the ATP conductance measured in this assay. However, the ATP conductance appears to be closely associated with CFTR. Because this channel is inhibited by gadolinium chloride and DPC and activated by a pressure gradient, this ATP-permeable channel may belong to the family of mechanosensitive anion channels. Although these data argue that CFTR itself is not likely to conduct ATP, the possibility cannot be ruled out that a regulatory cofactor required to induce CFTR to conduct ATP may be purified away from CFTR in these experiments.

**CFTR Expression, ATP Transport, Extracellular ATP Signaling, and Cell Volume Regulation Are Linked**—To provide a causal link between each of the entities described above, we tested the possibility that a mechanosensitive anion channel may be involved in conductive ATP transport out of cells and autocrine control of RVD. Focusing again on WT-CFTR-expressing fibroblasts, Fig. 6 shows the effect of ion channel-blocking drugs on basal and hypotonicity-induced ATP release and on RVD dynamics. DIDS, a broad specificity Cl$^-$ channel and transporter blocking drug, was used in lieu of DPC, because DPC has inhibitory effects on the luciferase detection reagent while DIDS and GdCl$_3$ do not (see Fig. 6 legend for data). The presence of DIDS blocked fully basal and hypotonicity-induced ATP release (Fig. 6A, left panel). DIDS also blocked RVD and caused cells to swell markedly (Fig. 6A, right panel). To confirm that DIDS was inhibiting RVD through effects on ATP release, ATP$_\gamma$S was added in the presence of DIDS. ATP$_\gamma$S rescued the effects of DIDS fully (Fig. 6A, right panel), consistent with an effect of DIDS as an inhibitor of autocrine ATP control of cell volume regulation. Similar experiments were performed with the mechanosensitive ion channel blocker, GdCl$_3$. GdCl$_3$ inhibited basal and hypotonicity-induced ATP release markedly (Fig. 6B, left panel). GdCl$_3$ also blocked RVD and caused cells to swell markedly (Fig. 6B, right panel). Again, exposure to ATP$_\gamma$S
were transiently transfected with WT-CFTR plasmid con-
est effects of
and hypotonicity pI
5
5
an intermediate response to both stimuli (forskolin pI
2.37)
versus
DIDS inhibited both basal and hypotonicity-induced ATP release fully (representative of six independent experiments). A: right panel, representative ATP release assay under isotonic and hypotonic conditions; DIDS inhibited both basal and hypotonicity-induced ATP release markedly (representative of nine independent experiments). B: right panel, RVD measurements in the absence of either drug, in the presence of DIDS, and in the presence of DIDS plus ATPγS (n = 3 each). DIDS blocked RVD; ATPγS rescued DIDS inhibition. B: left
panel, representative ATP release assay under isotonic and hypotonic conditions; GdCl3 inhibited both basal and hypotonicity-induced ATP release
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3 each). In contrast, DPC (200
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control, 189
6
M) inhibited luciferase activity 100-fold (data not shown).

**Fig. 6.** Ion channel blocking drugs inhibit hypotonicity-induced ATP release and RVD. WT-CFTR-expressing fibroblasts were assessed with both assays. A: right panel, representative ATP release assay under isotonic and hypotonic conditions; DIDS inhibited both basal and hypotonicity-induced ATP release fully (representative of six independent experiments). A: right panel, RVD measurements in the absence of either drug, in the presence of DIDS, and in the presence of DIDS plus ATPγS (n = 3 each). DIDS blocked RVD; ATPγS rescued DIDS inhibition. B: left
panel, representative ATP release assay under isotonic and hypotonic conditions; GdCl3 inhibited both basal and hypotonicity-induced ATP release
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3 each). In contrast, DPC (200
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control, 189
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M) inhibited luciferase activity 100-fold (data not shown).

**DISCUSSION**

**CFTR-dependent ATP Release and Signaling**—Potential interactions between ABC transporters and ATP signaling pathways have been difficult to define. The primary observations of these studies are that CFTR expression (1) enhances cell volume recovery from swelling or RVD, (2) stimulates both constitutive and volume-sensitive ATP release, and (3) facilitates
autocrine control of cell volume regulation via purinergic receptors. While the mechanism of ATP release is not fully defined, these effects appear to be due to CFTR-dependent modulation of a separate ATP channel. These results are in agreement with volume-sensitive ATP release and ABC transporter potentiation of that release in hepatocytes and biliary epithelial cells (5,52). These studies also synergize with evidence for other ATP channels besides CFTR. For example, because DIDS and DPC (2 units/ml each) (number of cells screened was 25; result representative of three independent transfections). E, comparison in 9HTE non-CF airway epithelial cells of hypotonicity-induced Cl\(^{-}\) permeability in the absence and presence of two different ATP scavengers, apyrase and hexokinase (2 units/ml each) (number of cells screened was 25; result representative of three independent sets of experiments). HYPO, hypotonic; P, cellular cyclic AMP levels. Based on these observations, our working hypothesis is that cell volume is sensed and transduced in an ABC transporter-dependent manner, leading to potentiation of ATP release through stimulation of a separate, yet tightly associated, ATP channel. In lipid bilayers, this ATP release pathway is stimulated by a transmembrane pressure gradient and is inhibited by the mechanosensitive ion channel blockers, the lanthanides (GdCl\(_3\) and LaCl\(_3\)), and the broad-spectrum Cl\(^{-}\) channel blockers, DIDS and DPC (Fig. 8). This working model is bolstered by the data that DIDS and GdCl\(_3\) do not inhibit CFTR Cl\(^{-}\) channel activity. The finding that cyclic AMP potentiates volume-sensitive ATP release only in wild-type CFTR-expressing cells underscores a key regulatory role for CFTR in this process. Because the sensitivity as well as the magnitude of extracellular ATP release is lost in CF epithelia or in heterologous cells that lack wild-type CFTR, it is attractive to speculate that the sensor for hypotonicity may be related to CFTR itself. The possibility, that CFTR as well as other ABC transporters may be “sensors” or receptors, has been suggested in a previous report (23).

ATP Release Channel Can Be Dissociated from CFTR—The specific cellular mechanisms whereby CFTR facilitates the release of ATP are not known and are limited by the lack of a molecular candidate other than CFTR (14, 19–30). Observations herein are consistent with the view that CFTR itself does not conduct ATP but, instead, is associated closely with a separate ATP-permeant channel that is expressed in both epithelial and heterologous cells. First, an ATP channel conductance was not reliably detected in a highly purified preparation of CFTR in lipid bilayers, consistent with other published work (28, 30). Second, in less purified systems containing many proteins, both Cl\(^{-}\) and ATP channels are observed. Third, ATP channels are still detectable after rigorous immunodepletion of CFTR. The fact that ATP channels are active independently of CFTR is intriguing and suggests that, in the bilayer, the cofactor regulatory machinery is no longer present. These findings are consistent with other studies that demonstrate that ATP release from intact cells is diminished but still detectable in the absence of CFTR. It is important to emphasize, however, that CFTR, as a member of the ABC transporter family, may transport ATP itself, albeit at rates that are nonconductive and more consistent with a transporter. Moreover, it is also possible that CFTR may transport ATP at conductive rates, in that purification of CFTR may have eliminated a regulatory protein that is essential for helping CFTR conduct ATP. These uncertainties underscore the need for identification of new molecular candidates for the ATP release pathway.

In this light, several anion channels beyond CFTR are candidates for ATP release channels. For example, because DIDS
inhibits ORCCs, because ORCC Cl\(^{-}\) channels are present in the
less purified protein material derived from bovine tracheal epithelial
membrane vesicles, and because ORCCs are stimulated
by membrane stretch (55), it is possible that ORCCs may
be inserted into the plasma membrane as ATP-filled vesicles
that fuse to release ATP by an exocytic mechanism (9).

**CFTR as a Cell Volume Regulator**—These considerations suggest a working model for ATP release and signaling that
places CFTR in a different context (Fig. 8). In non-CF cells, this
signaling system would be active under basal conditions, and
volume-sensitive ATP release would be augmented by hypotonicity
and would culminate in normal RVD in a manner governed
by CFTR at the apical plasma membrane (Fig. 8). However,
in CF cells with the \(\Delta F508\) mutation, the lack of functional
CFTR leads to impaired ATP release and a depletion of
this autocrine signaling system. The result is that cell volume
regulation would be desensitized or down-regulated under
both basal and hypotonic conditions (Fig. 8). Theoretically,
in the CF epithelium, this could lead to an impaired ability
to maintain normal cell volume under basal conditions and
a decreased ability to RVD under hypotonic conditions. Indeed,
abnormalities in ionic and osmotic strength and/or volume of
CF airway surface liquid have been measured in multiple studies
(35–38). The fact that airway surface liquid appears regulated
in its depth and in its composition implies a regulatory
role of the airway surface epithelium itself and an involvement
of cell volume regulatory processes. Thus, dysfunction of RVD
mechanisms, cell volume regulation, or both due to defective
CFTR and a lack of extracellular autocrine ATP signaling may
contribute to the pathogenesis of CF lung dysfunction.

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